THE ECOLOGY OF GREAT DIVING BEETLES (*DYTISCUS* SPP.) IN THE SOMERSET LEVELS AND MOORS

SUBMITTED BY
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Dytiscus marginalis — 1, Male; 2, Female; 3, Eggs; 4, Pupa; 5, Larva attacking a tadpole; 6, *Hydrocharis caraboides*; 7, Its larva; 8, *Acilius sulcatus*, Female. (All nat. size.)

Illustration from Miall, L.C. (1895) The Natural History of Aquatic Insects. Macmillan and Co., London.

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> "*As the species of the same genus usually have, though by no means invariably, much similarity in habits and constitution, and always in structure, the struggle will generally be more severe between them, if they come into competition with each other, than between the species of distinct genera…. We can dimly see why the competition should be most severe between allied forms, which fill nearly the same place in the economy of nature; but probably in no one case could we precisely say why one species has been victorious over another in the great battle of life*."

[Charles Darwin (1859) *The Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London. (This quote appears on page 59 in the sixth edition of 1872)]

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ABSTRACT

Dytiscid beetles are significant predators in freshwater aquatic ecosystems, playing a major role in structuring macro-invertebrate communities in some habitats (Cobbaert *et al* 2010). Great Diving Beetles (*Dytiscus* spp.) can be among the top predators, yet more than one species may be present in a particular physical location, prompting questions regarding how the *Dytiscus* species co-exist. This study investigated *Dytiscus marginalis* Linneaus 1758 and the much rarer *Dytiscus dimidiatus* Bergsträsser 1778 which occur together in drainage ditch ecosystems in the Somerset Levels and Moors in the United Kingdom. Estimates of niche breadths were made in relation to seasonal activity patterns, habitat usage and prey in order to gauge the degree of specialisation displayed by the two species. Findings broadly supported the view that *D. marginalis* is more of a generalist species than *D. dimidiatus*, however, a considerable degree of niche overlap was shown to exist. Evidence was found of a stronger preference in *D. dimidiatus* for shaded watercourses and for sections of ditch with less extensive coverage of duckweed (*Lemna* spp.) in the early part of the season. There were indications of both inter-specific and intra-specific predation of larvae by adults and larvae of *Dytiscus* spp. A major challenge overcome during the study concerned how to distinguish the larvae of the two species. Molecular ecological techniques (RAPD, PCRs and gene sequencing) were compared with morphological means to determine species identity. A relatively simple molecular method was found to distinguish the species based on species-specific sequences within a short fragment of the mitochondrial cytochrome oxidase 1 (CO1) gene. This technique successfully identified 90% of 108 individual larvae tested whereas morphology-based analysis failed to resolve them. The implications for conservation practice arising from these observations are discussed in relation to *D. dimidiatus,* which is considered at risk in the UK.

WORD COUNT = 298

KEY WORDS: *Dytiscus dimidiatus*; niche breadth; niche overlap; larval predation; larval identification; CO1 gene.

STATEMENT: WORK NOT SUBMITTED ELSEWHERE FOR EXAMINATION

I hereby declare that this thesis has not been and will not be submitted in whole or in part to another university for the award of any other degree

Signed:  A F Serjeant

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I am fortunate to have received generous support from my employers, Somerset County Council, not only financially through the award of a training grant, but also in allowing me access to the County Council laboratory and giving me the flexibility in my working hours without which this study would have been impossible to undertake. Yanina Pelegri-Fairfax patiently supervised my use of the equipment and instruments in the laboratory and made many helpful suggestions regarding the DNA work. The authority also allowed me to make use of its aerial photography collection for purposes connected with the study. My colleague, Paul Silvers, was kind enough to spend a considerable amount of his own time digitising aerial photographs and manipulating the Council's Geographical Information System (GIS) to measure the degree of tree and shrub cover in the vicinity of study ditches.

I am grateful to Natural England and to the Somerset Wildlife Trust for their kind permission to sample from ditches on their land. The following paid staff, volunteer nature reserve wardens and others greatly assisted in the selection of study sites, many giving generously of their own time to show me around sites and to advise: Simon Beard; Mark Blake; Phil Holms; David Northcote-Wright; David Reid; Tony Smith and Bill Urwin. Tony Price of Somerset Environmental Records Centre searched the SERC database for *Dytiscus* records.

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Lastly, I would like to thank Pamela Serjeant, who has assisted me during much of the fieldwork and who took many of the photographs that are reproduced in the Appendices. Without Pamela it is very doubtful whether this work would have been completed at all. It has been difficult for me at times to balance the competing demands of full-time work and home life with those of postgraduate study, but Pamela has supported me patiently through all of the difficulties with patience, kindness and love.

To my wife,
Pamela Serjeant

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Chapter 1: Introduction

The fundamental aim of this study was to investigate the ecological niches occupied by separate but related species of Great Diving Beetle (*Dytiscus* spp.) in the Somerset Levels and Moors. This chapter introduces some key concepts and themes that appear throughout this thesis, including those of niche, niche breadth and niche overlap. An overview is provided of the genus *Dytiscus* and of current knowledge regarding the ecology and distribution of *Dytiscus* species at a European, UK and Somerset scale. A brief description is given of the Somerset Levels and Moors where all of the fieldwork for this study was conducted. In the last section the objectives of the study are summarised.

1.1 Key concepts and themes

1.1.1 Autoecology and insect conservation biology

Autoecological studies examine the relationship of a single organism or a single species to its environment. This thesis reports the results of an investigation into the comparative autoecologies of species of *Dytiscus* water beetle that occur in the Somerset Levels and Moors wetland, primarily focusing upon two species – the Great Diving Beetle *Dytiscus marginalis* Linnaeus, 1758 and the King Diving Beetle *Dytiscus dimidiatus* Bergsträsser, 1778. [The common names used here are as suggested in Sutton (2008) while specific names follow those in Nilsson (2011).]

Some species of insect can be serious economic pests or vectors of disease while others are recognised as important plant pollinators or providers of products useful to humans (e.g. silk, honey). For these reasons, the large majority of autoecological studies of insect species cited in introductory texts on entomology focus upon species of recognised direct economic or social significance to human beings. For example, the bibliography in Gullan & Cranston (1994) contains 188 references, of which 31 (17%) can be said to be autoecological in the sense of dealing with a single species, group of species or

genus. Nearly two thirds (19) of these articles are concerning taxa of economic or social significance.

Insect conservation biology is a relatively new discipline that has arisen in response to what is perceived to be a catastrophic global-scale loss of insect biodiversity [Wilson (1992), Samways (1994)]. Because of the nature and scope of the problem, research in the field of insect conservation has tended to be at an ecosystem or landscape scale, but, nevertheless, it is recognised that autoecological studies can provide valuable information. Samways (*Op. cit*.) emphasises the importance of autoecological studies in elucidating "*the different vulnerabilities of the various life stages of insects*" and, as an example of this, cites the work of researchers such as J. A. Thomas that established the crucial role of particular ant species in the life cycles of a range of Lycaenid butterflies including the endangered Large Blue butterfly, *Maculinea arion* Linneaus, 1758. An account of the successful project to re-introduce the Large Blue in the UK can be read in Thomas, Simcox & Clarke (2009).

1.1.2 Flagship and Indicator Species

Autoecological studies connected with the conservation of particular insect species have often concentrated upon 'flagship species' such as the Large Blue or on species that are thought to be indicators to some degree of environmental quality. It has been argued that water beetles, and, particularly, the larger aquatic Coleoptera can be both 'flagship' and 'indicator' species.

According to the World Wide Fund for Nature (WWF), a 'flagship species' is "*a species selected to act as an ambassador, icon or symbol for a defined habitat, issue, campaign or environmental cause*" (WWF 2011). In terms of water beetles as 'flagship species', Beebee & O'Neil (2005) put forward a case for the Great Silver Water Beetle - *Hydrophilus piceus* Linnaeus, 1758 - to be considered as a UK Biodiversity Action Plan (BAP) Priority Species on account both of its status as "*surely one of Britain's charismatic insects*" and because of its reliance upon a threatened and declining habitat – well-vegetated drainage ditches in coastal and floodplain marshes.

Several authors have recommended the use of data on water beetles for the evaluation of the conservation value of wetland sites, notably Foster & Eyre (1992) and Davis *et al.* (1987). Basing their view on the assumption that "*the number of predatory invertebrates at any site is an important indicator of environmental quality*", Foster & Eyre (*Op. cit*.) suggested that: "…*assessment of subgroups of predatory species, particularly the Odonata and aquatic Coleoptera, rather than the entire range of macroinvertebrate predators, should result in considerable savings in time and cost of a biological monitoring programme*."

One objective of this study is to investigate whether the Great Diving Beetles possess the characteristics that would make them good candidates as 'flagship' and/or 'indicator' species. This is explored partly in Chapter 7 in which there is consideration of whether the occurrence of either *D. marginalis* or *D. dimidiatus* is a good predictor of faunal diversity within a ditch. The suitability of Great Diving Beetles as indicator or flagship species is discussed in the final chapter.

1.1.3 Ecological niche, niche breadth and niche overlap

One concept that has special relevance in the context of this study is the 'ecological niche' - i.e. "*the limits, for all important environmental features, within which individuals of a species can survive, grow and reproduce*" [definition from Begon, Harper & Townsend (1996)].

A key objective of this study was to try to distinguish the ecological niches occupied by *D. marginalis* and *D. dimidiatus* and the extent to which these niches might be separated in the Somerset Levels. If this can be done, the answer may cast light on why one species (i.e. *D. dimidiatus*) is rare and limited in its distribution while the other is widespread and abundant. It is intended that any better understanding of the autoecology of *D. dimidiatus* arising from the study should be used in order to inform and influence conservation practice in the areas where the species still occurs.

Mathematically, a species' fundamental niche has been formalised due to Hutchinson (1957) as the n-dimensional hyper-volume within which occur all the points which correspond to a state of the environment that would allow the species to exist indefinitely. Factors such as predation and competition for resources mean that a species rarely occupies its whole fundamental niche, but, in any given situation occupies a proportion of it known as the 'realised niche', which may vary from one locality to another.

In niche theory, the axes on which the hyper-volume is plotted can be regarded as 'resource states' that may relate to food resources, habitat resources, natural or artificial sampling units or other ecological categories assigned by researchers [Colwell & Futuyama (1971), Krebs (1999)]. Some species are more specialised than others and have a narrower, more constricted ecological niche compared with more 'generalist' species. In terms of the Hutchinsonian hyper-volume, niche breadth "*is the "distance through*" *a niche along some particular line in niche space*" [Colwell & Futuyama (1971)]. A generalist species would tend to have a broader niche breadth along axes compared to a specialist species. *D. marginalis* is assumed to be a generalist species on the account of its being widely distributed in a diverse variety of still, freshwater habitats. One might expect that this species would have a wider measurable niche breadth than, for example, *D. dimidiatus* and this idea was investigated by calculating measures of niche breadth for the two species from data collected during the study (see Chapter 8).

Another niche metric that is commonly investigated in cases where there are resources that are thought to be shared by one or more species is 'niche overlap'. In Hutchinson's conceptual model this would be the region of niche space that was shared by the niches of two species [Colwell & Futuyama (1971)].

Schoener (1974) reviewed 81 studies of niche differences between species from a diverse range of taxonomic groups. He concluded that the variables that served most often to differentiate the niches of closely related species were, in

order of relative importance: macrohabitat; microhabitat; food size & type and seasonal and diel time. For the purposes of this investigation, aspects of each of these niche variables were examined and the following paragraphs serve to describe where each aspect is covered within this thesis.

One possible way in which ecological niches may be differentiated is temporally, with different species exploiting the same environmental resources, but at different times of the day or the year. Examples of seasonal niche differentiation have been observed in large, predatory insects such as species of mantids that co-exist in the same areas over large parts of their range [Hurd & Eisenberg (1990) cited in Begon, Harper & Townsend (1996)]. Variation in diurnal and nocturnal activity patterns have been observed across a range of carnivorous Ground Beetles (Coleoptera: Carabidae) in UK woodlands [Lovei & Sunderland (1996)]. The results of an investigation into seasonal activity patterns of *Dytiscus* spp. at sites within the Somerset Levels and Moors are presented in Chapter 5.

Niche differentiation can be spatial in its basis. Samways (1994) made the point that, in relation to insects, it is important to be clear about the spatial scale under consideration. A full description of niche for an insect might well need to take account of a variety of environmental factors operating at landscape-scale, on an individual site basis, within a particular habitat or habitats at a site and at the micro-habitat level. The biotic and abiotic factors that might influence distribution of *Dytiscus* species at each of these spatial scales across the Somerset Levels and Moors are examined in Chapter 6, in particular the effects of macro-habitat availability, woodland cover and degree of shading.

With regards to food size and type, a complicating issue when considering the predatory behaviour of aquatic coleoptera is the recognition that a particular species is likely to exhibit different food preferences at different stages in its life cycle. Thus, for example, the larvae of *Hydrophilus piceus* are reported to be “exclusively carnivorous” whereas the adult beetles are said to be omnivores (Sutton 2008). Any autoecological study of water beetle prey preference must

take this into account in its methodology. Experiments to try to elucidate prey preferences of both adult and larval *Dytiscus* species are reported in Chapter 7.

1.1.4 Competition and predation between *Dytiscus* species and their relationships to ecological niche

Abrams (1987) recognised six broad categories of species interaction addressed by ecologists: competition; predation; herbivory; mutualism; disease and parasitism. Abrams acknowledged that many authors believe parasitism and disease to be essentially identical categories of interaction and some regard predation as a component of this category also.

In this study the possibility has been considered of both competitive and predatory interactions between *Dytiscus* populations in the Somerset Levels and Moors and, specifically, between *D. dimidiatus* and *D. marginalis* populations.

With regards to interspecific competition, a distinction can be made between 'resource competition' (including 'scramble competition' and 'contest competition' [Nicholson (1954)] and also called 'exploitative competition' [Park (1954)]) and 'interference competition' (Birch 1957). In resource competition, organisms compete for resources that are in short supply compared with the number of animals seeking access to them. In interference competition the resources are not in short supply. In both cases, some harm is assumed to befall one or both of the populations competing, usually in terms of an increase in mortality or a decrease in reproductive success or both. In interference competition between two species, the population of both species is reduced - that of Species A due to the interference from Species B and that of Species B due to the costs to it of its interference with Species B. Interference can take many forms including allelopathy (production of noxious or poisonous chemicals) and predation of young – both of which may be mechanisms operating in beetle populations. Amarasekare (2002) has proposed some models for this kind of competition that provides a potential explanation for

patterns observed in several natural systems, including ones involving aquatic invertebrates.

The mathematical models of interspecific competition that have been developed, whether these are based on the classical Lotka-Volterra equations of the early twentieth century [Lotka (1925), Volterra (1926)] or they have some other basis [e.g. Tilman (1982,1990)], all tend to suggest that:

- Interspecific competition can lead to one species driving another to extinction, or;
- to a situation where populations of the two competing species co-exist in the same place, sometimes in a seemingly stable equilibrium.

In localities where *D. dimidiatus* and *D.marginalis* both occur it is possible to find at least the adults together in the same section of ditch or area of similar freshwater habitat. This is true of the Somerset Levels and Moors (T. Beebee *pers. comm.*) and it is true of other wetland sites in the UK [e.g. at Wicken Fen for example according to Friday & Preston (1997)]. It is thought that in situations where two closely related species occur in the same area there are likely to be strong competitive interactions between them. Birch (1957) traces this idea back to Darwin (1859) (the quotes that this attribution is based upon are given in the title pages of this thesis).

If the mechanisms of the competitive interaction between two species can be identified, it may be possible to discern whether the interaction represents 'resource competition', 'interference competition', or a mixture of both. However, in practice it is often difficult to establish that competition of any form is occurring between two species [Krebs (1999)]. Authors, such as Wiens (1989), have been critical of the readiness of some ecologists to identify competitive interactions without, as the authors see it, sufficient strong evidence. Wiens himself has proposed criteria for establishing if there is interspecific competition in a given situation. These criteria are given in Table 1.1 below.

Table 1.1: Wiens' criteria for establishing if inter-specific competition occurs between species [Source: Wiens (1989)]. The criteria are weighted from 1 to 5 in ascending order with 1 representing 'weak evidence' and 6 representing 'convincing evidence'

1. There are observed checkerboard patterns of distribution consistent with predictions
2. The species overlap in resource use
3. Intraspecific competition occurs
4. Resource use by one species reduces its availability to another species
5. One or more species is negatively affected
6. Alternative process hypotheses are not consistent with patterns

Later in this work (chapter 8) there will be an attempt made to draw some conclusions regarding possible competition between *D. marginalis* and *D. dimidiatus* using Wiens' criteria to decide whether there is a strong case to believe that competitive interactions are occurring.

Mention has already been made of the measurement of niche overlap. Many of the first ecologists who investigated niche overlap hoped that its measurement would enable them to identify instances where inter-specific competition was occurring and that the metric would help them to quantify the intensity of the interaction [e.g. Schoener (1974)]. However, there are theoretical reasons for believing that degree of niche overlap is not always a reliable indicator of inter-specific competition [MacArthur (1968), Abrams (1980), Holt (1987)].

So far as predation is concerned, *Dytiscus* species are portrayed in popular literature as "fearless" and "voracious" carnivores that tackle many different types of prey, including animals of a similar, or even, greater, size [e.g. Fitter & Manuel (1986)]. For the purposes of this study it was assumed that both larvae and adults of *Dytiscus marginalis* and of *D. dimidiatus* species would be likely to prey upon individuals of the same species or another *Dytiscus* sp. if the opportunity arose. This does not seem to be an unwarranted assumption given the evidence of cannibalism among other predatory aquatic invertebrates [e.g. Rajavel (1992)]. There is discussion within Chapter 8 about whether any data collected during the study supports the idea that either intra-specific or inter-specific predation actually occurs among the *Dytiscus* spp. living in the Somerset Levels and Moors.

For the purposes of biological monitoring and conservation, if the number of predatory invertebrate species at a site is to be taken as a reliable indicator of overall community diversity - as Davis *et al.* (1987) suggested – then there must be a degree of niche differentiation between the predators in terms of prey preferences. If all the predators shared an equal preference for one particular species of prey, and if that prey occurred in sufficient abundance within the site to support all of its predators, then a community could be envisaged comprising of a comparatively rich predatory fauna but an impoverished herbivorous fauna. In this situation, overall community species diversity would be lower than one in which a wide range of predators preyed upon a wide range of prey.

Where two closely related species are found co-existing in the same area, without obvious signs of decline in the fortunes of one species as opposed to the other, it is assumed usually that the two species have achieved equilibrium because they have partitioned the available environmental resources in such a way that intense inter-specific competition is avoided. Another way of stating this theoretical principle is to say that coexisting competitors exhibit a differentiation of realised niches.

1.1.5 Rarity and conservation status

Rabinowitz *et al.* (1986) distinguished seven different forms of rarity. Although their paper was written about the British flora, the seven forms of rarity that its authors identified are applicable to the study of insects [see Samways (1994)]. Table 1.2 summarises the paper's findings.

Table 1.2: Seven forms of rarity. Source: Rabinowitz *et al.* (1986) modified after Samways (1994)

Form	*Geographical range*	*Habitat (Biotope) Specificity*	*Local population size*
1	Wide	Broad	Somewhere large
2	Wide	Broad	Everywhere small
3	Wide	Restricted	Somewhere large
4	Narrow	Broad	Somewhere large
5	Narrow	Broad	Everywhere small
6	Narrow	Restricted	Somewhere large
7	Narrow	Restricted	Everywhere small

There are a number of points that arise from this categorisation:

- Not every rare species is in imminent danger of becoming extinct. A species that is rare in the sense of Form 1 may be very scarce in a particular locality but cannot be said to be threatened with extinction on a global scale. If a local population disappears there is a good chance that the locality may be re-colonised;
- Rarity may be due to a high degree of specialisation or reliance on a resource that is by its nature scarce. A classic example would be that of some cave insects which may display rarity in the sense of Form 3. Species that are able to tolerate only a restricted range of habitats or ecological conditions are termed ‘stenotopic’. A species which is ‘eurytopic’ can tolerate a wide range of habitats or conditions;
- For many species of insect, population size fluctuates considerably from year to year. This means that a rare insect species might move between categories within a relatively short space of time compared, say with a plant species. One corollary of this is that an insect species might become threatened within a locality within a very short timespan.

An objective of this study is to consider in what precise sense *D. dimidiatus* is ‘rare’ and what the consequences of this should be for conservation practice. The problem is addressed in the final chapter from the standpoint of different spatial scales – European, UK and local (Somerset Levels and Moors).

At the national scale, assessments of rarity and conservation status tend to be based on recorded occurrence within 10km squares (hectads) of the British Ordnance Survey National Grid. The most recent comprehensive review of the conservation status of the British beetle fauna [Hyman & Parsons (1992)] used the status categories given in Table A.1 in Appendix A1. These definitions of vulnerability and rarity are those used in this study, for example in the discussion below in section 1.3.3 of the status of the *Dytiscus* spp. within Britain.

1.2. Why study *Dytiscus* spp. water beetles?

Dytiscus spp. water beetles tend to be large and conspicuous members of the freshwater aquatic fauna [Beebee (1991)]. They are thought to be very significant predators in freshwater systems (see discussion in Chapter 7). *Dytiscus* spp. can be serious pests of commercial fisheries [Bhimachar & Tripathi (1966)] but also exert significant control over vectors causing disease in humans [Reddy *et al.* (1967), Nelson (1977), Rondelaud (1979)].

As well as being sizeable, *Dytiscus marginalis* seems to be relatively widespread and common throughout its range and, these traits combined with the ease with which it may be kept in aquaria [Barker (1984), Bauer (1984)] have made it a popular subject for scientific research. In the early 1920s Korschelt wrote two lengthy monographs on the species [Korschelt (1923), (1924)] and aspects of the biology of *D. marginalis* continue to be investigated into the present day. A search through one North American on-line bibliography of papers published on aquatic coleoptera to 1996 revealed nearly fifty references to papers on *D. marginalis* on a range of themes such as diel activity patterns, biochemistry, cellular biology, developmental biology, locomotory studies, genetics, sexual dimorphism, neurobiology, phenology, predatory behaviour and taxonomy (Jasper 1996).

Much less is published concerning the rest of Britain's resident *Dytiscus* species compared with the literature about *D. marginalis*. Far less information is available, for example, about *Dytiscus dimidiatus*. While there are 48 papers on aspects of the biology of *D. marginalis* in the on-line database cited above this compares with five on *D. dimidiatus*. Papers on the latter are predominantly about species records and taxonomic status rather than fundamental aspects of autoecology.

1.3 The genus *Dytiscus* – The 'Great Diving Beetles'

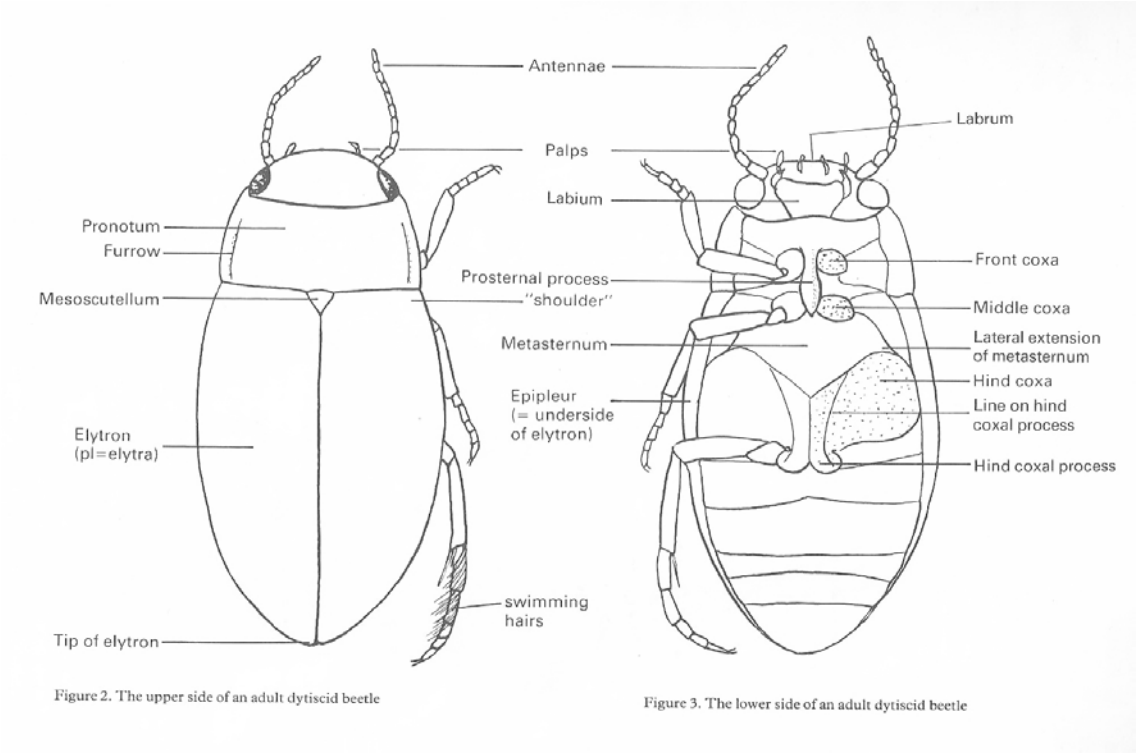
1.3.1 Characteristics of the genus *Dytiscus*

Adults of the genus *Dytiscus* Linnaeus, 1758 display clearly the characters that define the family Dytiscidae Leach, 1815: Threadlike antennae with 11 segments; long, flattened hind legs with five-segmented, tapering tarsi bearing swimming hairs and hind coxae with rounded, pointed or truncate processes. The metasternum may have a medial transverse groove or suture but this never extends as far as the middle coxa [Friday (1988), du Chatenet (2005), Foster & Friday (2011)]. These features are illustrated in Figure 1.1 on page 13.

The sub-family Dytiscinae Leach, 1815 comprises a group of large, dytiscid water beetles in which the adult males possess front tarsi with the middle sections modified into round or oval, flat plates equipped on the ventral surface with an array of rounded suckers [Friday (*Op. cit*.), du Chatenet (*Op. cit*.), Foster & Friday (*Op. cit*.)]. The tarsal plates (or 'palettes') perform an important function in the mating behaviour of Dytiscinine beetles as they help the males to cling tightly to the female. The suckers allow the male's fore legs to adhere to the smooth pronotum of the female [Blunck (1912, 1913), Adis (1974) both cited in Wichard, Arens and Eisenbeis (2002)].

Dytiscus species tend to be larger in size than others of the Dytiscinae (at least 20mm) and can be distinguished by having a visible mesoscutellum and equal-sized claws on the hind legs [Friday (1988)]. The females may have nine or ten deep groves on their elytra, however, this is not an entirely reliable diagnostic feature as the females of some species may occur as varieties with smooth elytra.

Figure 1.1 Dorsal and ventral views of an idealised dytiscid water beetle. After Friday (1988)



D. marginalis is the type species for the *Dytiscus* genus and sexual dimorphism within this species is indeed typical of many species in the group. Adult male beetles possess front tarsi in which three segments have become modified in the way described above in order to grasp females during copulation (see Figure 1.2 below).

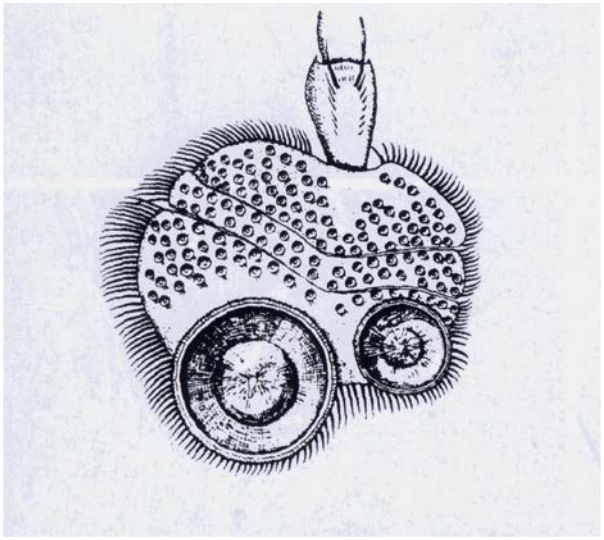


Figure 1.2: Tri-sectioned adhesive organ on the fore-leg of male *Dytiscus marginalis.* After Wesenberg-Lund (1943) reproduced in Wichard *et al.* (2002). The organ would be about 1 – 2 mm across in life.

The great majority of female *D. marginalis* have 'sulcate' elytra with deep grooves and this is in contrast to the male's wing cases that are invariably smooth. It has been postulated that this dimorphism has arisen as part of an 'evolutionary arms-race' between the sexes [e.g. Bergsten *et al.* (2001)].

Very occasionally, female *D. marginalis* are found with no sulci or only faint striations on the elytra. One form or variety of the species with this characteristic has been called '*conformis*' Kunze, 1811. The occurrence of smooth female varieties of *D. marginalis* has led to some taxonomic confusion with specimens wrongly identified as the female of a separate, new species (such as '*D. conformis').*

1.3.2 General life cycle

Dytiscus marginalis is the type species for the *Dytiscus* genus and there are many accounts of its life cycle in popular literature on natural history [e.g. Miall (1895), Clegg (1967), Fryer (1991)] as well as in more technical scientific literature [e.g. Wichard *et al.* (2002)].

Dytiscus species are reported to be univoltine in temperate regions (i.e. they have one brood of young per year) [Aiken & Wilkinson (1985)]. Both adults and larvae are carnivorous. In *Dytiscus marginalis*, eggs are laid in slits cut in the submerged portions of aquatic vegetation (Régimbart, 1875, cited in Miall, 1895).

Larvae within the family Dytiscidae vary significantly in their morphology and this variation seems related to the differing strategies adopted to capture prey [Wesenberg-Lund (1943), Galewski (1971)]. The larvae of *Dytiscus* species are all very similar in appearance and their characteristic form, with long legs with swimming hairs, is supposed to suit their lifestyles as relatively unspecialised, yet active predators. The eyes of these larvae are only moderately developed, so it is thought that prey location is primarily by tactile and/or chemical means.

Pupation takes place on land in a "roundish cell" excavated in moist earth beside water. Fryer (1991) reports that if pupation begins the summer adults can emerge within three weeks, but if it is delayed until later then the beetle can over-winter as a pupa. Individuals that pupate over winter emerge as adults at the onset of spring onwards [Miall (1895)]. *Dytiscus* beetles can also over-winter as adults. The adults may live for up to 18 months [Edgar & Shepley (2004)].

1.3.3 *Dytiscus* spp. in the Palearctic region and Europe

Nilsson (2011) distinguishes seventeen species of *Dytiscus* living in the Palearctic zoogeographical region. All these species are listed in Appendix A2 along with details of synonomy and distribution. Of the species listed, several, such as *Dytiscus delictus* Zaitzev, 1906 and *Dytiscis sinensis* Feng, 1935, have a distinctly Asian distribution with some, like *Dytiscus dauricus* Gebler, 1832, extending from East Asia into the Nearctic region. Other Great Diving Beetle species like *Dytiscus mutinensis* Branden, 1885 and *Dytiscus pisanus* Laporte, 1835 are confined to southern Europe and North Africa. From Nilsson's catalogue it is possible to identify a few species with very wide distributional ranges, such as *Dytiscus circumflexus* Fabricius, 1801 which has records reported from across Europe, North Africa and the Middle East.

Dytiscus marginalis is similar to *D. circumflexus* in respect of its being one of the more widespread *Dytiscus* species according to Nilsson's catalogue [Nilsson (*Op. cit*.)]. Two sub-species of *D. marginalis* have been distinguished: *Dytiscus marginalis marginalis* Linnaeus, 1758 and *Dytiscus marginalis czerskii* Zaitsev, 1953. The latter has an eastern distribution being recorded from China and the Russian Far East. *D. d. marginalis* is found as far west as Ireland, as far north as Northern Russia and Finland and as far south as Spain, Italy and the Balkans (including Greece). Some distributional details are given in Table A.2 in Appendix A2, while maps of the distribution within Western Europe of selected species are reproduced in Appendix A3.

Dytiscus dimidiatus has a similarly wide distribution as *D. marginalis* although there are fewer countries in which it is found (see Table A.2 in A2 and Appendix A3). Perhaps significantly, the species is not found in Ireland and is at the edge of its westerly range in South West England and South Wales. This has prompted some authorities such as Balfour-Browne to suggest that *D. dimidiatus* is extending its range westwards and is a relatively recent colonist of Britain [Balfour-Browne (1950)].

1.3.4 *Dytiscus* spp. in Britain

The following concerning the current status of the species in Britain is based largely on accounts given in Sutton (2008). Six species are currently resident and these are listed in Table 1.3 below along with the varieties that are thought to occur in Britain.

Table 1.3: A summary of the *Dytiscus* species resident in Britain. Source: Sutton (2008)

Species	Varieties recorded	Form of ♀ elytra
circumcinctus Ahrens, 1811	Nominate	Sulcate
	Var. *flavocinctus* Hummel, 1813	Smooth
circumflexus Fabricius, 1801	Nominate	Smooth
	Var. *dubius* Audinet-Serville, 1830	Sulcate
dimidiatus Bergsträsser, 1778	Nominate only	Sulcate
lapponicus Gyllenhal, 1808	Nominate only	Sulcate
marginalis Linnaeus, 1758	Nominate	Sulcate
	Var. *conformis* Kunze, 1811	Smooth
semisulcatus Müller, O.F., 1776	Nominate only	Sulcate

Distribution maps for the species in Britain are reproduced in Appendix A4. As may be seen from these maps, only *D. marginalis* and to a lesser extent *D. semisulcatus* can be said to be widespread throughout the British Isles. *D. lapponicus* has a distinctly northern, montane distribution and is thought to be a cool-climate specialist that particularly favours stony lochs more than 250m above sea level [Beebee (1991)]. Foster (2010) postulated that there was a correlation of the Scottish distribution with the 22º C maximum summer summit isotherm.

Until relatively recently, *D. circumflexus* was rarely recorded away from coastal areas especially in the south and east of England. It was thought to be a brackish water specialist but, increasingly, there have been records of it occurring inland in freshwater habitats, especially in newly-created waterbodies. It is possible that this species is taking advantage of climate change to expand its range westwards.

D. circumcinctus has a patchy distribution in the British Isles that is difficult to explain except perhaps in terms of an association with areas like the Cheshire plains with abundant long-established and well-vegetated ponds or ditches [Denton (2007)].

Sutton (2008) describes *D. dimidiatus* as "*almost certainly the rarest, and probably the most threatened of the* Dytiscus *species in the British Isles*". Its strongholds are the Somerset Levels and Moors, the Cambridgeshire Fenland, Norfolk and East Sussex.

Of the *Dytiscus* species that occur in Britain only *D. dimidiatus* is accorded Red Data Book status at present (RDB3 – Rare). The species was not listed in the first British Red Data Book [Shirt (1987)] while an earlier work that had attempted to identify invertebrates that would qualify as RDB species [Ball (1986)] designated it as 'Notable A'. This status was changed to RDB3 in 1992 due to Foster [in Hyman & Parsons (1992)].

Three species of *Dytiscus* are designated as nationally notable. The status of *D. lapponicus* was changed from 'Notable A' to 'Notable B' in Foster's 1992 review of water beetle statuses because of new records [in Hyman & Parsons (O*p. cit.*)]. The statuses of *D. circumflexus* (Notable B) and *D. circumcinctus* (Notable A) have remained the same since 1986 [Ball (*Op. cit*.), Hyman & Parsons (*Op. cit.*)].

1.3.5 Identification of the British *Dytiscus* spp.

The adults of the *Dytiscus* species that occur in Britain may be told apart from a suite of characters based on size, colouration and shape of the postcoxal processes on the hind legs. Keys to the adults are to be found in Balfour-Browne (1950), Friday (1988), Beebee (1991), Sutton (2008) and Foster & Friday (2011). The key which accompanied Beebee's article is reproduced as Appendix A6 [Beebee (*Op. cit*.)] since this was the one used primarily in this study.

The larvae of the species which occur in Britain are more difficult to assign to species based on external morphology than are the adults. Balfour-Browne comments: "…*the distinguishing characters are largely measurements and minute differences by no means easy to make out*" [(*Op. cit.*)].

The most modern key to larvae that could be found for this study was that in Klausnitzer (1991). Once this was translated it was found to rely heavily on small differences in features that require very close examination. Therefore, I thought it likely that the key would not be capable of being used reliably in the field on live specimens. For this reason, the need for a different means to identify larvae was appreciated early in the study. I decided to employ the techniques of DNA analysis to see if these would distinguish between larvae of *D. marginalis* and *D. dimidiatus*.

Chapter 4 explains in detail how the issue of larval identification was tackled in this study and, in particular, the means by which the techniques of molecular ecology were deployed to assist in determinations of larval identity. A comparison is made between identifications made using morphological traits and those obtained by DNA analysis.

1.3.6 *Dytiscus* spp. in Somerset

Dytiscus lapponicus has never been recorded in Somerset, while *Dytiscus circumcinctus* is known only from sub-fossil records and is regarded as extinct in the county [Duff (1993)]. Girling (1984) gives an account of sub-fossil remains of insects found during archaeological digs in the Somerset Levels. *D. circumcinctus* is stated by her as having been found as a sub-fossil at Shapwick Heath. Maps showing the current distribution of the remaining four *Dytiscus* species within Somerset are given in Appendix A5.

1.3.6.1 *D. marginalis*

According to Duff (1993) *D. marginalis* is "*resident*" and "*widespread*" within Somerset and found in "*streams, drainage ditches and ponds*". Duff lists records from twenty-five 10 km squares of Somerset (out of

sixty-four 10km squares covering Watsonian Vice Counties 5 & 6). Appendix A5 contains a summary of records of the species from the Levels and Moors.

1.3.6.2 *D. dimidiatus*

Records of *D. dimidiatus* within Somerset indicate a distribution which is less widespread than that of *D. marginalis*. Duff (*Op. cit*.) recognises *D. dimidiatus* as "*resident*" but restricted only to the Levels. An analysis of known records is to be found in A4. These records fit within seven 10km squares of VCs 5 and 6.

1.3.6.3 *D. semisulcatus*

Duff (*Op. cit*.) gives *D. semisulcatus* as: "*Resident, with sub-fossil records*". Although Duff cites records from only ten 10km squares, he regards the species as "*widespread*" within Somerset compared with *D. dimidiatus* that is described as "*Local*" in its distribution.

1.3.6.4 *D. circumflexus*

Four out of the five 10km squares from which records are reported by Duff (*Op. cit*.) are on Somerset's coast. The species has been recorded in freshwater and brackish water habitats according to the same author Duff (*Op. cit*.).

1.4 The Somerset Levels and Moors

1.4.1 A wetland of international importance

The Somerset Levels and Moors is an area of low-lying land bounded by the Bristol Channel and the high ground of the Mendips, the Dorset Hills, Quantocks, Blackdowns and Brendons [Storer (1985)]. This area contains the UK's largest remaining area of lowland wet grassland and associated wetland habitats, comprising some 35,000 hectares in the floodplain of the rivers Axe, Brue, Parrett, Tone and their tributaries [JNCC (2006)]. A map of the area is provided in Appendix A7.

The area first gained a reputation as an important area for water beetles following a survey of its beetle fauna by F. Balfour-Browne in 1915 [Duff (1993)]. About one fifth of the Levels and Moors area is designated currently as a Ramsar Wetland of International Importance, in part, because it "*supports an outstanding assemblage of aquatic invertebrates, particularly beetles*" [JNCC (2006)]. Selected extracts from the Ramsar citation are reproduced in Appendix A8.

1.4.2 Key habitats for freshwater invertebrates

The drainage channels that are so much a feature of the Levels and Moors landscape (known locally either as 'rhynes' or 'ditches', depending on size) connect into larger channels ('drains') or into main rivers that carry water westwards to the coast. As well as having a drainage function, the channels act as 'wet fences' that assist the management of livestock (mainly cattle) which is the dominant agricultural activity on the Levels and Moors. Much of the water beetle interest of the Levels and Moors is associated with its rhynes and ditches.

1.4.3 Study areas within the Levels and Moors

The Levels and Moors wetland comprises of a number of discrete units of land, their drainage systems hydrologically-isolated one from another. A distinction is sometimes made between the 'Levels' - taken to be the belt of coastal claylands extending five or six miles inland from the sea – and the 'Moors' – being the river valleys further inland with thick layers of peat sometimes overlying, sometimes overlain by clay [Williams & Williams (1992), Hill-Cottingham *et al.* (2006)]. This distinction is not reflected very well in actual place names, so that, for example, Wick Moor is on the coast and Westhay Level is further inland.

A full description is given in Chapter 2 of how the study sites were chosen for the fieldwork underpinning this autoecological investigation, but, essentially, since *D. dimidiatus* was to be the focus of particular attention, it was decided to concentrate fieldwork effort on areas of the Levels and Moors from which there

are recent records of the beetle. This resulted in all the study sites being located in the Brue Valley in the northern part of the Levels and Moors area. I have assumed that, because of the basic underlying similarity of landscapes and land management systems within the Levels and Moors, findings regarding the ecological preferences and requirements of *Dytiscus* species in the Brue Valley have wider applicability to the Levels and Moors as a whole.

1.5 Summary – The objectives of the study

The primary aim of this investigation was to ascertain whether ecological niche separation could be observed in populations of *Dytiscus* beetles living in the Somerset Levels and Moors and, if it could, to determine the degree and nature of the separation and distinguish how it was maintained. The main foci of attention were the closely related species *D. marginalis* and *D. dimidiatus*.

As discussed above, niche separation can be temporal or spatial in nature. If spatial, the separation may be at the landscape, habitat and/or micro-habitat scales. For carnivorous species like *Dytiscus* beetles niche separation might be on the basis of differences in the types of prey taken. Different stages in the lifecycle of an organism can have different requirements and, therefore, this has to be taken into account in the study.

In order to ascertain whether there was any temporal niche separation I decided to investigate the patterns of abundance of adults and larvae over the course of a year at a study site where it was known that both *D. marginalis* and *D. dimidiatus* occurred. The site I selected for this purpose was Shapwick Heath. Details concerning the site and the reasons for choosing it are provided in section 2.1 and, particularly, in section 2.1.1.

The hypothesis has been advanced that *D. dimidiatus* displays a marked preference for shaded waterbodies compared with *D. marginalis* [Beebee (1991) Boyce (1994)]. I decided to test this idea by examining frequencies of the two species at sites with substantial tree and shrub cover as opposed to sites without. Section 2.1 outlines how the study sites were selected to

investigate if any differences could be discerned between the two species in terms of broad habitat preferences.

One of the key problems concerned how to collect sufficient numbers of beetles to enable meaningful comparisons to be made between sites. Two main methods were available to capture beetles and larvae – netting and trapping. An attempt was made to compare the two methods in order to determine which of the two might be the more useful in the context of this study. This aspect of the investigation is the subject of its own chapter (Chapter 3) but there is a description (in section 2.2) of the equipment and methods used when the techniques of netting and trapping were employed during fieldwork.

The fieldwork at each site aimed at elucidating not only whether niche separation was evident at the broad habitat level but also whether influences at the micro-habitat level played a role. The parameters measured and the means of measurement and recording are detailed in section 2.3.

The final type of work that was based in the field (as opposed to the laboratory) involved experiments looking into possible predator-prey relationships in case this was a significant factor in niche separation. The predatory behaviour of *Dytiscus* spp. adults and larvae was studied by introducing individual beetles into glass tanks with known numbers of potential prey. The numbers of potential prey left after a period of time was compared with the numbers at the beginning of the experiment in tanks to which beetles had been added as well as in control tanks with no beetles. A description of the methodology employed is given in section 2.4.

The larvae of *Dytiscus* species are very similar in form. Because there was considerable doubt that the larvae could be assigned to particular species in the field, DNA analysis was used for identification. An account is given in section 2.5 of the various genetic techniques employed. A subsidiary objective of this study was to compare the identifications of larvae made using morphological traits with those obtained from DNA analysis. This was to investigate whether

there was close agreement between the results obtained using the two approaches. Section 2.6 contains an explanation of how the biometric measurements were made that formed the basis of the morphological analyses.

To summarise, the main objectives of the study were:

i. To investigate whether ecological niche separation can be observed in populations of *D. marginalis* and *D. dimidiatus* living in the Somerset Levels and Moors;
ii. If niche separation can be demonstrated, to identify how separation is achieved. Is the separation primarily on a temporal basis; is it habitat-based, or is it due to food preferences? To what extent can it be said that either species display the traits of a 'generalist' or 'specialist'? (Answering these questions will involve measurements of niche breadth and niche overlap of the two species.);
iii. To determine whether there is good evidence to suggest that there is inter-specific or intra-specific competition occurring between *D. marginalis* and *D. dimidiatus.* (A special case of competition would be inter-specific and/or intra-specific predation and this is one aspect of competition that is assessed);
iv. To evaluate the *Dytiscus* species that occur in the Somerset Levels and Moors to judge if any possess suitable characteristics that would make them good candidates as 'flagship' and/or 'indicator' species. This will include consideration of the conservation status of *D. dimidiatus* at an international, national (UK) and local (Somerset Levels and Moors) scale.

Two important methodological objectives, necessary to achieving (i) to (iii) were to determine:

(a) The best technique to capture relatively large numbers of the subject animals for study; and
(b) A sound basis to identify the larvae to species level given the similarity in gross appearance of the larvae of *D. marginalis* and *D. dimidiatus*.

Ultimately the goal of this research is to use the results to improve conservation and land management practice in the Somerset Levels and Moors.

Chapter 2: Methods

Introduction

This chapter describes the various methods used to achieve the study's objectives as set out in section 1.5.

Fieldwork

2.1 Study sites

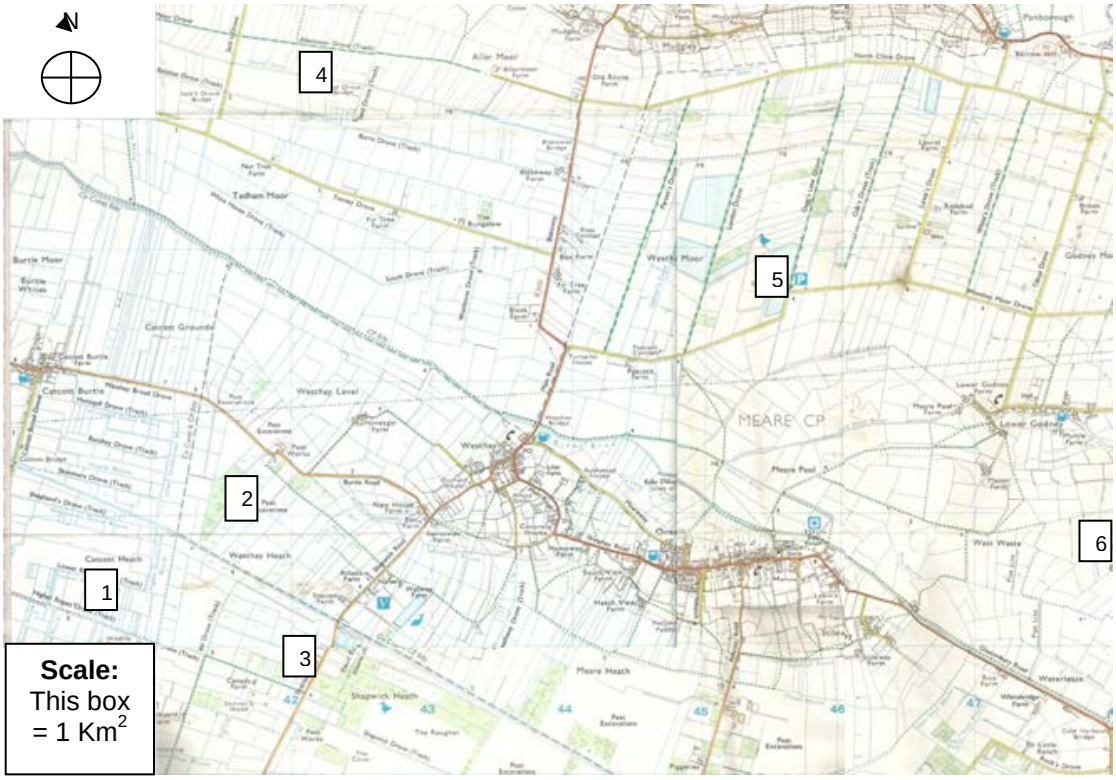
The ditch habitats selected for study all occurred within six discrete sites in the eastern half of the floodplain of the River Brue, namely:

- Shapwick Heath
- Westhay Moor
- Westhay Heath
- Catcott North
- Tadham Moor
- East Waste

I decided to investigate sites that were a relatively short distance apart in the Brue valley in order to minimise influences due to abiotic factors such as weather, geography, soils, etc, so that it might be easier to distinguish effects on beetle distribution due to differences in habitat (primarily amount of tree cover and shading). The two sites that are furthest apart - East Waste and Catcott North - are separated by about 8 km.

The location of the Brue Valley in relation to the rest of the Somerset Levels and Moors is shown on the map in Appendix A7. The locations of the individual sites are illustrated in Figure 2.1 below. A larger version of this map showing the sites in relation to Glastonbury is Map B1a in Appendix B1.

Figure 2.1: Map showing locations of study sites within the Somerset Levels and Moors: 1= Catcott North; 2 = Westhay Heath; 3 = Shapwick Heath; 4 = Tadham Moor; 5 = Westhay Moor; 6 = East Waste



All the study sites chosen were below sea level as measured against Ordnance Datum Newlyn (ODN). The lowest lying site - Catcott North - was the westernmost. This is about 8m below ODN according to the SSSI citation (see Appendix B2). The overlying soils into which ditches have been cut in all of the sites are predominantly peat. Across the Somerset Levels and Moors where peat occurs its depth varies between 1 m and 8 m [Somerset County Council (2009)].

The peat in the Westhay Moor, Westhay Heath and Shapwick Heath study sites is principally of the 'Westhay Turbary Moor series acid peats' which is derived from bog rather than fen vegetation [Wolseley *et al.* (1984), JNCC (2011)]. In the other three sites the peat is classified as belonging to the Sedgemoor/Altcar series of reed peats. In none of the sites can the peat be regarded as being in a totally pristine condition, it having been modified in most places by cutting, drainage or agricultural management (mostly livestock rearing). The Turbary peat soils tend to have a lower pH at their surface than do the

Sedgemoor/Altcar series soils and this is mentioned because the soil might exert some influence on the water chemistry of the ditches cut though it [Wolseley *et al.* (1984)]. However, in practice a greater influence will be the nature of the waters feeding the ditches and in the Somerset Levels and Moors the waters are predominantly base-rich [Wolseley *et al.* (*Op. cit*.)].

All the sites with the exception of East Waste are SSSIs managed for nature conservation, with an emphasis on trying to maintain high water levels all year round. The part of East Waste in which I worked was within a Raised Water Level Area (RWLA). RWLAs are areas where water levels are maintained at higher levels than the prevailing pen level in order to encourage breeding waders and other wetland birds. The pen level is the target set by the internal drainage boards for each moor for water levels within the ditch systems.

The SSSI citations for the first five sites are included in Appendix B2 and there is an excerpt there also from a report of a 2010 habitat survey conducted in the East and West Wastes [Somerset Wildlife Trust (2010)]. I considered it beneficial to select sites where there is some ecological information readily available and where (for the nature reserves at least) management practices are relatively well-documented.

Aerial photographs of each site are provided in Appendix B1 with the ditches marked upon them that were sampled. These are Somerset County Council (SCC) aerial photographs taken in 2007 and they show the vegetation cover prevalent in the area at about the time that fieldwork was undertaken.

Three of the sites from the list above – Shapwick Heath, Westhay Heath and Westhay Moor – were selected to represent sites with considerable tree and shrub cover over and around ditches, the others as examples of sites with ditches in more open habitats. The actual tree and shrub cover within 100 m and 1000 m of the ditches studied at each site was estimated from the 2007 SCC aerial photos using the County Council Geographical Information System (GIS). I chose the distances of 100 m and 1000 m in order to investigate

whether landscape-scale effects influencing the distribution and abundance of *Dytiscus* beetles could be detected. The results are reported in Chapter 6 and are discussed in the analysis of the effects of tree cover and shade on species distribution.

2.1.1 Shapwick Heath

This study site was used throughout the investigation. As well as being one of the six sites compared one with another, it was here that the trapping methods were tested and this was the site used for fortnightly sampling to establish seasonal activity patterns of the two focal species. The majority of specimens used in DNA analysis came from Shapwick Heath.

Shapwick Heath was chosen as a study site principally because ditches within it could be pin-pointed where *D. dimidiatus* had been netted in the recent past [T Beebee (*pers. comm.*)]. Since particular attention was to be paid to *D. dimidatus*, it was considered important to investigate at least one site that was believed to support a thriving population of this species.

Numerous mentions of Shapwick Heath as a locality for *D. dimidiatus* have been reported [Blachford (1932), Balfour-Browne (1936), Walton (1943), Boyce (2004)].

An important reason for the selection of Shapwick Heath as a study site was the presence within the SSSI of substantial blocks of established wet woodland. Agricultural practices in the area and the cut-over nature of the peat surface in places (due to mechanised peat extraction) have both contributed to the drying out of areas of the SSSI. Some drier areas have become colonised by fen woodland and scrub dominated by Alder (*Alnus glutinosa)* and willows (*Salix spp)*. Substantial specimens of these trees and of Downy Birch (*Betula pubescens)* and Pedunculate Oak (*Quercus robur)* occur within the woodland, suggesting that at least some of the fen woodland at Shapwick Heath has been established for several decades. Aerial photographs from 1947 show an extensive tree cover on parts of the present SSSI (see Appendix B1). The main

areas of woodland appear to coincide with peat cuttings and it is thought that the oldest extant woodland probably stands within areas of peat cut by hand in Victorian times [David Reid (*pers. comm*.)].

Another factor that was considered as being in favour of Shapwick Heath as a study site included ease of access to relatively undisturbed ditches. The value of the SSSI's ditches for invertebrates is recognised within the SSSI Citation (see Appendix B2).

2.1.2 Westhay Moor

Most of the Westhay Moor SSSI comprises grassland and there are substantial areas of reedbed and open water habitats in former peat workings. However, there is also an area of birch (*Betula* spp.) and willows *(Salix* spp.) carr woodland in the south of the site and this is where attention was focussed in this study. This carr woodland is associated closely with an area of relict raised bog, and it is possible that the woodland has developed on relatively undisturbed peat although there is evidence on the raised bog of areas hand cut for peat. The area under discussion was wooded in the 1947 aerial photographs (see Appendix B1).

Within the SSSI there are a variety of watercourses and waterbodies including field ditches, rhynes (the drainage channels into which field ditches empty) and flooded peat workings. The SSSI Citation mentions the diverse aquatic and bankside flora as being a reason for SSSI designation as well as *"a nationally outstanding community of terrestrial and aquatic invertebrates*" (see Appendix B2).

Dytiscus dimidiatus was recorded at Westhay Moor in the 2004 survey for *Hydrochara caraboides* that is cited above [Boyce (2004)]. *D. dimidiatus* was also captured there in a 1984 invertebrate survey [Drake *et al.* (1984)].

2.1.3 Westhay Heath

The blocks of scrubby wet woodland that occur at Westhay Heath are dominated by Grey Willow (*Salix cinerea*) which can tolerate waterlogged conditions. In drier areas of the site, such as on drove banks, birch trees (*Betula* sp.) and even some significant examples of Pedunculate Oak (*Quercus robur*) can be found.

No information regarding invertebrate species is given in the SSSI Citation although the SSSI is one of the areas of land that has been designated as a Ramsar wetland partly on the grounds of its invertebrate fauna (see Appendix A8). Duff (1993) cited a 1988 record from A J Parsons for *D. dimidiatus* at Westhay Heath apparently obtained in September of that year.

2.1.4 Catcott North

Catcott North is a portion of the landholdings belonging to the Somerset Wildlife Trust that, collectively, comprise the Trusts 'Catcott complex' of nature reserves. The complex of reserves lies within the Catcott, Edington and Chilton Moors SSSI which, according to the SSSI Citation: "*form part of the extensive grazing marsh and ditch systems of the Somerset Levels and Moors*" (see Appendix B2). The SSSI citation describes the water beetle fauna as being "*of exceptional interest, with the nationally rare species Haliplus mucronatus and Hydrophilus piceus present*".

The Catcott North site is an area of 8.1 ha of unimproved grassland cut for hay. It is grazed in mid summer (June/July). Access is by permit from Somerset Wildlife Trust. Although the site was selected to represent ditches in open habitats, there is significant block of wet woodland habitat within the 'Catcott complex', to the immediate south of Catcott North. Many of the ditches beside droves are also lined by mature Alder (*Alnus glutinosa*).

The particular field in which were situated the study ditches was a meadow that conformed closely to the description within the SSSI Citation of unimproved grassland habitats swards "*dominated by species-rich mire-type communities*".

Such plant communities are characterised by the presence of Meadow Thistle (*Cirsium dissectum)*, Meadow Rue (*Thalictrum flavum)*, Quaking-grass (*Briza media)*, Heath-grass (*Danthonia decumbens)*, Carnation Sedge (*Carex panacea)*, Common Sedge (*Carex nigra)* and Southern Marsh-orchid (*Dactylorhiza praetermissa)*.

Boyce recorded *Dytiscus dimidiatus* from a ditch shaded by Alder (*A. glutinosa*) to the west of the field in Catcott North during his survey for *H. caraboides* [Boyce (2004)]. In 1999, Godfrey caught two specimens of the beetle in a ditch to the north of the study site [Godfrey (1999a)]. Earlier, in 1994, *D. dimidiatus* was obtained from the Catcott, Edington and Chilton Moors SSSI, but there is no indication within the body of the report regarding the location of the catch [Gibbs (1994)].

2.1.5 Tadham Moor

Tadham Moor comprises mainly grazing marsh with associated ditch systems. There are few significant blocks of wet woodland or scrub. The site is part of the Tealham and Tadham Moors SSSI.

The particular ditches chosen for the study form the boundary ditches of a field managed as hay meadow by Somerset Wildlife Trust. The field was floristically more diverse than many fields in the vicinity that were dominated by Meadow Fescue (*Festuca pratensis*) or Perennial Rye-grass (*Lolium perenne*). The field contained Common Knapweed (*Centaurea nigra*), Crested Dog's-tail (*Cynosurus cristatus*) and Meadow Rue (*Thalictrum flavum*) which are mentioned in the SSSI Citation as indicators of more species-rich swards. There were also areas within the field suggestive of wetter conditions with rushes (*Juncus* spp.), Lesser Spearwort (*Ranunculus flammula*) and stands of Pond-sedges (*Carex riparia/acutiformis*). As at Catcott North, the field was grazed and cropped for hay.

According to the SSSI citation: "*The water beetle fauna is exceptionally rich, with the nationally rare species Hydrophilus piceus and Hydrochara*

caraboides…. together with the rare soldier flies Stratiomys furcata and Odontomyia ornate [sic]". The occurrence of "*good numbers*" of Hairy Dragonfly (*Brachytron pratense*) and the Variable Coenagrion (*Coenagrion pulchellum*) is also mentioned in the SSSI Citation.

Duff (1993) cites some old records for *D. marginalis* (1925-35) from Frank Balfour-Browne at "*Tealham-Tadham*", but there is no mention of *D. dimidiatus* at this locality. The species was not recorded during invertebrate surveys that sampled ditches on the Moors between 1984 and 2011 (See List of Surveys in Appendix C1).

2.1.6 East Waste

The East and West Wastes taken together provide a good example of open habitat in the Somerset Levels and Moors that is grazed by cattle and, to a lesser degree, by sheep. Hay cuts are usually taken from the fields in which the ditches are located which were sampled.

An account of a botanical study undertaken at the site by Somerset Wildlife Trust is provided in Appendix B2. An invertebrate survey commissioned by Somerset County Council [Keystone Environmental (2011)] recorded 30 taxa from one ditch on East Waste but none of the *Dytiscus* species were caught. The surveyors concluded that ditches in the locality "*had low invertebrate diversity*" and were "*below Local value for aquatic invertebrates*".

2.2 Methods for capturing *Dytiscus* spp.

2.2.1 Netting

The same wooden-handled, aluminium D-framed pond net of mesh size 1 mm was used for all fieldwork involving netting. The frame measured 20cm by 25 cm. The protocol used for netting and subsequent sorting of material varied depending on the objectives for fieldwork.

2.2.1.1 Testing netting versus trapping

Where the objective was primarily to compare netting versus trapping (see Chapter 3) a standardised approach was adopted to sample each section of ditch occurring between pairs of bottle traps set. Five sweeps were made per ditch section (i.e. 45 sweeps in the spaces between 10 sets of paired traps or c.450 seconds netting per survey visit), each sweep of approximately ten seconds duration. Sweeps were conducted vigorously, avoiding bottom sediments, but attempting to sample as many microhabitats between the locations of paired traps as possible, paying particular attention to in-channel vegetation. Material collected in the net bag was deposited into a yellow sorting tray and the material was examined in order to pick out *Dytiscus* adults and larvae. Where considerable amounts of duckweed and/or other plant matter were collected this was washed through with ditch water in order to ensure that all larvae were picked out. The time taken to sort samples varied for this reason, but all samples were examined until I was convinced no individual *Dytiscus* sp., either adults or larvae, remained to be found. In each instance netting took place before traps were set so as to ensure that the animals caught by netting were not ones that had already been attracted to the area and captured in traps

2.2.1.2 Netting to gather data regarding ditch faunae

Where the intention was to gather data to compare the invertebrate fauna of different ditches a different netting protocol was followed to that outlined in the preceding paragraph. At each study site three ditches

were selected for sampling with five trap locations established at each. The ditches were sampled one at a time, netting being carried out between the trap locations for ten counted seconds, meaning that time spent netting was kept to approximately 50 seconds per ditch (c.150 seconds per survey visit). Material collected from the five bouts of netting was placed together in a sorting tray and sorted through for a timed period of 15 minutes (i.e. 45 minutes sorting per study site).

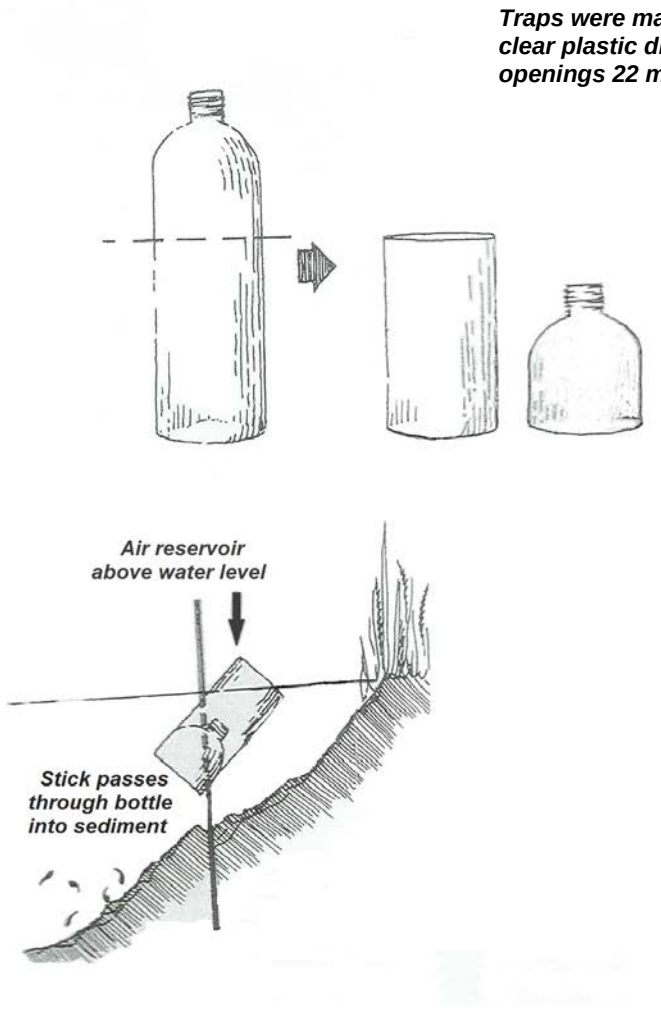
The time allocated to netting for gathering general faunal data was only one third per survey visit of that spent when methods (netting versus trapping) were being compared as means to capture *Dytiscus* spp. By contrast, although no record was kept of the exact time spent sorting through material collected to compare sampling methods, substantially more time was spent in sorting through material to gather the faunal data.

2.2.2 Trapping

Traps used in this study were made from 1.5 litre plastic drinks bottles according to the design as shown in Figure 2.2 [based on Griffiths (1985)]. If baited, the bait used was one half of a rasher of unsmoked bacon from 250g or 500g supermarket packs. The traps were fixed in place using bamboo canes as shown in Figure 2.2 with the bottles orientated so as to be roughly at a right angle to the line of the ditch with the trap opening furthest away from the near bank side. There are photographs in Appendix E of traps in operation.

The numbers of traps used are given in Chapters 3, 5 and 6 that report the results of fieldwork. The distance between traps was roughly 30 m in each case. Traps were set during daylight hours and collected in 24 hours after having been set. The order in which traps were set at sites was varied as described in the Chapters that follow in order to ensure that there was no bias to results because of traps always being set at a particular location at a specific time of day or due to some being run for a longer period on average than others.

Figure 2.2: Bottle trap used in this study. Redrawn after Griffith (1985).



2.3 Investigation of abiotic parameters within study sites

The means by which abiotic environmental parameters were measured is outlined in this section.

2.3.1 Temperature

During trapping, Brannan maximum and minimum mercury thermometers were deployed at selected trap locations. The thermometers were attached to the same cane used to secure the bottle trap at the trap location (see Figure 2.2 above). A photograph in Appendix E demonstrates the arrangement. The thermometer was submerged and positioned to one side of the trap in order not to obstruct the trap entrance.

2.3.2 Width and depth of water body

The width and depth of linear water features were measured using 3 m ranging poles graduated in red and white 50 cm segments. The width of the water body was taken to be the width of the water present measured at the trap location at right angles to the ditch. The depth was measured by holding the ranging pole vertically at arm's length then lowering it into the water until it met resistance from the bottom substrate.

2.3.3 Water Chemistry

2.3.3.1 Oxygen and electrical conductivity

Oxygen levels and electrical conductivity were measured using a portable combined conductivity and dissolved oxygen tester (model DiST4 EC Hannah Instruments). This was held with electrodes approximately 5 cm below the water surface. The values were recorded once the temperature readings had stabilised after a few seconds.

2.3.3.2 Measurement of pH

A portable pH tester with 0.1 pH unit resolution was used to measure pH (model pHEP4 Hannah Instruments). Readings were obtained in the same way as described in paragraph 2.3.3.

2.4 Investigation into predator/prey relationships

The predatory behaviour of *Dytiscus* spp. adults and second or third instar larvae was studied by introducing individual beetles into glass tanks (15.5 cm wide x 30 cm long x 15 cm tall) with known numbers of potential prey. The numbers of potential prey left after a period of time was compared with the numbers at the beginning of the experiment in tanks to which beetles had been added as well as in control tanks with no beetles. The species that were added are given in Chapter 7 where there is also an account of the numbers and the configuration of tanks with and without beetles.

Twenty-one tanks were kept in a shaded area at Natural England's Peat Moors Centre near Shapwick (see Map B1a in Appendix B1). The tanks were arranged in a single block in an experimental array comprising of three rows with seven tanks per row. The arrangement is shown in photographs and diagrams in Appendix G.

The outside of the array was covered to about two thirds the height of a tank with grey, stretchable woollen fabric to provide some insulation and to minimise light penetration from the sides of the array. It was thought desirable that light should fall on the water mainly from above as would be the case in ditch habitats.

A layer of substrate from the bottom of Shapwick ditches was added to each tank. Samples of this substrate were netted from ditches, spread out on a plastic sheet and left in the sun to dry thoroughly for a period of at least 24 hours. This was done in order to make sure no living aquatic organisms were transferred inadvertently to the tanks amongst the substrate.

In order to try to ensure that amount of re-wetted substrate added to each tank was roughly the same, the material was collected in a sieve and emptied into a plant pot of diameter c.12cm until the pot was completely filled with loosely packed material. This generated a tank sediment layer about 3 - 4 cm deep. Once the substate had been added to all twenty-one tanks then water from a rain-fed pond at the Peat Moors Centre was poured into the tanks through a 1 mm sieve, to a depth of 10 cm and topped up as necessary during the experiment.

Some submerged plants were added to each tank, partly to provide food for phytophagous animals introduced as potential prey, but also to allow the water to be oxygenated. Strands of Canadian Pondweed (*Elodea canadensis*) and Rigid Hornwort (*Ceratophyllum demersum*) were collected from the artificial pond at the Peat Moors Centre, washed to remove animals and introduced to

each tank. An attempt was made roughly to standardise the amount of plant material placed in each tank.

Once substrate and submerged plants had been added clear plastic covers were placed on the tops of the tanks to prevent colonisation by flying insects, to limit water losses due to evaporation and prevent escapes. The tanks were allowed to settle over a period of at least 24 hours before any animals were added.

Laboratory Work

2.5 Molecular Ecology

2.5.1 DNA Extractions and Assays

2.5.1.1 Sources of tissue for extractions

Muscle tissue is a potential source from which DNA can be obtained for subsequent procedures, but the amounts, purity and integrity of the DNA extracted are very dependent on the nature of the muscle as well as on the extraction technique [Saunders (1999) in Saunders & Parkes (1999)].

Edwards & Ruska (1955) examined sections of muscle-fibre from *Dytiscus* spp. under electron microscope. The red muscle fibre of the thoracic flight muscles contained more and larger mitochondria than did white fibres from the coxal levator leg muscles. These observations suggest that in *Dytiscus* adults flight muscle may be a richer source of DNA, and particularly of mitochondrial DNA, than leg muscles. However, for larvae this was not an option and individual legs were used as sources of DNA.

Based on the work with the Great Silver Water Beetle (*Hydrophilus piceus*) sufficient DNA should be extractable from a single leg from a *Dytiscus* larva to enable subsequent amplification by PCR [Beebee

(2007)]. Removal of a single leg should represent a minor risk to the individual's subsequent survival, borne out by the occasional capture of individuals lacking one or more limbs during the course of fieldwork.

Legs from larvae or adults were removed using a pair of dissecting kit scissors, cutting cleanly through the femur as close to its junction with the coxa as possible. Normally, the middle leg from the beetle's left side was taken. Harvested legs were placed immediately into tubes containing 70% or 100% Industrial Methylated Spirit (IMS) B.P. 66 OP (William Ransome & Son PLC). No legs were taken from any captured individual that already lacked limbs in order to ensure that all material collected was from definitely separate individuals.

Where whole adults or larvae were taken in the field, these were transferred as soon as possible to 70% or 100% IMS to fix tissues. To minimise the number of specimens killed, most of the whole animals taken were ones found dead or dying in traps.

Tissues were stored at room temperature in glass tubes with screw top lids. The tubes were checked at intervals to make sure that the sample was immersed in an adequate level of IMS. Where found necessary tubes were topped up with 100% IMS.

2.5.1.2 Preliminary DNA extraction experiment

An experiment was conducted to test whether sufficient DNA could be extracted from larval legs and to compare yields of DNA from larval legs and from adult flight muscle. Details of this experiment are given below and in section 4.2.1 where the results and conclusions are reported.

The extractions were undertaken using a DNeasy Blood and Tissue Kit manufactured by Qiagen (http//www.qiagen.com/). This kit uses spin column technology to isolate DNA from samples. The basis of this technology is that DNA is selectively bound onto a membrane within the

column and later released after contaminants have been removed by successive washing and centrifugation steps. Unless otherwise stated below, the procedure followed was the 'Animal Tissue (Spin Column) Protocol as per kit instructions in the DNeasy Blood and Tissue Handbook July 2006 [Qiagen (2006)].

Sample Preparation – The larval legs were removed from the IMS and patted dry using paper tissue before transfer to a 1.5 ml microcentrifuge tube. To liberate soft tissue the legs were placed in 180 µl of Kit lysis buffer 'ATL' and manually ground using a glass rod for two minutes. Samples using flight muscle were prepared by dissecting out some of the muscle from preserved whole beetles. The removed muscle tissue was cut into small pieces and placed directly into 'ATL buffer' with no grinding. According to the Qiagen Handbook, blocks of muscle tissue which are approximately $2mm^3$ in volume typically weigh between 10mg and 15mg. Since the volume of muscle that was dissected out exceeded 2 mm^3, twice the recommended volume of buffer (i.e. 360 µl) was added to the muscle preparations.

Lysis – To liberate cellular and organelle contents including DNA, 20 µl of 10 mg/µl Proteinase K solution was added to the larval leg preparations and 40 µl to the flight muscle preparations. The resulting volumes were mixed thoroughly by vortexing for 15 seconds and then incubated overnight at 55 ºC in a water bath.

Isolation and purification of genomic DNA – Following incubation samples were vortexed for 15 seconds to re-mix the contents of the 1.5 ml tubes. 'Buffer AL' was added (200 µl to leg preparations, 400 µl to muscle preparations) followed by 100% ethanol (in the same volumes as 'Buffer AL'). After each addition the sample was vortexed to ensure good mixing.

The entire contents of the 1.5 ml tubes were pipetted into DNeasy mini spin columns placed in 2ml collection tubes. The mixtures were centrifuged for 1 minute at 8,000 rpm. The collection tube with flow through was discarded following centrifugation and the mini spin column (with adsorbed DNA) was placed in a new 2 ml collection tube.

500 µl of 'Buffer AW1' was added to each sample and the spin column and collection tube centrifuged for 1 minute at 8,000 rpm. The collection tubes and the flow throughs were once again discarded. The spin columns were placed in new 2 ml collection tubes and 500 µl of 'Buffer AW2' was added to each sample. The samples were centrifuged for 3 minutes at 13,000 rpm). The collection tubes and flow throughs were discarded and the spin columns placed in 1.5 ml microcentrifuge tubes.

To release adsorbed DNA 100 µl of elution buffer ('Buffer AE') was pipetted onto the DNeasy membrane at the bottom of each spin column and the columns and 1.5 ml tubes left to incubate at room temperature for 1 minute. Following incubation the columns and 1.5ml tubes were centrifuged at 8,000 rpm for 1 minute to elute the extracted DNA into the 1.5 ml tubes.

Assay - The DNA concentration in 5µl of elution product obtained was calculated from UV absorbance readings at a wavelength 260 nm measured on a HE λ 10S Spectrograph spectrophotometer. It was assumed that a sample with a DNA concentration of 50 µg/ml has an absorbance of 1.00 at 260nm. The results of the assay are given in section 4.1.

2.5.1.3 Further extractions using magnetic beads

A technique employed routinely in 2009 -10 in Somerset County Council's (SCC) laboratories to extract DNA for food analysis purposes was based on selectively binding DNA onto magnetised beads rather than on spin column membranes as above [Tepnel Biosystems (2006)].

DNA extractions from *Dytiscus* beetles were performed using the magnetised beads from a Tepnel Biosystems' 'BioKit GMO and Allergen DNA Extraction Kit' (http://www.neogeneurope.com/). Unless otherwise stated the procedure followed the kit instructions set out in the manual for BioKits DNA Extraction Kit (Speciation) 201388Xv03 Rev:11.06 Cat No. 901040N [Tepnel BioSystems (2006)]. The extraction protocol and the way that it was applied in this instance are described below.

Sample Preparation – Extractions using this protocol were conducted on legs (from both adults and larvae) and on pieces of flight muscle. The majority of extractions were performed on legs from adult beetles in order to obtain DNA from individuals of known species identity.

Where legs were used, each leg was removed from IMS preservative and patted dry using paper tissue before being transferred to a 1.5 ml Eppendorf tube. 400µl of Kit lysis buffer 'Tissue Extraction Solution I' was added and the leg was ground manually using a glass rod for two minutes. Flight muscles removed from whole beetles were treated in a same way except that the material was not ground but was cut into smaller pieces.

Lysis – 20 µl of Proteinase K enzyme solution (10mg/ml) solution supplied with the kit was added to the sample preparations. The resulting volumes were mixed thoroughly by vortexing for 15 seconds and then incubated for 1 hour at 65 ºC. A control with no tissue was prepared and incubated also comprising 400µl of Kit lysis buffer 'Tissue Extraction Solution I' and 20 µl of the Proteinase K solution.

Isolation and purification of DNA – The samples and the control were centrifuged at 8,000 rpm for 5 minutes to spin down the cellular debris left following lysis. While centrifugation was occurring the magnetic beads were prepared.

50 µl batches of the kit solution containing the beads was dispensed into new 1.5 ml Eppendorf tubes and the beads in the solution were immobilised on the sides of the tubes by placing the tubes against a magnetic strip attached to a magnetic tube rack. The liquid in which the beads had been suspended was removed using a pipette. The tubes were relocated away from the magnetic strip and re-suspended in 500 µl of Molecular Biology Grade Water (MBGW). The beads were again immobilised by placing the tubes against the magnetic strip and the MBGW removed using a pipette. In this fashion the beads were cleaned ready for use.

The Eppendorf tubes with the beads were moved away from magnetic strip and as much as possible of the liquid from the centrifuged samples and control added. When pipetting liquid from the samples, care was taken to avoid transferring debris from the bottom of the centrifuged sample.

400 µl of the kit 'DNA Binding Solution' was added to tubes with beads. The tubes were agitated by flicking to ensure the beads became thoroughly re-suspended. The tubes were incubated with the Binding Solution at room temperature for 5 minutes. During this time the tubes were flicked occasionally tubes to ensure good mixing. The beads (now with DNA bound to them) were immobilised by placing the tubes against the magnets in the magnetic rack. The entire rack was then inverted several times to wash beads from the sides of the tubes. The rack of tubes was left to stand for 2 minutes with the tubes next to the magnetic strip. After this any supernatant was discarded, care being taken not to discard any beads.

The beads were then washed with Ethanol to remove any contaminants that might be adhering to the bead/DNA complexes. This was done by moving the tubes away from the magnets and re-suspending the beads in 500 µl 75% Ethanol. Each tube was flicked and inverted several times

to ensure good re-suspension. The beads were next immobilised once again by placing the tubes against magnetic strip. As much as possible of the Ethanol was then removed and discarded using a pipette. This washing process was repeated once.

In order to remove residual Ethanol, tubes were incubated in an oven with their lids open at 65 ºC for 5 minutes. Any liquid remaining after incubation was removed by pipette and discarded. It was important to ensure as little as possible Ethanol remained as it is a potential inhibitor of PCR reactions [Tepnel BioSystems (2006)].

To release the DNA from the bead/DNA complexes, 50 µl of the kit's 'TE Buffer' was added to each tube to re-suspend the beads. The tubes were flicked gently to ensure good mixing. The mix with the re-suspended beads was incubated at 65 ºC for 10 minutes. After 5 minutes, the tubes were taken out of the oven and flicked to ensure the beads were still suspended. The tubes were then returned to the oven for the last 5 minutes. Upon removal of the samples from the oven the tubes were flicked once again to ensure the beads were properly suspended then the tubes were centrifuged for 5 seconds to collect any condensate that might have formed post incubation. The beads were immobilised in the magnetic rack and the supernatant (containing released DNA) pipetted into a clean Eppendorf tube.

Following the Biokits' protocol, the process of incubation with 'TE Buffer' was repeated once to remove as much as possible of any residual DNA still adhering to the beads. In a minor departure from the protocol, 50 µl of the TE buffer was used each time rather than 100 µl in order to create a more concentrated DNA extract for subsequent amplification.

Extracts awaiting PCR were stored at 4ºC. Before extracts were refrigerated they were checked to see that they were bead- free. This was done by placing the combined eluate against magnetic strip in the

magnetic rack, allowing it to stand for 2 minutes. If a pellet of beads was observed then the supernatant was removed to another clean Eppendorf tube.

Assay – Assays were carried out using a Cecil Instruments CE1021 Spectrophotometer to measure absorbance at a wavelength of 260 nm. It was assumed that a sample with a DNA concentration of 50 µg/ml has an absorbance of 1.00 at this wavelength.

2.5.1.4 Further extractions using spin columns

DNA extraction from the majority of larval legs was carried out using the kit in the Agilent DNA Fish ID Ensemble (part number 5500-0100) utilising spin column technology (http://www.genomics.agilent.com/). This works on the same principle as the spin column technology used in connection with the initial extractions as described above in section 2.5.1.2.

The Fish ID Ensemble was a kit that came into use in the SCC laboratories for food testing. It was recommended to the author by the laboratory's senior scientist in charge of DNA analyses as a potentially faster method for processing *Dytiscus* material than the magnetic beads technique described above [Yanina Pelegri-Fairfax (*pers. comm*.)]. The key advantage in terms of time saving was the reduction in incubation time at the sample preparation stage from 1 hour to 10 minutes. The protocol for this spin column method of extraction was based on that of Formosa *et al.* (2010). The protocol followed is described below.

Sample preparation – No extractions from flight muscle or from adult legs were attempted using this protocol. Only extractions from larval legs were undertaken. As in previous extractions, each leg was removed from IMS preservative and dried before being transferred to a 1.5 ml Eppendorf tube. 200 µl of Kit lysis buffer 'Proteinase K Digestion Buffer'

was added. The leg was ground in the buffer for two minutes using a glass rod. The buffer and the leg were not incubated for five minutes at 65 ºC as the Agilent protocol demands, since it was considered unnecessary given the incubation step in the next stage.

Lysis – 20 µl of the Proteinase K enzyme solution (10 mg/ml) supplied with the kit was added to the sample preparations. Each mixture was then incubated for 10 minutes at 65 ºC.

Isolation and purification of DNA – The samples were centrifuged at 10,000 rpm for 5 minutes to spin down the cellular debris left following lysis. 150 µl of the supernatant from each centrifuged sample was pipetted to a separate 1.5 ml microcentrifuge tube, care being taken to avoid transfer of debris.

500 µl of the kit 'Nucleic Acid Binding Buffer' was added to each tube and mixed with the sample by pipetting up and down once or twice. Each 650 µl mixture was removed to its own 'DNA Binding Spin Cup' seated in a 2 ml 'Receptacle Tube'. The spin cup and receptacle tube were as supplied with the kit and were designed so that the tube's cap could be snapped shut on top of the spin cup. The spin cups in their receptacle tubes were centrifuged with caps shut for 1 minute at 13,000 rpm. At this stage DNA was adsorbed onto the fibre matrix in the spin cups. The filtrate from each spin cup was discarded and the cups placed back in their respective receptacle tubes.

Each samples was then washed through to remove potential contaminants firstly with a High Salt Buffer and then with 80% Ethanol. Firstly, 600 µl of the High Salt Wash Buffer was added to each spin cup and these were centrifuged with caps shut for 1 minute at 13,000 rpm. The filtrate in each receptacle tube was discarded and 500 µl of 80% Ethanol added to each spin cup. The spin cups were centrifuged with caps shut for 1 minute at 13,000 rpm and the filtrates were discarded.

The addition of 80% Ethanol and subsequent centrifugation and disposal of filtrate was repeated twice more. After the third wash with Ethanol the cups and receptacle tubes were centrifuged at 13,000 rpm for 2 minutes to remove any residual Ethanol.

In order to remove adsorbed DNA, the spin cups were placed in 1.5 ml Eppendorf tubes and 100 µl of 'Elution Buffer' was pipetted onto the fibre matrix in each spin cup. For each sample the tube cap was closed over the spin cup and the sample left at room temperature for 1 minute before being centrifuged at 13,000 rpm for 1 minute. The eluted samples with the liberated DNA were retained in the Eppendorf tubes and the spin cups were discarded.

Assay – DNA assays were carried out using a GeneQuant 1300 Spectrophotometer. This instrument gave automatic readouts of absorbance levels at 230nm, 260nm, 280nm and 320nm and gave a value for DNA concentration in ng/µl.

2.5.2 Amplification of DNA by Polymerase Chain Reaction (PCR)

A number of analytical techniques based on PCR are available to researchers investigating genetic variation between individuals, populations and species [Beebee & Rowe (2004)]. I used three such techniques to attempt to identify and distinguish *D.dimidiatus* larvae from *D. marginalis*:

I. Randomly Amplified Polymorphic DNA (RAPD) analysis;
II. Use of species-specific primers based on known sequences in the mitochondrial CO1 genes in *D.dimidiatus* and *D. marginalis*;
III. DNA sequencing of PCR products obtained from amplification of part of the CO1 gene.

An account is given in the following three sections of the methodology employed using these techniques, including details of master mixes and of thermal conditions programmed into the automatic thermal cycling machines used.

2.5.2.1 RAPD Analyses

Theory - In RAPD analyses, single randomly selected 10-mer oligonucleotides of known sequence are used as primers in PCR reactions [Williams *et al.* (1990)]. Amplification only occurs if the oligonucleotides can bind to primer sites which are relatively close together (usually under 1000 base pairs apart) and in opposite orientation [Beebee & Rowe (2004)]. When there is little or no DNA sequence information available on which to base the choice of primers, an assortment of them is used to build up a banding pattern that can enable species, populations within species or even individuals within populations to be identified by the presence or absence of particular amplified DNA bands on an agarose gel.

At the outset of the study, I had no information regarding species-specific sequences in the *Dytiscus* genomes, so I considered RAPD analysis to be a good molecular ecological technique with which to begin. Starting with DNA sourced from adult beetles (known to species level), I hoped that 10-mer oligonucleotide primers could be found that generated PCR products from *D. dimidiatus* DNA but not from *D. marginalis* DNA and vice-versa. The presence or absence of specific bands when particular primers were used in the PCR reaction would become a diagnostic tool for determining whether a particular larva was *D. marginalis* or *D. dimidiatus*.

Experiments Conducted - Initially, amplification was attempted using four commercially available 10-mer oligonucleotide primers: OPD_9 (CTCTGGAGAC); OPD_{10} (GGTCTACACC); OPD_{19} (CTGGGGACTT) and OPD_{20} (ACCCGGTCAC).

PCRs were conducted using flight muscle DNA from adults that had been identified to species level. In order to ascertain whether DNA from larval legs might also generate bands, PCRs were conducted with the particular primers being investigated.

Initial experiments were conducted with the extracts diluted by 1 in 5 or 1 in 10 so as to give notional DNA concentrations in the 30-50 ng/µl range based on the starting concentrations indicated by spectrophotometric assay. Subsequent experiments used undiluted extracts.

The PCR reaction mixes and PCR conditions used are summarised respectively in Tables 2.1 and 2.2 below. Separate master mixes were made up on ice for each 10-mer primer to be tested. The reaction buffer used came complete with sources of Magnesium and Potassium ions included. The Taq Polymerase was added to each master mix after all other reagents and, following this, each mix was vortexed to ensure good mixing and placed back on ice prior to use.

Table 2.1: Details of PCR mix used in the RAPD experiments. The figures in represent the final concentrations of each reactant in a single PCR volume.

1 x Reaction buffer (including magnesium and potassium ions)
0.1 mM Deoxyribonucleotides
0.2 µM Oligonucleotide primer
2.5 units Taq Polymerase (Source: New England Biolabs)

Table 2.2: PCR conditions used in the RAPD experiments.

Step	Treatment	No. of cycles
Denaturation	95ºC for 5 minutes	1
Denaturation	94ºC for 1 minute	35
Annealing	36ºC for 1 minute	
Elongation	72ºC for 1 minute	
Elongation	72ºC for 4 minutes	1
Storage	4ºC for up to 99 hours	N/A

2.5.2.2 Use of species-specific primers

Theory - In studies aiming to assign individuals to species, a focus is required on loci with high interspecific variation but low intraspecific variation. A candidate for the role as 'species-specific barcode' in aerobic eukaryotic organisms is the CO1 gene in mitochondrial DNA [Alberts *et al.* (2008), Hebert *et al.* (2003)]. CO1 codes for subunit 1 of the Cytochrome Oxidase enzyme which is essential in aerobic respiration.

CO1 sequences have been used by researchers in freshwater macro-invertebrate ecology to solve taxonomic problems such as the identification of larval stages of freshwater invertebrates to species level [Pfrender *et al.* (2010)] and in assigning individual mayflies accurately to species [Ball & Hebert (2005)].

Assuming at least partial knowledge of the CO1 sequences from closely related organisms it should be possible to identify two sections of the CO1 gene relatively close together that are likely unique to each species. Primers can be designed to bind to these two sections and, given the correct PCR conditions, initiate amplification of a section of the CO1 gene of predictable size during PCR. The presence or absence of amplified DNA fragments at or around the expected size of the target region of the CO1 can then be used as a diagnostic tool for determining the species identity of larvae. For example, the occurrence in PCR products of a DNA fragment of the size that would be obtained if species-specific primers were used that work with *D. dimidiatus* but not *D. marginalis* would be good evidence that the amplified DNA came from the former species.

Primer Design – During 2009 CO1 sequences for *Dytiscus* beetles became available to the author [Johannes Bergsten (pers. comm.)] and this enabled primers to be designed with the potential to distinguish *D. marginalis* from *D. dimidiatus*.

Appendix D1 contains the sequences that were reported by Bergsten [at Appendices D1(i) and D1 (ii)]. These sequences have not been published and, as of July 2012, they had yet to be submitted to the GenBank database [Benson *et al.* (2011)]. Appendix D1 contains also sequences for sections of the CO1 gene in *D. marginalis* and in *D. dimidiatus* that had been reported by July 2012 to GenBank, but which were not available in 2009 when species-specific primers were first used.

Primer pairs complementary to sections of the sequence reported by Bergsten were designed and tested for species-specific amplification. Details of these are given in Table 2.3 below.

Table 2.3: Species-specific CO1 primers used in this study. All are shown in 5' – 3' direction. Key: A = Label given to primer; B = Primer Sequence; C = Oligonucleotide length in base pairs (bp); D = Melting temperature; E = Expected product size from PCR using the primer pair

A	B	C	D	E
DDF1	F1: AGGATTTGGAATAATTTCACAT	22 bp	58ºC	275 bp
DDR1	R1: CTCATAATAAAGATGGGCTATAA	23 bp	56ºC	
DDF2	F2: TCAAATTAGTTATAGCCCATCTTTAT	26 bp	59ºC	113 bp
DDR2	R2: GAAGAATAATATCAATTGATGAA	23 bp	56ºC	
DMF1	F1: AGGGTTTGGGATAATTTCTCAC	22 bp	62ºC	279 bp
DMR1	R1: AATGCTCATAGTAAAGAAGGACTATAT	27 bp	54ºC	
DMF2	F2: TCAAATTAGATATAGTCCTTCTTTAC	26 bp	55ºC	113 bp
DMR2	R2: GAAGAATAATATCAATCGAAGAG	23 bp	55ºC	

Figures 2.3 to 2.4 inclusive below show the species-specific sequences targeted for amplification by the primers in Table 2.4.

Figure 2.3 shows that the primer sequences in each species differed by 4 nucleotides in both the forward and reverse binding sites. Because of this level of mis-match over such a relatively short sequence, one would be reasonably confident that the DDF1/R1 combination of primers would facilitate the amplification of mitochondrial DNA from *D. dimidiatus* but not DNA from *D. marginalis*. Conversely, it would be expected that the

DMF1/R1 primer combination would have the opposite specificity, amplifying *D. marginalis* DNA but not *D. dimidiatus* DNA.

Figure 2.3: Partial CO1 Sequences targeted by DDF1 & DDR1 compared with those targeted by DMF1 & DMR1. Primer binding sites are highlighted. Loci within the binding sites where there are differences between the species are underlined. (Source of CO1 sequence data: J Bergsten.)

Partial CO1 Sequence for *D. dimidiatus* with DDF1 & DDR1 binding sites

AGGATTTGGAATAATTTCACATATTATTAGACAAGAAAGAGGAAAAAAGGAAACTTT
TGGTTCTTTAGGAATAATTTATGCTATACTAGCAATTGGTTTATTAGGGTTTGTTGTA
TGAGCACATCATATATTTACTGTAGGGATAGATGTAGACACACGAGCATATTTTACT
TCTGCTACTATAATTATTGCCGTACCCACAGGAATTAAAATTTTTTCTTGATTAGCAA
CTCTTCATGGATCTCAAATTAGTTATAGCCCATCTTTATTATGAG

Partial CO1 Sequence for *D. marginalis* with DMF1 & DMR1 binding sites

AGGGTTTGGGATAATTTCTCACATTATTAGACAAGAAAGAGGAAAAAAGGAAACTTT
TGGTTCTCTAGGTATAATTTATGCTATATTAGCAATTGGTCTATTAGGATTTGTTGTA
TGAGCACATCATATATTTACTGTAGGAATAGATGTAGACACACGGGCATATTTTACT
TCTGCTACTATAATTATTGCTGTACCCACAGGAATTAAAATTTTTTCTTGGTTAGCAA
CTCTTCATGGATCTCAAATTAGATATAGTCCTTCTTTACTATGAGCATT

As can be seen from Figure 2.4, similar specificity could be expected reasonably from the DDF2/R2 and DMF2/R2 primer pairs. In this case, comparing the *D. dimidiatus* CO1 sequence with that for D. marginalis, there are 4 different nucleotides between the forward binding sites and 3 nucleotides difference in the reverse sites.

Figure 2.4: Partial CO1 Sequences targeted by DDF2 & DDR2 compared with those targeted by DMF2 & DMF2. Primer binding sites are highlighted. Loci within the binding sites where there are differences between the species are underlined. (Source of CO1 sequence data: J Bergsten.)

Partial CO1 Sequence for *D. dimidiatus* with DDF2 & DDR2 binding sites

TCAAATTAGATATAGTCCTTCTTTACTATGAGCATTAGGGTTTGTATTTTTATTTACT
GTAGGGGGTTTAACAGGAGTAGTATTAGCTAACTCTTCGATTGATATTATTCTTC

Partial CO1 Sequence for *D. dimidiatus* with DDF2 & DDR2 binding sites

TCAAATTAGTTATAGCCCATCTTTATTATGAGCATTAGGATTTGTATTTTTATTTACT
GTAGGGGGTTTAACAGGAGTAGTATTAGCTAATTCATCAATTGATATTATTCTTC

Testing the primers – As explained, it was anticipated that The DM series (i.e. the DMF1/R1 and DMF2/R2 pairs) would facilitate amplification of *D. marginalis* DNA but not the DNA of *D. dimidiatus* whereas the DD series (DDF1/R1 and DDF2/R2) should have the opposite specificity. In practice, it is possible for even large (17 – 25 mer) primers to generate PCR products other than those expected because a minor mis-match in base sequence will not necessarily stop primers binding. Optimisation of PCR conditions (in terms of salt conditions and annealing temperatures) should minimise but may not altogether remove the possibility of amplification of DNA from non-target species [Beebee and Rowe (2004)]. For this reason, before using them to identify a particular target species (such as material from *Dytiscus* larvae), it was important to test candidate primers on DNA of known provenance to make sure they were capable of yielding accurate and consistent results.

In this study, the four sets of paired primers were tested (i.e. DDF1 & DDR1, DDF2 & DDR2, DMF1 & DMR1 and DMF2 & DMR2) using template DNA extracted from adult beetles.

Preliminary tests were conducted to discover whether optimal PCR conditions could be identified for PCRs involving the four primers. The effects were investigated firstly of varying the Magnesium ion concentration.

Magnesium is an enzyme co-factor required by the thermostable DNA Polymerase (Taq) enzyme used in PCR amplification (McDowell, 1999) and amplification success is sensitive to magnesium concentration. PCR Master mixes were made generating differing final PCR concentrations of $MgCl_2$ as follows: 1.0 mM; 2.0 mM; 3.0 mM; 4.0 mM; 5.0 mM and 6.0 mM. With the exception of the Magnesium ions and the concentrations of primer (1.0 µM of forward and reverse primers) the concentrations of reactants in the final PCR mix were as in Table 2.2. The primer pair DDF1/R1 was selected to facilitate amplification of template DNA from an

adult *D. dimidiatus* beetle. PCRs were conducted in duplicate with annealing temperatures of 52ºC and 55 ºC. The thermal conditions used in the PCR are summarised in Table 2.4 below.

Table 2.4: PCR conditions used in Mg^{2+}optimisation experiments.

Step	Treatment	No. of cycles
Denaturation	95ºC for 5 minutes	1
Denaturation	94ºC for 1 minute	35
Annealing	52ºC or 55 ºC for 1 minute	
Elongation	72ºC for 1 minutes	
Elongation	72ºC for 1 minutes	1
Storage	4ºC for up to 99 hours	N/A

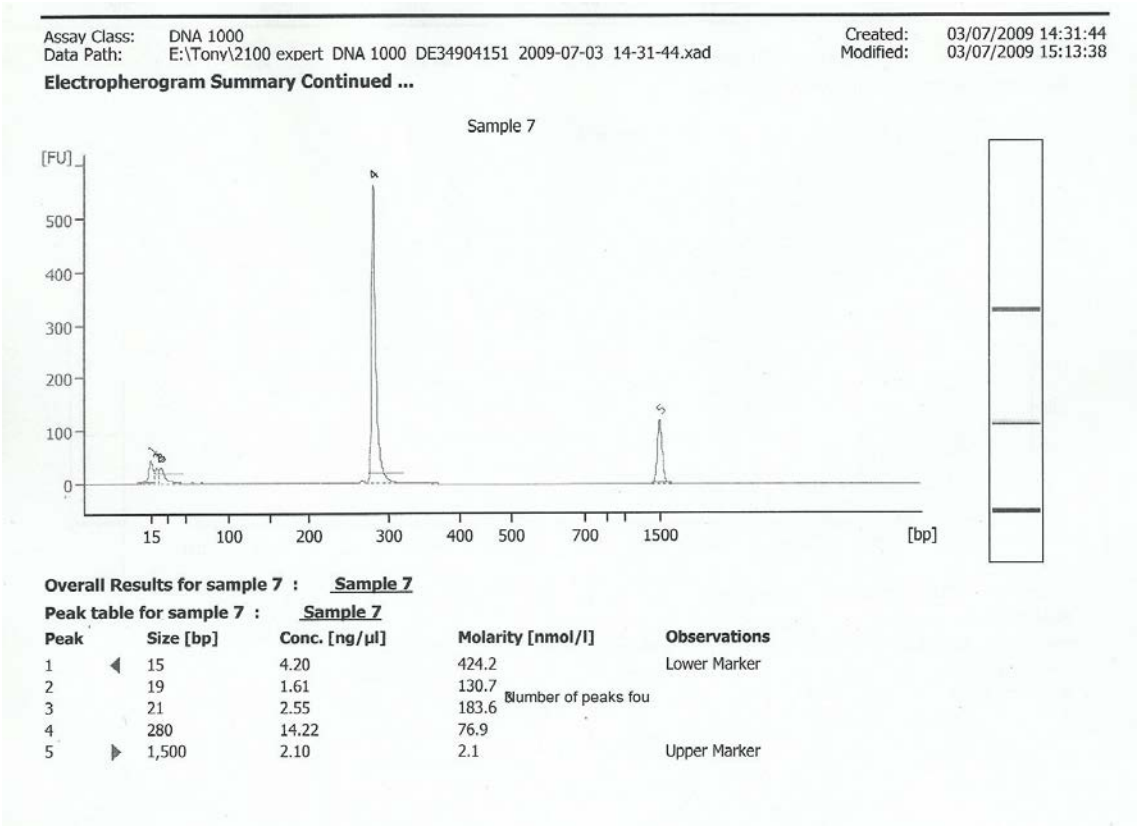
The results of the optimisation experiments at 52 ºC and 55 ºC are reported and discussed in Chapter 4. Once the optimal Magnesium ion concentration was established, further experiments were carried out to investigate PCRs outside of the 52 ºC to 55 ºC range and in order to test the effect of annealing temperature on PCRs using each primer pair combination. Annealing temperatures are usually 15 ºC to 25 ºC lower than the melting temperatures of a DNA duplex but increasing annealing temperature usually increases amplification specificity [Hames and Higgins (1985)]. In the context of this study, if 'DM primers' and 'DD primers' amplified non-target DNA at annealing temperatures towards the lower end of the temperature range, they might cease to do so at higher values of T Anneal.

To investigate the effect of temperature the DM and the DD primer pairs were tested in PCRs with an $MgCl_2$ concentration of 3.0 mM at various values of T Anneal between 50 ºC and 65 ºC. Template DNA from single adults of both *D. dimidiatus* and *D marginalis* was prepared by diluting neat extracts 1:5 with MGBW. Where possible, the experimental treatments were duplicated in order to account for chance amplification failure.

Assay - PCR products were separated on micro-chips rather than on agarose gels. The chip-based system depended on capillary

electrophoresis [Dooley *et al.* (2005) and the electrophoresis was conducted on an Agilent 2100 Bioanalyser device using 12-well chips [Dooley & Garrett (2005)]. Visualisation of the results was achieved using the '2100 Expert' software supplied with the 2100 Bioanalyser. This programme is capable of displaying the results either as an electropherogram or as an interpretation of how the results would appear if separation had been done on an agarose gel, the peaks in the electropherogram being interpreted by the software as bands in a 'virtual gel'. An example of the electropherogram format is shown in Figure 2.5 (below). Results are displayed in Chapter 4 only as electropherograms.

Figure 2.5: Example of DNA assay interpreted by Agilent 2100 Bioanalyser



2.5.2.3 DNA Sequencing

Theory – Automated methods exist to elucidate the sequence of bases in a DNA strand and these may be exploited to investigate polymorphisms down to the single nucleotide level [Hunkspiller *et al.* (1991)]. Sequencing techniques were employed here to distinguish between *D. dimidiatus* larvae and *D. marginalis* larvae. This was done by sequencing a fragment of the CO1 gene from a larval specimen and comparing the sequence with that from the same fragment of the CO1 gene from beetles of known species identity (i.e. adults).

In the first instance, the sequence of part of the CO1 gene was obtained from adult beetles.

In the previous section it was reported that sequence information had been obtained [Bergsten (pers. comm.)] for an 803 bp segment of the CO1 gene in *D. marginalis* and *D. dimidiatus* individuals from Oland in Sweden (see Appendix D1 for full sequences). It was possible using these sequences to identify two almost identical portions of more than 25 bp length, for primer design, in the CO1 gene of both species. These are illustrated in Figure 2.6 below.

Figure 2.6: 159 bp Portion of CO1 gene in *D. dimidiatus* with sections highlighted that are similar or identical between this species and *D.marginalis.* In the latter species the underlined Cytosine (C) nucleotide is replaced by a Thymine (T).

GAGGAAAAAAGGAAACTTTTGGTTCTTTAGGAATAATTTATGCTATACTAGCAATTG
GTTTATTAGGGTTTGTTGTATGAGCACATCATATATTTACTGTAGGGATAGATGTAG
ACACACGAGCATATTTTACTTCTGCTACTATAATTATTGCCGTAC

From Figure 2.6 it can be seen that the first highlighted section (comprising 25 bp) of DNA in both the *D. dimidiatus* and *D. marginalis* sequences is precisely the same in each species. The second highlighted section (27 bp in length) differs by a single base (underlined in the Figure) between the two species. Forward and reverse primers based on these sequences were designed and used in PCRs to obtain

amplified fragments of the CO1 gene of predictable size (c.150 bp) as follows:

Forward - GAGGAAAAAACGAAACTTTTGGTTC;

Reverse – GTACGGCAATAATTATAGTAGCAGAAG.

The fragments that would be expected from PCRs using this primer are given in Figure 2.7. It was assumed that the difference of one base pair in the reverse primer compared with the known sequence for *D. dimidiatus* would not prevent amplification from taking place of material from this species.

Figure 2.7: Expected 159 bp fragments from CO1 genes of *D. dimidiatus* and *D. marginalis*. Primer sequences are underlined and nucleotide differences with *D. marginalis* are highlighted in blue and red.

***D. dimidiatus* sequence**

GAGGAAAAAAGGAAACTTTTGGTTCTTTAGGAATAATTTATGCTATAC
TAGCAATTGGTTTATTAGGGTTTGTTGTATGAGCACATCATATATTTA
CTGTAGGGATAGATGTAGACACACGAGCATATTTTACTTCTGCTACT
ATAATTATTGCCGTAC

***D. marginalis* sequence**

GAGGAAAAAAGGAAACTTTTGGTTCTCTAGGTATAATTTATGCTATAT
TAGCAATTGGTCTATTAGGATTTGTTGTATGAGCACATCATATATTTA
CTGTAGGAATAGATGTAGACACACGGGCATATTTTACTTCTGCTACT
ATAATTATTGCTGTAC

Larval DNA that was amplified using the Forward and Reverse Primers and then sequenced was compared with the expected sequences for the two species and assigned to one of the two.

PCR of larval DNA extracts – Samples of larval DNA for sequencing were prepared using a double PCR process followed by purification of the product. The aim was to generate clean samples for sequencing with

large amounts of the 159 bp CO1 fragment. The PCR protocol that was followed is described below.

A PCR mix was made up on ice with reactants in the proportions shown in Table 2.2 with the Magnesium ion concentration at 3.0 mM. The conditions under which the PCRs were conducted are shown in Table 2.5.

Table 2.5: PCR conditions used to prepare larval DNA samples for sequencing.

Step	Treatment	No. of cycles
Denaturation	94ºC for 4 minutes	1
Denaturation	94ºC for 1 minute	35
Annealing	53ºC for 1 minute	
Elongation	72ºC for 1 minute	
Elongation	72ºC for 4 minutes	1
Storage	4ºC for up to 99 hours	N/A

The process described above was repeated adding 4 μl of each first-round PCR products to a set of new tubes with16 μl of fresh PCR mix. The second round PCR products were purified using a Qiagen MinElute PCR Purification Kit as set out below.

PCR Purification – The procedure followed in order to purify second round purification products was that which is documented in the 'MinElute PCR Purification Kit Spin Protocol' contained in the 'MinElute Handbook March 2008' accompanying the Purification Kit [Qiagen (2008)].

100 µl of Kit Buffer 'PB' was added to each 20 µl volume of second round PCR product and the mixture transferred to a MinElute spin column nested in a 2ml collection tube (one per PCR sample to be purified). The spin columns were centrifuged in their collection tubes at 13,000 rpm for 1 minute. The flow-through in each collection tube was discarded and the spin columns put back in the collection tube.

To wash each sample, 750 µl of Kit Buffer 'PE' was added to each spin column in its collection tube and the column and tube centrifuged at 13,000 rpm for 1 minute. The flow throughs were discarded and the columns and tubes centrifuged once more for 13,000 rpm for 1 minute. This was done in order to remove any residual PE Buffer as the buffer contains Ethanol which could interfere with PCRs conducted in sequencing.

To elute DNA the spin columns were placed in fresh 1.5 ml Eppendorf tubes and 10 µl of Buffer 'EB' (10 mM Tris-Cl, pH 8.5) added to each column. Care was taken to apply the buffer to the centre of the spin column membrane. Each sample was allowed to stand for 1 minute before the columns were centrifuged in the tubes at 13,000 rpm for 1 minute. According to Qiagen, on average 9 µl of elute is obtained for every 10 µl of EB Buffer applied.

The Eppendorf tubes with eluted, purified DNA were stored at -20ºC prior to being sent for sequencing.

Sequencing – Samples to be sequenced were sent with a small volume of the forward primer to Macrogen Inc. The company employs a 3730XL DNA Automatic Sequencer to conduct sequencing that utilises capillary electrophoresis (Source: http://www.macrogen.com/eng/sequencing).

Sequences were obtained from Macrogen in various computer file formats including in .txt files. The .txt sequence files were searched by eye and by using the 'Find' function in Microsoft Word 2003 to look for the 5 – 6 bp sequences with single base pair differences that distinguish *D. dimidiatus* from *D. marginalis*. The sequences used to distinguish the two species are given in Table 2.6 below.

Table 2.6: Sequences (5 – 6 bp) within the amplified 158 bp CO1 fragment used to distinguish between species.

D. dimidiatus	GTTTA	GGTTT	GGATA	GAGCAT
D. marginalis	GTCTA	GATTT	GAATA	GGGCAT

The positions of these identifier sequences within the 159 bp sequence are shown in Figure 2.8 below.

Figure 2.8: Expected 159 bp fragments from CO1 genes of *D. dimidiatus* and *D. marginalis* with identifying sequences highlighted. Primer sequences are underlined and identifier sequences are highlighted in blue and red.

***D. dimidiatus* sequence**

GAGGAAAAAAGGAAACTTTTGGTTCTTTAGGAATAATTTATGCTATAC
TAGCAATTGGTTTATTAGGGTTTGTTGTATGAGCACATCATATATTTA
CTGTAGGGATAGATGTAGACACACGAGCATATTTTACTTCTGCTACT
ATAATTATTGCCGTAC

***D. marginalis* sequence**

GAGGAAAAAAGGAAACTTTTGGTTCTCTAGGTATAATTTATGCTATAT
TAGCAATTGGTCTATTAGGATTTGTTGTATGAGCACATCATATATTTA
CTGTAGGAATAGATGTAGACACACGGGCATATTTTACTTCTGCTACT
ATAATTATTGCTGTAC

It was considered possible that one 5 bp sequence corresponding to one of those in Table 2.5 and Figure 2.8 might occur in the amplified sample by chance and therefore no positive identifications were made unless at least two segments of DNA that had sequences matching the species-specific 5-6 bp sequences occurred.

2.6 Biometrics of *Dytiscus* spp. larvae

During the investigation specimens of *Dytiscus* larvae were taken in the field and preserved in 70% or 100% Industrial Methylated Spirit (IMS). These specimens were used for identification using morphological criteria. In order to decide what morphological features would be used for this purpose, I investigated what keys were available for the identification of *Dytiscus* larvae and then analysed the keys to see which diagnostic features would be most useful to identify the species most likely to occur in the study areas (i.e. *D.*

dimidiatus, *D. marginalis*, *D. semisulcatus* and possibly, *D. circumflexus* – see Chapter 1 for details of historic records from the Somerset Levels and Moors). The results and conclusions of this part of the investigation are presented in Chapter 4.

2.6.1 Larval keys

Three dichotomous identification keys were found for identification of the larvae of northern European *Dytiscus* species, those of Klausnitzer (1991), Rozkošnȳ (1980) and Nilsson (1982).

Kausnitzer's key was published in German and was based on two much earlier keys [Blunck (1923) for first instar (L_1) larvae and Blunck & Klynstra (1929) for third instar (L_3) larvae]. A translation of Klausnitzer's key was undertaken for the author by Inga Zeisset and a modified version of this is reproduced in Appendix D along with a copy of the original.

Rozkošnȳ's key appeared in a work written in Czech on the larvae of aquatic insects of the former Czechoslovakia. Like Klausnitzer, Rozkošnȳ cited Blunck and Klynstra's 1929 paper and a rough translation by the author of Rozkošnȳ's key suggested that it uses many of the same diagnostic features as those in Klausnitzer's key and, presumably, therefore, of those due to Blunck and Klynstra (Op. cit.). The main difference between the keys of Rozkošnȳ and Klausnitzer appeared to be in the way that the dichotomous couplets were arranged. For example, *Dytiscus circumflexus* was identified sooner using Rozkošnȳ's key, but, otherwise there was a great deal of similarity between the two keys particularly in terms of the diagnostic features used.

I obtained the key produced by Nilsson (1982) only towards the end of the investigation. It did not influence the choice of diagnostic features to be used in this investigation but it is discussed in the results section in Chapter 4.

2.6.2 Diagnostic features

The keys separate the larvae into species of *Dytiscus* on the basis of a number of diagnostic features that are outlined below. Particular attention is paid in this account to the features that supposedly distinguish *D. dimidiatus* from *D. marginalis*. According to Blunck (1923), there are three larval instars in *Dytiscus* before pupation. From earliest to latest these may be designated L_1, L_2 and L_3 to distinguish them.

2.6.2.1 Overall size

According to Blunck (1923), *D. dimidiatus* larvae tend to be larger than *D. marginalis* larvae in both the L_1 and L_3 stages. Blunck (Op cit.) gives measurements indicating that L_1 larvae of *D. dimidiatus* may be up to 27 mm long but that those of *D. marginalis* do not exceed 23 mm. Rozkošnȳ (1980) states that L_3 larvae of *D. marginalis* grow to 50 mm in length but D. *dimidiatus* can be up to 58 mm. According to Rozkošnȳ (1980), the L_3 larvae of other *Dytiscus* species recorded historically in Somerset range in maximum size from *D. semisulcatus* (46.5 mm) to *D. circumflexus* (48 mm), suggesting that the larvae of these species tend to be smaller than either *D. dimidiatus* or *D. marginalis*. The size order (smallest to largest) reported in the larvae of the species mentioned – i.e. *semisulcatus, circumflexus, marginalis, dimidiatus* – mirrors that of the adult beetles as given by Foster & Friday (2011), except insofar as *D. marginalis* and *D. circumflexus* are said to attain the same maximum size as adults. The final stage larvae of the beetles that are largest in adulthood tend to be relatively large themselves.

2.6.2.2 Shape and dimensions of head

In the L_1 and L_3 larvae of most of the six species of *Dytiscus* that are native to the UK, the front edge of the head (frontoclypeus) is strongly convex in shape. The one exception to this is *D. semisulcatus*. In this species, when its larvae are viewed directly from above, the frontoclypeus is only weakly convex in comparison with the other species

as can be seen in the Figures accompanying the translated key in Appendix D.

D. semisulcatus larvae may also be distinguished from the other species by the width of the neck compared with the width of the head. The neck is at least three quarters the width of the head in this species whereas, at its widest point, the head is much broader than the neck in all the others.

The L_3 larvae of *D. marginalis* and *D. dimidiatus* supposedly can be distinguished from each according to by the comparative length and width of their heads. According to Klausnitzer's and Rozkošnȳ's keys, the width of the larval head does not exceed 7 mm in *D. marginalis* , but can be up to 8.3 mm wide in *D. dimidiatus*. The same keys quote head lengths of 8.6 mm for *D. dimidiatus* and 7.3 mm for *D. marginalis*.

The L_3 larvae of *D. circumflexus* possess heads that are more similar in overall shape to those of *D. dimidiatus* and *D. marginalis* than they are to *D. semisulcatus*, but the dimensions of the head are smaller than either *D. dimidiatus* or *D. marginalis*.

2.6.2.3 Length of antennae

The antennae of L_3 *D. dimidiatus* larvae exceed 6.5 mm in length according to Klausnitzer and Rozkošnȳ and are shorter in *D. marginalis* by up to 1 mm or more. They are shorter still in *D. circumflexus* and *D. semisulcatus* according to measurements presented in Klausnitzer (1991) from Blunck and Klynstra (1929).

2.6.2.4 Length of jaws

The length of the larval jaws is not a diagnostic feature in Klausnitzer's key, but it is used in Rozkošnȳ's. There, it is one of the features that are used to distinguish *D. dimidiatus* larvae from those of *D. marginalis* as well as from *D. circumcinctus* and *D. circumflexus*. According to

Rozkošnỹ, the jaws of *D. dimidiatus* larvae exceed 6.5 mm in length while those of *D. marginalis* are over 5 mm but do not exceed 5.5 mm.

2.6.2.5 Length of maxillae

The length of these mouthpart appendages is used in Klausnitzer's key in the identification of both L_1 and L_3 larvae after the couplets that separate out *D. semisulcatus* and *D. dimidiatus*. According to this key, *D. marginalis* differs from *D. circumflexus* in that the maxillae of the former can exceed 5 mm whereas those of the latter do not.

2.6.2.6 Relative length of urogomphi compared with abdominal segment 8

Both early and late instar larvae of *D. semisulcatus* possess urogomphi that are long in comparison with the final abdominal segment to which they are attached. According to Klausnitzer (1991), it is a unique feature to *D semisulcatus* that the urogomphi of the L_1 larvae are longer than abdominal segment 8. In the L_3 instar, abdominal segment 8 is hardly, if at all, more than 1.5 times as long as the urogomphi.

2.6.2.7 Swimming hairs on front legs

Observation of the swimming hairs on the front legs of larvae offers an alternative means to distinguish *D. semisulcatus* larvae from those of the other species. Illustrations reproduced in Klausnitzer's key from Blunck and Klynstra (1929) indicate that the hairs on the tarsi of the front legs are grouped distally (i.e. furthest away from the body) in *D semisulcatus* but proximally in all other species. This diagnostic feature is used in both the Klausnitzer and Rozkošnỹ keys.

The illustrations referred to do not appear to the author to suggest any means by which the larvae of the other species could be separated readily on the basis of swimming hairs on the front tarsi.

2.6.2.8 Length of hind legs

Rozkošnȳ and Klausnitzer both use hind leg length as a feature to distinguish *D. dimidiatus* from other species. It is stated that, for the L_3 larvae, the length of the hind leg is about 19.5 mm (excluding claws).

The length of the hind femur is supposedly a means to distinguish *D. marginalis* larvae from those of *D. circumflexus* according to Klausnitzer (1991). If its femur is longer than 4.5 mm the larva will be that of *D. marginalis*, according to the key, shorter and it will be *D. circumflexus*.

2.6.2.9 Summary regarding diagnostic features

From the above it can be seen that, according to the keys, a larva of *D. semisulcatus* ought to be readily distinguishable from the other *Dytiscus* species likely to be encountered on the Somerset Levels by the thickness of its neck compared to the breadth of its head, by the shape of the frontoclypeus and by its large urogomphi in relation to abdominal segment 8. A further diagnostic feature available is the position of swimming hairs on the tarsus of the front legs.

The keys rely largely on size differences to separate the other *Dytiscus* species that are likely to be found. The overall size of the larva, the length and breadth of the head as well as the length of hind legs, jaws, maxillae and antennae contribute to the identification.

The suite of larval features that were measured during this investigation is listed in Table 2.6 below and is illustrated in Figure 2.9. Neither the maxillae nor the jaws of larvae were measured, because I judged that none of these features are crucial to a positive determination of species using the keys. Given the curving nature of the jaws there was also an issue regarding how the measurement would be obtained in a consistent manner.

Table 2.7: Larval Biometry – Features measured and calculations undertaken to obtain quantitative numerical data on *Dytiscus* larvae

	Feature
A	Length of antenna
B	Length of head capsule
C	Width of head capsule
D	Width of neck
E	Length of thoracic segment 1 (T1)
F	Length of thoracic segments 2 & 3 (T2 – T3)
G	Length of abdominal segments 1, 2 & 3 (A1 – A3)
H	Length of abdominal segments 4, 5 & 6 (A4 – A6)
I	Length of abdominal segment 7 (A7)
J	Length of abdominal segment 8 (A8)
K	Length of urogomphus
L	Length of tarsal claw of hind leg
M	Length of tarsus of hind leg
N	Length of tibia of hind leg
O	Length of femur of hind leg
P	Length of coxa of hind leg

Calculations: For each specimen (where possible):

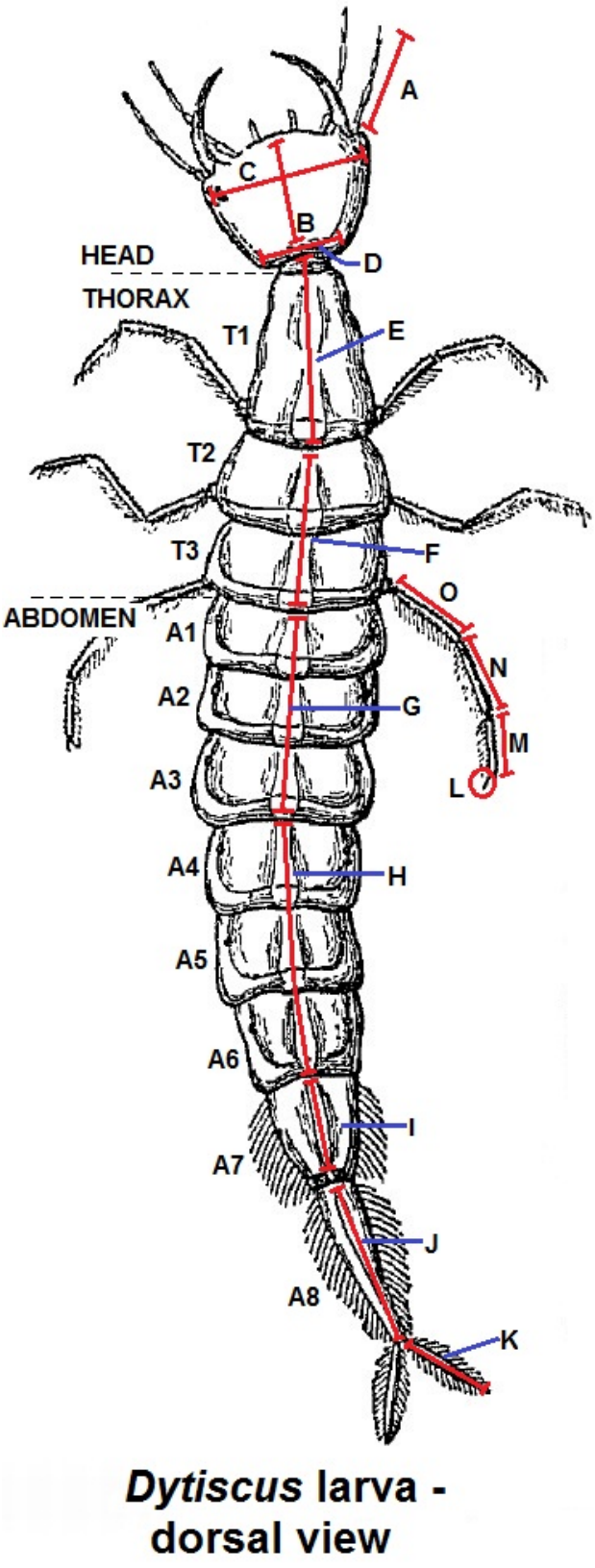
Body Length = B+D+E+F+G+H+I

Leg length = L+M+N+O+P

The ratios C/D, P/M and J/K were also calculated

All the features labelled A – O in Table 2.7 are shown on Figure 2.9. It is not possible to see the coxa of the hind leg because a dorsal view of the larva is presented in Figure 2.9 and the coxa would be underneath the animal. Wherever possible the urogomphus was measured with the larva on its side since it is usually partially obscured when viewed directly from above.

Figure 2.9 – *Dytiscus* larva showing features measured



KEY

HEAD
A = Length of antenna
B = Length of head capsule
C = Width of head capsule
D = Width of neck

THORAX (T)
E = Length of T1
F = Length of T2 – T3

ABDOMEN (A)
G = Length of A1 – A3
H = Length of A4 – A6
I = Length of A7
J = Length of A8
K = Length of urogomphus

HIND LEG
L = Length of tarsal claw
M = Length of tarsus
N = Length of tibia
O = Length of femur

(N.B. Coxa on ventral surface)

Values for F, G and H will depend on the amount of extension allowed by the inter-segmental membranes which could be affected by preservation in ethanol.

2.6.3 How measurements and observations were made

Measurements were made using an eyepiece graticule in a Meiji Techno EMZ Zoom binocular microscope. The graticule divisions were calibrated against a Phillip Harris Micrometer (0.1mm divisions) before measurements were begun. The results of the calibration are summarised in Table 2.7 below

Table 2.8: Calibration of Meiji Binocular Microscope. (4/8/2010)

Power	Magnification	Mm per eyepiece graticule division
0.7	7x	0.150
1.0	10x	0.100
1.5	15x	0.068
2.0	20x	0.050
2.5	25x	0.041
3.0	30x	0.033
3.5	35x	0.029
4.0	40x	0.025
4.5	45x	0.022

In each case the lowest magnification was used which enabled the measurement to be made using the graticule. Generally, it was found that body and head measurements could be made using 7x or 10x magnification and leg measurements using the 20x magnification.

All body measurements (i.e. measurements A to I inclusive) were taken wherever possible looking down onto the dorsal surface of the larva. In cases where the specimen had become fixed in a doubled-up position, certain measurements had to be taken from the side.

In order to make it easier to take the leg measurements, a single hind leg was removed from the specimen by cutting the leg from the body as close as possible to the base of the coxa. The legs that were removed were used to extract larval DNA as described in section 2.5.1.4 above. All measured larvae have been retained stored in 100% IMS. Legs from which DNA was to be extracted were kept in 100% IMS until they were macerated during the extraction process (see 2.5.1.4).

A number of observations were made of certain characteristics of each specimen in addition to the measurements. Firstly, the condition of the larva was noted and these notes were used to assign the specimen to one of four categories: 1 – Whole; 2 – Damaged but intact; 3 – Exoskeleton only and 4 - Fragments only. These data are used later in Chapter 7 to assess the possible causes of trap mortality and the possible levels of inter-larval predation in the traps.

In addition to the condition assessment, the larvae were examined to see whether swimming hairs could be observed on the sides of abdominal segment 7 (A7). This was because the absence of hairs is regarded by authorities as indicating that a larva is in the first larval instar (L_1) stage [Klausnitzer (1995)]. Keys such as Klausnitzer's utilise the shape of the frontoclypeus and the position of swimming hairs on the tarsi of the front legs to separate examples of second or third instar larvae (L_2 and L_3) into species [Kausnitzer (O*p Cit*.)]. For this reason, if the larval specimen did possess swimming hairs on A7, further observations were made to decide whether the frontoclypeus was 'concave' or 'convex' in shape and if the swimming hairs on the front tarsi were located 'proximally' or 'distally' in relation to the body.

Desktop Study

2.7 Statistical analyses

2.7.1 'Standard' Statistical Tests

Unless stated otherwise in the text, the statistical software programme used to analyse most of the data collected was 'QED Statistics' (version 1.1.3.450 © Pisces Conservation Ltd, 2007).

2.7.2 Sequential Bonferroni Corrections

Where many correlations (or other inferential statistical tests) are attempted from the same dataset it is possible for the null hypothesis to be rejected when it is in fact true. For example, if a confidence level (α) of 0.05 is set, this implies that in 1 in 20 correlations from the same dataset a false significant result might arise due to random chance. The simplest (but most conservative way) to account for the possibility of acceptance of false null hypotheses is to reduce the confidence level by applying the Bonferroni Correction as developed by Dunn (1961). This is the corrective device used in this study.

The Bonferroni Correction is applied sequentially. The P values obtained from n statistical tests are placed in numerical order with the lowest first and highest last. Each value of P is in turn compared with a reduced confidence level α/n for the first comparison, α/n-1 for the second comparison, α/n-2 for the third, etc. The null hypothesis is rejected if the P value obtained from the test statistic is less than the adjusted confidence level. Critics of this method have noted that, although the Bonferroni Correction guards against the acceptance of false positives, it is possible for null hypotheses to be accepted where, a statistically significant result should be recognised [see, for example Moran (2003)].

2.7.3 Multivariate techniques

Two types of multivariate statistical analyses were conducted and each is described below.

2.7.3.1 Principle Components Analysis (PCA)

PCA is an ‘explanatory’ multivariate method used to summarise datasets in order to show the relationships between variables. A dataset comprising of many variables may be seen as a set of coordinates in a multi-dimensional space with one axis per variable. The data is mathematically transformed [by a method

devised by Pearson (1901)] so that the greatest amount of variance is projected onto the first axis of a graph (called the first principal component), the second greatest variance on the second axis, etc, etc. Because multi-dimensional graphs are difficult to visualise, when the results of PCA are displayed graphically this is usually in a 2 dimensional form (or 'biplot'), which is the convention used in this thesis. Points on the plot with the greatest similarity are closest together in multi-dimensional space.

A measure of the amount of variance represented within a particular principle component is its 'eigenvalue'. Because each axis resolves a diminishing amount of variance, it is usually the case that the first few principle components only are meaningful in the sense that they 'explain' a reasonable percentage of the total variance within the dataset. For this reason, Henderson & Seaby (2008) suggest that "*you should only interpret principle components if the corresponding eigenvalue is larger than the mean of all the eigenvalues*".

PCA is most effective when all the variables in a dataset are normally distributed; however, according to Henderson and Seaby (2008) PCA "*gives good results with non-normal data provided none of the variables is highly skewed or has extreme outliers*".

The PCAs were undertaken using Community Analysis Package (CAP) software version 4.0 (Pisces Conservation Ltd 2007). The CAP software allowed individual animals to be assigned to particular groups (e.g. larval stages or species). In order to test whether the groups assigned had any statistical significance an Analysis of Similarity Test (or ANOSIM') was performed using the CAP software.

The ANOSIM employed was developed as a test of the significance of groups that have been defined *a priori* [Clark

(1988, 1993)]. If the assigned groups reflect real, underlying associations between specimens, then individuals within groups should be more similar in composition than those from different groups. The test uses the Bray-Curtis (or 'percentage difference') measure of similarity [Bray & Curtis (1957)]. The null hypothesis is that there are no differences between the members of the various groups.

Strictly speaking the Bray – Curtis Measure (B) is a measure of dissimilarity, while its complement (1/B) is a similarity measure. Formulae for calculating the Measure are given in Krebs (1999) along with a commentary on its applicability to ecological problems. According to Krebs, some authors (e.g. Wolda 1981) counsel against using this measure in situations where sample size (n) is large and the samples are highly diverse.

The test statistic (R) is calculated to measure the differences between the groups according to the following formula:

$$R = \frac{\bar{r}_B - \bar{r}_W}{n\,(n-1)\,/\,4}$$

Where: $\bar{r}_B$ is the mean of ranked similarity between groups, and

$\bar{r}_W$ is the mean ranked similarity within groups.

Values of R can be from +1 to -1. +1 indicates that all the most similar samples are within the same groups. R = 0 occurs if the high and low similarities are perfectly mixed and bear no relationship to the group. A value of -1 indicates that the most similar samples are all outside of the groups.

To test for significance, the ranked similarity within and between groups is compared with the similarity that would be generated by random chance. The samples are randomly assigned to groups

1000 times and R calculated for each permutation. The observed value of R is then compared against the random distribution to determine if it is significantly different from that which could occur at random.

2.7.3.2 Canonical Correspondence Analysis (CCA)

This form of analysis, largely due to ter Braak (1986, 1994), is one in which multiple regressions are carried out of the dependent variables onto the independent. It was used here to investigate relationships in situations where possible explanatory, independent variables were included in the ordination alongside the dependent variables [Henderson & Seaby (2008)].

According to Henderson & Seaby (*Op. cit*.), "*for sufficiently large sample sizes CCA is robust to deviations from normality*" but these authors advise that the use of extreme outliers be avoided, while the technique should not be applied to data with heavily skewed distributions. Henderson & Seaby (Op. Cit) stress the need not to use independent variables in the analysis that are correlated one with another. In order to check whether one variable can be used with another it is customary to run a correlation first to see if any variables should be discarded from the CCA.

One advantage of CCA over most ordination methods is that it allows hypothesis testing so that, not only can one tell the proportion of the variability that is explained by the independent variables, but also whether or not this is statistically significant.

In order to test for significance 'ECOM II' runs a Monte Carlo simulation. Following the 'real' CCA, a series of further CCA plots are simulated one after the other for a specified number of trials. These trials use the same data, but the order of the samples is

shuffled randomly in each trial before the simulation is started. For each simulated CCA the eigenvectors are calculated as they would be normally. (In 'ECOM II' this is done only for the 3 axes with the largest eigenvalues as these are most likely to describe the bulk of the variation). Assuming that there was a strong correlation between the independent and dependent variables in the first instance, then shuffling the order of the samples randomly will produce mostly plots which lack any true relationship between the variables. The computer programme calculates the probability that an eigenvalue as large as that observed in the 'real' CCA plot could have occurred by chance. The null hypothesis is that there is no significant relationship between the variables and the eigenvalue could have arisen by chance.

CCAs and subsequent statistical tests were performed using the 'Ecological Community Analysis' software package 'ECOM II' (version 2.1.3.137 © Pisces Conservation Ltd, 2007).

2.8 Niche measures

2.8.1 Niche breadth

It was noted in Chapter 1 that: "*In niche theory, the axes on which the* [niche] *hypervolume is plotted can be regarded as 'resource states' that may relate to food resources, habitat resources, natural or artificial sampling units or other ecological categories assigned by researchers [Colwell and Futuyama (1971), Krebs (1999)]*". Niche breadth may be considered as the degree to which the subject organism uses all the particular resource states that are available to it. A generalist species tends to use all resource states evenly and has a wide niche breadth, while a specialist concentrates on a few resources and has a narrow niche breadth.

Levins' Measure estimates niche breadth by quantifying the uniformity with which individual organisms distribute themselves across resource states [Levins (1968)]. Levins' Measure (*B*) is calculated according to the formula:

$$B = \frac{1}{\sum p_j^2}$$

Where: p_j = Proportion of total individuals sampled found in or using resource state j.

Levins' Measure does not take account of the fact that resources can vary in abundance and availability but is satisfactory in situations where resource states are thought to be equally abundant or available, such as when the states comprise traps (artificial sampling units) that are in theory equally accessible to all individuals of the population being sampled or when the states are particular days of the year or habitats within the home range of a mobile species.

In order to compare estimates of niche breadth it is convenient to 'standardise' them to a scale of 0 to 1 (Krebs 1999). Hurlbert proposed a way to standardise Levins' Measure by applying the following formula [Hurlbert (1978)]:

$$B_A = \frac{B - 1}{n - 1}$$

Where: B_A = Levins' Standardised Measure of niche breadth;
B = Levins' Measure of niche breadth; and
n = Number of possible resource states.

2.8.2 Niche overlap

To investigate the degree of niche differentiation between two species it is customary to estimate niche overlap. Numerous means of estimating niche overlap have been proposed (Krebs 1999). Studies using both computer simulations and real data have investigated the relative merits of the commonly used measures in relation to such considerations as ease of calculation, effect of sample size and whether or not resource use can be expressed directly in terms of numbers of individuals [Ricklefs & Lau (1980), Wolda (1981), Smith and Zaret (1982), Chao *et al.* (2006)]. The consensus from these studies

seems to be that Morisita's Measure [Morisita (1959)] is the least susceptible to bias due to low sample size and is preferred where actual numbers of individual animals using each resource state are available.

Morisita's Measure (C) (or Morisita's Index of Similarity) is calculated according to the formula:

$$C = \frac{2 \sum^{n}_{i} p_{ij}\, p_{ik}}{\sum^{n}_{i} p_{ij}\, (n_{ij} - 1)/(N_j - 1) + \sum^{n}_{k} p_{ik}\, (n_{ik} - 1)/(N_k - 1)}$$

Where: *C* = Morisita's Measure of niche overlap between species j and k;
p_{jj} = Proportion resource i is of the total resources used by species j;
p_{jk} = Proportion resource i is of the total resources used by species k;
n_{ij} = Number of individuals of species j that use resource category i;
n_{ik} = Number of individuals of species k that use resource category i;
N_j = Total number of individuals of species j in the sample; and
N_k = Total number of individuals of species k in the sample.

Morisita's Measure has a value of 0 when there is absolutely no overlap and a value approximating to 1 when there is complete overlap. Theoretically, the maximum value of C can be slightly over 1 but in his study of similarity indices Wolda concluded that: *"The uncertainties in not having a fixed upper limit for the* [Morisita] *index equal to one are outweighed by the problems of correcting the other indices for effects of sample size and diversity"* (Wolda 1981). As with Levins' Measure, *C* does not take account of varying abundance or accessibility of resources and this limits the situations to which it can be applied readily.

All similarity indices are sensitive to a greater or lesser degree to the effects of sample size. Even Morisita's Measure is not completely immune to bias (in the sense of poorly estimating the true value of the similarity index) if sample sizes are small.

Chapter 3: The efficacy of different *Dytiscus* capture techniques

Introduction

In order to conduct the fieldwork most effectively, it was important to identify the most efficient way to capture sufficient numbers of the target species for study purposes. This chapter addresses the issue of capture techniques.

A literature review was undertaken to ascertain what techniques might be available to catch *Dytiscus* beetles for study. The review took two forms. Firstly there was an investigation of the popular/specialist and the scientific literatures relating to capture methods available for water beetles, focussing particularly on *Dytiscus* spp. There was secondly an examination of the techniques employed in various unpublished reports of contract surveys of aquatic invertebrates conducted on the Somerset Levels and Moors and in similar localities. The results of the two forms of literature review are presented in sections 3.1, 3.2 and 3.3.

Two commonly-used techniques – pond netting and bottle trapping - were compared experimentally at Shapwick Heath. There is a description of the techniques in Chapter 2 (section 2.2) and an account is given in section 3.4 of how they were tested against each other. The results are presented in this section with statistical analyses.

Conclusions concerning the relative merits of capture methods are discussed in section 3.5.

Results

3.1 The popular and entomological literature

This section summarises the results of a search through literature including books and non-peer reviewed journals for information regarding techniques used to capture dytiscid beetles.

3.1.1 Netting

Many older entomological works that give advice on methods to collect water beetles advocate only active methods such as netting or hand searching [e.g. British Museum (Natural History) (1974), Smithers (1982)]. However, few authors mention any potential disadvantages of netting or mention alternatives. Netting can be highly disruptive to habitats, modifying vegetation and stirring up and re-distributing bottom sediments. Not only this, but it is thought to require a relatively large amount of effort to capture large and mobile members of the aquatic invertebrate fauna such as dytiscid beetles. The adult *Dytiscus* in particular are strong swimmers and, will dive quickly to the bottom once the water column is disturbed [T Beebee (*pers. comm*.)].

3.1.2 Bottle trapping

The use of submerged traps can be used to catch water beetles, but such methods have not found widespread favour with British coleopterists, despite live-trapping being employed commonly by workers elsewhere in Europe [Denton (1996)]. Foster (1991) stated that: "*Abroad it is common practice to operate underwater traps to capture the larger dytiscines*". He attributed the lack of popularity of trapping with British coleopterists to low water temperatures in Britain that "*demand that these traps are operated for weeks rather than days to have any success*".

This view is not supported by Denton, who has found simple bottle traps of the sort recommended by Griffiths for amphibian monitoring [Griffiths, (1985)] to be *"a useful and non-destructive monitoring method, especially for the larger Dytiscidae"* [Denton (1996)]. He found that even unbaited traps *"are somehow positively attractive to large beetles and/or that these individuals are better able*

to avoid the net." His conclusion is based on a comparison of the effectiveness of bottle trapping as against netting to capture a range of beetle species from heathland ponds in Southern England. For the purposes of this study, it is interesting to note that, during the course of his investigation, Denton caught adults of three species of *Dytiscus* including one, *D. circumflexus*, that was only captured by trapping. At each sample site, where individuals of any of the three species were found, more individuals were taken in traps than were netted [Denton (1996)].

3.1.3 Other methods

Duff stated that both *D. marginalis* and *D. semisulcatus* are attracted to light [Duff (1993)] and these and, possibly, other *Dytiscus* species may be among the "*large water beetles*" that have been recorded in light traps set in recent years in and around the Somerset Levels and Moors by members of the Somerset Moth Group [D Miller (*pers. comm*.)]. The use of aquatic light traps, including floating traps is said to be on the increase in studies of aquatic faunae [Southwood and Henderson (2000)] and an example of such a trap used to capture water beetles is illustrated in one recently published field guide to European beetles [du Chatenet (2005)].

Bratton compared pond netting against sieving as a technique for monitoring assemblages of aquatic beetles and bugs in a ditch on Anglesey, Wales [Bratton (2001)]. His findings – that sieving tended to catch more species over a season than netting – while interesting are probably not directly helpful to this study, since *Dytiscus* species were not caught by either technique during his research. In any case, if it is suspected that pond netting may be relatively ineffective because of the strong swimming tendencies of the target species, then sieving is unlikely to be any better.

3.2 Scientific papers

The scientific literature is replete with papers on the ecology of aquatic macro-invertebrates. Researchers have employed a plethora of techniques to collect samples from standing waters, from active searching (e.g. using nets or grabs of various types) through to methods relying on more passive trapping techniques (e.g. box traps, funnels, bottles, etc). Since most aquatic bugs and beetles can also fly, capture methods are available also for these groups that are employed in studying winged insects (e.g. flight interceptors and light traps). The sheer variety of different methods used can make it difficult to compare results across studies and there are few papers, relative to the number published in this field, that compare techniques one with another. It was decided to focus the literature search on three types of paper: (a) Ones that compared more than two techniques in order to establish which combination of sampling methods produced the largest inventory of macro-invertebrate species for least survey effort; (b) Ones that compared hand netting against another technique, and; (c) Papers which aimed specifically at establishing the best methods to use to capture dytiscid water beetles.

3.2.1 Multi-technique comparisons

Most studies in which more than two techniques have been compared have sought to identify the most effective sampling protocol to obtain a list of species present that is as comprehensive as possible for the least amount of survey effort [e.g. Klečka & Boukal (2011) Turner & Trexler (1997)]. An issue for these studies has been how to standardise the measurement of survey effort. For example, Klečka & Boukal (2011) found that if survey effort was measured by the average number of specimens per sample the conclusions concerning which method was most effective differed from those reached if survey effort was measured in terms of time taken on average to obtain a specimen.

3.2.2 Hand-netting versus other techniques

Hand-netting using some type of long-handled pond net is a very commonly used technique employed in the study of aquatic macro-invertebrates and it was

one that I seriously considered as the primary means by which data would be collected for this study. For this reason it was thought particularly important that attention should be paid to studies comparing hand-netting with other methods.

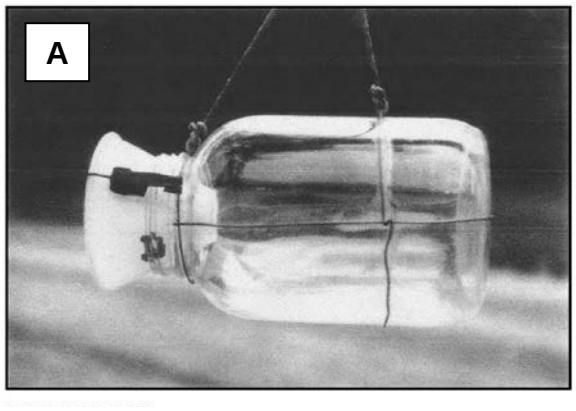
In the literature review conducted several examples were found of studies that compared hand netting against another technique in an attempt to establish which might be the best to employ in particular circumstances. For example, Becerra Jurado *et al.* (2008) compared hand-netting versus bottle traps to assess species diversity in heavily vegetated ponds in Ireland, while, earlier, Murkin *et al.* (1983) contrasted the same two techniques as a means to monitor secondary production in shallow lakes in Canada. O'Connor *et al.* (2004) investigated the macro-invertebrate faunae of Irish turloughs (seasonal waterbodies filled by groundwater). They used box traps fixed to the bottom substrate and hand nets. Garcia-Criado & Trigal (2005) examined sampling techniques applicable in a shallow Mediterranean lake, comparing hand-netting with the use of a Kornijów Sampler [Kornijów (1998)], a device designed for taking macro-invertebrate samples from emergent macrophytes. Muzaffar & Colbo (2002) evaluated hand-netting against the use of artificial substrates in Newfoundland ponds.

Bercerra Jurado *et al.* (2008) found that a mean average of 19.9% of the taxa captured was exclusive to bottle traps, 26.2% were exclusive to nets and 53.9% were common to both methods. They noted that: "*Highly mobile macroinvertebrates such as Dytiscus marginalis (L.) or Acilius sulcatus (L.) were generally exclusive to traps.*" This observation tends to support the earlier work of Murkin *et al.* (1983) which found that for many taxa there was a significant correlation between the species richness of samples from the same lakes caught by traps as compared with those caught by pond net. One exception to this proved to be dytiscid water beetles which were represented more in the bottle trap samples than in the netted samples. This finding was common to other predaceous groups (Coenagrionidae, Ceratopogonidae, Chaoboridae) and this led Murkin *et al.* to hypothesise that this: "*may be due to the attraction of these predators to the activity traps by the presence of the*

entrapped invertebrates. They may therefore be represented in the traps out of proportion to their actual densities" [Murkin *et al.* (1983)]. It is interesting that the two sets of researchers viewed their results in radically different ways, Becerra Jurado *et al.* (2008) interpreting their results as suggesting a negative avoidance of the net by dytiscids, Murkin *et al.* (1983) positing instead a positive attraction to the traps.

It should be noted that the studies of Becerra Jurado *et al.* (2008) and Murkin *et al..* (1983) cannot be compared directly not least because the type of bottle trap used differed profoundly. Those used by Murkin *et al.* were modelled on a design by Whitman (1974) comprising a 3.8 litre glass jar with a wide diameter plastic funnel at the entrance suspended by wire in the water column. The bottle traps used by Becerra Jurado *et al*. (2008) resembled much more those plastic ones described by Griffiths (1985) for amphibian research derived from modified 2 litre drinks containers. There were also differences in the type of net used in the two studies. Despite these differences it is noted that the studies did reach the same conclusions regarding the efficacy of a passive trapping method for catching dytiscids as opposed to active searching with a pond net. Other studies have also contrasted active (pond netting) with passive trapping techniques. A range of traps used in reviewed studies is illustrated in Figure 3.1.

Figure 3.1: A range of traps used to capture aquatic macro-invertebrates

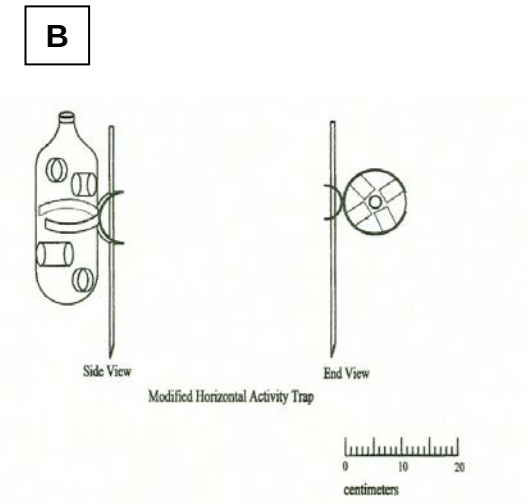


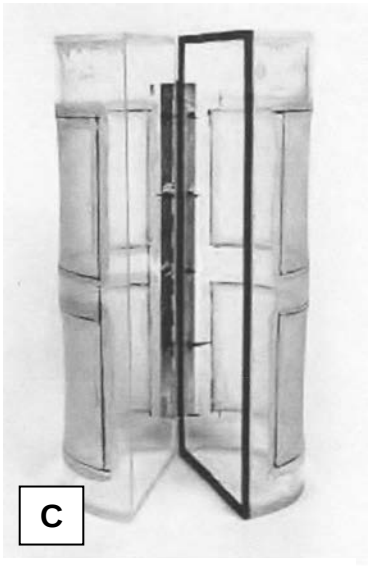
A = Activity trap after Whitman (1974)

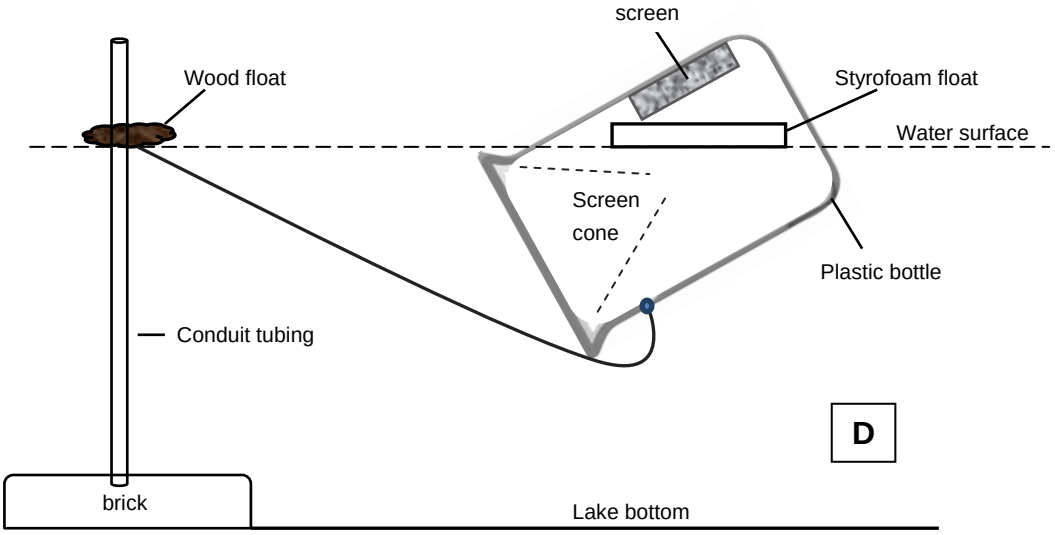
B = Horizontal activity trap after Becerra Jurado et al (2008)

C = Kornijów sampler after Kornijów (1998)

D = Bottle trap after Aiken & Roughley (1985)







O'Connor *et al* (2004) caught nineteen species of Dytiscidae during their study of three turloughs. Over half of the species recorded (9 out of 19) were captured only using a box trap while only two species were exclusively caught by netting. A box trap is a device to physically isolate a known volume of the bottom substrate and water column in a standing water habitat. The trap is deployed for a length of time that allows it to be colonised by macro-invertebrates after which it is retrieved and all the trapped animals are netted or sieved. The shape, design, area of bottom habitat enclosed and volume of water sampled has varied widely between studies [e.g. Goodwin & Eyles (1942), James & Nicholls (1961); O'Connor *et al.* (2004)]. The finding of the study comparing catches from one type of box trap with those from pond nets [i.e. O'Connor *et al.* 2004)] supports the view that passive traps are better at catching the more mobile elements of the water beetle fauna. However, it is noted that the only *Dytiscus* spp. caught - single specimens of *D. circumcinctus* and *D. semisulcatus* - were captured in pond net samples. The failure of the box trap to capture *Dytiscus* spp. that were present is perhaps a function of the low density at which it is likely that a large, predaceous water beetle will occur.

Muzaffar & Colbo (2002) reported catching dytiscid larvae with both pond net and through the use of artificial substrates. However, the only adult dytiscids caught - *Agabus*, *Hygrotus* and *Hydroporus* spp. – were netted. In this case, the artificial substrate consisted of coarse gravel taken from the bottom of the study ponds which was cleaned and returned to the ponds in 'rock bags' made of expansible plastic webbing in tube form. The artificial substrate was left in place for a time to allow colonisation by invertebrates and later collected and processed to obtain faunal samples. Sutton (2008) observes that *Dytiscus* larvae are buoyant and they "*must attach themselves to submerged material to prevent themselves floating to the surface*". This might explain why dytiscid larvae were found associated with the 'rock bags', but, because numbers of individuals captured is not given in the paper, it is not possible to comment on whether the 'rock bag' artificial substrate represented a really viable alternative to netting in terms of capturing reasonable numbers of beetle larvae.

In the context of ditch systems within the Somerset Levels and Moors, the use of ‘rock bags’ would be inappropriate given that most ditches lack rocky bottoms. Other artificial substrate traps could be designed perhaps for use in the particular circumstances that pertain.

An example of a technique involving artificial substrates which was evolved to meet specific requirements is that provided by Macan (1977a). He sampled invertebrates from a moorland tarn in Cumbria using ‘artificial *Litorella* mats’ made of polypropylene rope mimicking clumps of Shoreweed (*Litorella uniflora*) that grew in the tarn. Aside from two species of *Deronectes*, however, Macan does not mention that any other dytiscids were caught in four years by either pond-netting or through the use of the mats (Macan 1977a). This is despite the fact that *Dytiscus marginalis* at least is known from Cumbrian tarns [Fryer (1991)]. The possible absence of larger dytiscids in this instance may have been linked to the introduction of fish (Trout *Salmo Trutta*) to the tarn. Macan himself noted that: “*Only small invertebrates can survive predation by fish in the open water*” [Macan (1977b)].

The only dytiscid beetles caught by Garcia-Criado & Trigal (2005) were of the genus *Laccophilus*.

3.2.3 Studies specifically focussing on dytiscid beetles

Using the design of bottle trap shown in Figure 3.1, Aiken & Roughley (1985) reported trapping *“a maximum of 81 specimens of Dytiscus alaskanus J. Balfour-Browne (Coleoptera: Dytiscidae) in a single bottle over two days”*. They stated that the at times of peak population, the mean catch per bottle trap was 21.5 specimens of *D. alaskanus* while dip net collecting yielded fewer than five beetles per day.

The paper cited above reports an effective method for catching some species and remarks on how the technique compared with trapping, however, the authors did not set out deliberately to test trapping against netting as a method of catching dytiscid water beetles. Hilsenhoff (1987), however, purposely

undertook fieldwork to see which method yielded the greatest numbers of different taxa of dytiscids. He compared the total catch of adult beetles from 48 days' worth of trapping with that obtained during an equal number of days of netting. His conclusions were that trapping was the more effective of the two techniques for collecting larger-sized taxa, but netting caught more of the smaller beetles. So far as *Dytiscus* spp. were concerned, Hilsenhoff caught 7 different species from his study area comprising a total of 906 specimens from this genus. Of the total catch only 12 were obtained using a D-framed pond net (or 1 every 4 days) whereas the rest (98.7%) were caught in traps based on those designed by Whitman (1974) (see Figure 3.1). The catch of *Dytiscus* spp. in the traps was an average of 18.7 per day.

In a later study Hilsenhoff supplemented this research by investigating the relative effectiveness of D-framed pond nets and bottle traps in the capture of larvae. So far as Dytiscidae were concerned he concluded that traps caught more of the "*actively swimming*" dytiscid larvae but fewer larvae of taxa that are "*poor swimmers*" compared with the use of the pond net [Hilsenhoff (1991)]. Every one of the 109 adults and 93% of the larvae of the 5 *Dytiscus* species Hilsenhoff caught in this study were obtained using traps.

The papers discussed above all relate to studies undertaken in North America. One Northern European study [Nilsson & Söderberg (1996)] that compared netting with trapping as a method to capture dytiscid water beetles did not find any strong relationship as suggested by Aiken & Roughley (1985) or Hilsenhoff (1987, 1991) between body size and most effective trapping method. According to Nilsson and Söderberg (*Op. cit*.), the species caught in greater numbers in traps were not on average larger than those more abundant in the net samples, although the authors reported also that very large beetles were "*over-represented*" in the trap samples. 16 individual specimens of 3 *Dytiscus* spp. were caught over a season's sampling (3 *D. latissimus*, 12 *D. circumcinctus* and 1 *D. lapponicus*). So far as *D. circumcinctus* was concerned, individuals were captured both in net samples and in bottle traps, but trapping yielded the

greatest number of specimens. How the other *Dytiscus* species were caught is not reported in the paper.

The extensive comparison of literature on different trapping methods in Klečka & Boukal (2011) concluded that "*Adults and larvae of large Dytiscidae (Dytiscus, Acilius and Hydaticus) and Hydrophilidae (Hydrochara) were much better sampled by ATs* [Activity Traps] *than by BT* [Box Traps] *and HN* [Hand Netting]". The authors point out that Nilsson & Söderberg's (1996) work that appears to suggest there is little difference between activity traps (such as bottle traps) and hand netting compares data collected from studies that employed the techniques at two different sets of lakes. To this I would add that the netting and trapping were undertaken by Nilsson and Söderberg at slightly different times during the summer period.

Klečka & Boukal (2011) suggested that: "*Large Dytiscidae were mostly missed by BT* [Box Traps] *and HN* [Hand Netting] *either because they are primarily nocturnal and hide during daytime, as hypothesized by Hilsenhoff (1987), or because they were at lower densities than small species*". The first hypothesis advanced here seems reasonable when it is considered that observations made by Aiken (1986) indicated that fifty times more *D. alaskanus* were caught in traps during hours of darkness than from the same traps in daylight. It would be difficult, however, to test this hypothesis against the idea that the comparatively low catch might be due to low densities because we rarely have reliable population estimates that may be used for assessment.

3.3 Surveys of aquatic invertebrates in the Somerset Levels and Moors 1984 - 2011

Over the course of the study reports were collected of surveys for aquatic invertebrates from the Somerset Levels and Moors study area and also from the North Somerset Levels (sometimes called the 'Avon Levels'). The North Somerset Levels comprise of grazing marsh systems that are very similar to those on the Somerset Levels and Moors but which are separated from them by

the Mendip Hills (see map in Appendix A7). Fifteen reports of surveys conducted between 1984 and 2011 were analysed to obtain information regarding survey techniques. The reports consulted are referenced in Table C1 in Appendix C1. The methods employed were noted, particular attention being paid to the number of samples taken and the amounts of time spent in collecting and sorting material. This was done in order to gauge the efficiency of survey methods in relation to *Dytiscus* species.

Efficiency was measured in two ways: (a) Frequency of occurrence of *Dytiscus* spp. in samples, and; (b) Number of *Dytiscus* spp. caught per unit time of survey effort expended.

All fifteen surveys analysed relied almost exclusively upon sampling with a pond net supplemented in a few cases [e.g. Gibbs (1994), Hill-Cottingham & Smith (1998a)] by 'puddling' – trampling of marginal mud to force out some sediment-dwelling species [for a description of the technique see Gibbs (1994)]. Although better than many other available techniques in these regards, pond netting is not necessarily an easily quantifiable or readily standardised technique, as several of the authors cited in the literature review have pointed out [e.g. Klečka & Boukal (2011) Turner & Trexler (1997)]. Nevertheless, there are a number of ways in which a pond net survey can be standardised so that at least the samples taken within the same study can be compared and statistically analysed. Some variables that may be controlled are listed in Table 3.1 below. Where the information is available from the survey reports, the manner in which each variable was controlled or not in the separate surveys is given in Tables C2 (i) – (iii) in Appendix C2.

Table 3.1: Variables that can be standardised in aquatic macro-invertebrate surveys using a pond nets

Sampling Methodology	Sorting Methodology
A. Type of net B. Time of year C. Length of ditch netted D. Number of sweeps per sample E. Pattern of sweeping F. Time spent sweeping	A. Equipment used (e.g. sheet, tray, sieve, bucket) B. Time spent sorting C. Material taken for later ID D. How counted

By examining just the variables in Table 3.1 alone, it became clear that surveys were not readily comparable because of differences in methodology. Each variable is analysed in turn below:

Sampling

A. Type of net – With the exception of Drake (2005), the survey reports are not very specific about the type of pond net used, so it is difficult to be sure that the equipment used was similar in every case;
B. Time of year – Some surveys took place in spring or early summer [e.g. Keystone Environmental (2011)], some in the late summer or autumn [e.g. Anderson *et al*. (1991)];
C. Length of ditch netted – This varied from 20 metres [Drake *et al.* (1984)] to 50 metres [e.g. Drake (1989)];
D. Number of sweeps per sample – In some cases this was not recorded, suggesting there was no standardisation, while, in some surveys [e.g. Hill-Cottingham & Smith (1996)] three sweeps per sample was chosen, in others it was six sweeps [e.g. Drake *et al.* (1984)];
E. Pattern of sweeps – In many instances the surveyors stated that an attempt was made to sample all micro-habitats judged to occur in the length of ditch surveyed suggesting no fixed pattern. Only Drake (2005) adopted a definite fixed pattern of sweeps in order to test the results versus time limited netting;
F. Time spent netting – A few surveys adhered strictly to time limited netting (Godfrey 1999a & 1999b, Keystone Environmental 2011) choosing three minutes as the standard per sample.

Sorting

A. Equipment used – Some workers favoured tipping netted material onto a plastic sheet [e.g. Gibbs (1994)] and some preferred to use a sorting tray [e.g. Hill-Cottingham & Smith (1996, 1997, 1998a & 1998b)];

B. Time spent sorting – This varied from 10 minutes per sample [Drake (2005)] to up to one hour (including netting time) [e.g. Anderson *et al.* (1991)];
C. Material taken for ID – In most surveys a proportion of the material collected was taken away for positive identification at a later date;
D. How counted – Only Godfrey reported actual numbers of individual animals caught in his surveys {Godfrey (1999a & 1999b)]. In most cases abundance was recorded on a pre-defined scale.

The lack of standardisation demonstrated above may be regrettable in terms of limiting our ability to compare the species inventories obtained, but how far should it prevent an evaluation of netting as a means to catch *Dytiscus* species?

The methods selected for each survey were chosen to suit the objectives of that particular survey and none of the surveys were designed specifically to capture as many specimens of *Dytiscus* as possible. Nevertheless, all the surveys involved netting by relatively experienced surveyors. The *Dytiscus* species are conspicuous components of the macro-invertebrate fauna and it is considered likely that, if either adults or late instar larvae had been caught, these would have been identified in the samples within a short space of time irrespective of the sorting methods practiced. The numbers of samples in which *Dytiscus* were recorded in each survey is far more likely to have been influenced by sampling methodology therefore than by the method chosen for sorting samples.

In order to gauge whether there were big differences between surveys with regards to the frequency of occurrence of *Dytiscus* species in samples the number of samples was noted in which *Dytiscus* spp. were found either as adults or as larvae. The results of this are presented in Table 3.2 below.

Table 3.2: Numbers of samples positive for *Dytiscus* species collected during contract aquatic invertebrate surveys conducted in the Somerset Levels and Moors 1984 – 2007

Survey	**Total No. Samples**	**Number and percentage of samples positive for *Dytiscus* (i.e at least one individual caught)**							
		D.marginalis		*D. dimidiatus*		*D. semisulcatus*		*Dytiscus* larvae	
		No.	%	No.	%	No.	%	No.	%
1. Drake *et al*.. (1984)	243	16	6.6	6	2.5	3	1.8	-	-
2. Drake (1989)	46	4	8.7	1	2.2	-	-	23	50.0
3. Anderson *et al*. (1991)	40	2	5.0	-	-	-	-	-	-
4. Hill-Cottingham (1993)	30	1*	3.3	-	-	-	-	2*	6.6
5. Anderson *et al*. (1994)	14	-	-	-	-	-	-	-	-
6. Gibbs (1994)	120	+	+	4	3.3**	+	+	-	-
7. Hill-Cottingham & Smith (1996)	21	-	-	-	-	-	-	7***	33.3
8. Hill-Cottingham & Smith (1997)	23	-	-	-	-	-	-	8	34.8
9. Hill-Cottingham & Smith (1998a)	12	-	-	-	-	-	-	6	50.0
10. Hill-Cottingham & Smith (1998b)	36	-	-	-	-	-	-	-	-
11. Godfrey (1999a)	120	1	0.8	2	1.7	-	-	57***	47.5
12. Godfrey (1999b)	69	-	-	-	-	-	-	29***	42.0
13. Boyce (2004)	37	+	+	3	8.1	-	-	-	-
14. Drake (2005)	44	2	4.5	1	2.3	-	-	-	-
15. Keystone (2011)	34	6	17.7	-	-	-	-	-	-

* The report does not contain a comprehensive list of beetles caught. This information is culled from notes for each ditch surveyed. It may be that not every capture was recorded.

** The report does not record frequencies of all beetles caught. There are accounts for RDB species which is the source of the information concerning *D. dimidiatus. Both D. marginalis and D. semisulcatus* were caught but it is not clear in what numbers or in how many samples

*** 'Dytiscidae larvae' or 'dytiscid larvae' reported here are all assumed to be *Dytiscus* spp.

The first thing that should be noted with regards to the results displayed in Table 3.2 is that in a striking number of surveys (six out of fifteen) no adult *Dytiscus* beetles at all were recorded as having been netted. Where adult *D. marginalis* were caught they were recorded in less than one in ten of samples except in the case of the most recent of all the surveys [Keystone

Environmental (2011)] where the beetle was recorded in nearly one fifth of all samples. So far as *D. dimidiatus* was concerned, in those surveys where it was recorded the percentage of positive samples was similar (around 2 - 3%) with the exception of Boyce (2005) who recorded it in just over 8% of samples. *D. semisulcatus* was only recorded in two out of fifteen surveys and in the one survey in which figures are available it was recorded in only 1.8% of samples.

In only three surveys (those in which the sampling time was fixed) was there sufficient information contained in the reports to be able to estimate a capture rate for *Dytiscus* adults and larvae measured in terms of animals caught per hour's survey effort.

Klečka & Boukal (2011) compared methods for water beetle collecting based on the amount of survey effort required to capture and process one individual beetle (T_i) where:

$T_i = T_{i,1} + T_{i,2} + T_{i,3} + T_{i,4} + T_{i,5}$ and

$T_{i,1}$ is time required for travel;
$T_{i,2}$ is time required for collecting in the field;
$T_{i,3}$ is time required for handling (sorting and storing);
$T_{i,4}$ is time required for databasing; and
$T_{i,5}$ is time required for identification.

For the purposes of the analysis here, time spent in travel ($T_{i,1}$) and in "databasing" (i.e. recording results) ($T_{i,4}$) were ignored. It was assumed also that adult *Dytiscus* could be readily distinguished in the field and *Dytiscus* larvae could be recognised almost immediately as *Dytiscus* spp. Thus $T_{i,5}$ was taken to be negligible and was ignored also. Therefore, in this context, survey effort was taken to be the time taken to collect the sample ($T_{i,2}$) plus the time to sort through material collected (a proportion of $T_{i,3}$ to be defined here as $T_{i,3sort}$). A further assumption was that whether or not a sample contained *Dytiscus* adults or larvae could be ascertained in the first minute of sorting (i.e. in all cases

$T_{i,3sort}$ = 1 minute). In my experience this is usually the case whether one is considering a sample collected in a pond net or in a bottle trap.

Therefore:

$T_i = T_{i,2} + 1/60n$

Where T_i is the total survey effort measured in hours,

$T_{i,2}$ is the time (in hours) expended by the surveyor in collecting samples,

n = Number of samples.

Results from the analysis of survey reports are presented in Table 3.3.

Table 3.3: Capture rates (catch per hour survey effort) for target taxa from three contract surveys 1999 – 2011.

Survey	**Survey Effort (T_i)**	**Number caught and rate of capture for *Dytiscus* spp.**							
		D.marginalis		*D. dimidiatus*		*D. semisulcatus*		*Dytiscus* larvae	
		No.	Rate	No.	Rate	No.	Rate	No.	Rate
11. Godfrey (1999a)	8.0	1	0.13	2	0.25	0	-	57	7.13
12. Godfrey (1999b)	4.6	0	-	0	-	0	-	29	6.30
15. Keystone (2011)	2.3	6	2.61	0	-	0	-	-	-

From Table 3.3 it will be noted that the capture rates for adult beetles achieved by Godfrey in his first survey of 1999 are about the same as those reported by Hilsenhoff (1987) (see above). The twentyfold difference between these rates of capture and those obtained by Keystone Environmental's surveyors might be due to a netting technique that ensured greater success at catching Dytiscus spp. or simply to luck. Had Hilsenhoff been able to match these rates over his 48 days of sampling he would have caught several hundred adults, but his catch by netting would still have been appreciably smaller than that he gained through trapping.

The hourly capture rates for larvae reported by Godfrey do encourage the view that a reasonable number of specimens of larvae might be obtained for study by netting.

3.4 Experiments to compare the efficacy of bottle trapping versus pond netting in catching *Dytiscus* spp.

3.4.1 Methods

To investigate whether similar results to those reported above would be obtained if the two methods were tried in the study area, the relative effectiveness of bottle trapping versus netting was tested in sections of ditch at Shapwick Heath National Nature Reserve (NNR).

Four visits were made to Shapwick Heath NNR at fortnightly intervals in May and June 2006. At each visit ten sets of paired bottle traps (baited and unbaited) were set up at various points roughly equidistant one from another (about 30 metres) along a chosen section of ditch. These were run for approximately 24 hours before being collected in the following day and the contents sorted. In all cases baited traps were baited with pieces of unsmoked, raw bacon. The trap design is illustrated and described in section 2.2.2.

For three of the visits, on the day that the traps were set, the sections of ditch between the traps were swept using a standard Freshwater Biological Association (FBA) pond net as described in detail in 2.2.1.1. Material was deposited into a sorting tray and examined in order to pick out *Dytiscus* adults and larvae. Where considerable amounts of duckweed were collected this was washed through with water in order to ensure that all larvae were picked out. The time taken to sort samples varied for this reason, but all samples were examined until the sorter was convinced no individual *Dytiscus* sp, either adults or larvae, remained to be found.

3.4.2 Results

If one takes each bottle trap to be a sample and likewise each section of ditch swept then it is possible to derive some figures for the numbers and percentage of samples positive for *Dytiscus* beetles. The results from such an analysis are presented in Table 3.4.

Table 3.4: Numbers of samples positive for *Dytiscus* species collected during fieldwork at Shapwick Heath National Nature Reserve 7/5/06 to 9/6/06.

Sampling Method	**Number of Samples**	**Number and percentage of samples positive for *Dytiscus* (i.e at least one individual caught)**					
		D.marginalis		*D. dimidiatus*		Larvae	
		No.	%	No.	%	No.	%
Baited Traps	40	18	45.0	3	7.5	20	50.0
Unbaited Traps	40	5	12.5	0	-	5	12.5
Pond netting	30	3	10.0	0	-	8	26.7

In Table 3.5 the results are broken down for sampling sessions.

Table 3.5: Comparison of trapping and netting as method of capturing *Dytiscus* species from ditches at Shapwick Heath NNR. May – June 2006

Date	**Species**	**Sex/stage**	**Numbers caught**		
			Pond Net	**Baited Trap**	**Unbaited Trap**
6-7/5/06	*D. marginalis*	Adult ♂	-	7	1
	D. marginalis	Adult ♀	-	6	1
	D.dimidiatus	Adult ♂	-	2	0
	D.dimidiatus	Adult ♀	-	1	0
	Dytiscus sp.	Larval	-	2	0
13-14/5/06	*D. marginalis*	Adult ♂	0	6	3
	D. marginalis	Adult ♀	1	2	1
	Dytiscus sp.	Larval	1	3	0
28-29/5/06	*D. marginalis*	Adult ♂	0	6	0
	D. marginalis	Adult ♀	0	2	0
	Dytiscus sp.	Larval	4	14	1
5-6/6/06	*D. marginalis*	Adult ♂	2	1	0
	D. marginalis	Adult ♀	0	1	0
	Dytiscus sp.	Larval	4	20	5
TOTALS	*D. marginalis*	Adult ♂ & ♀	3	31	6
	D.dimidiatus	Adult ♂ & ♀	0	3	0
	Dytiscus sp.	Larval	9	39	6

It should be noted that, although adult *D. dimidiatus* were caught at Shapwick Heath on one visit and subsequently following these visits, none were caught during the period that the pond netting was tested alongside trapping.

Adult *D. marginalis* were at least as abundant as and usually more frequent in trap samples than in net samples. The same pattern was observed in the

numbers of larvae captured, with more individuals represented in the samples collected by trapping than in netted samples.

In Table 3.6 the performance of baited and unbaited traps is compared. The figures represent frequencies of a particular outcome obtained from baited and unbaited traps set in pairs along ditches at Shapwick Heath NNR between May and June 2006. Figures in brackets in second and third columns show the number of times that the trap indicated was the only one of the pair that caught a beetle.

Table 3.6: Summary of trapping success in capturing adult *D. marginalis* and larvae of *Dytiscus* species.

Date collected	Baited trap caught more	Unbaited trap caught more	Traps caught equal numbers	Traps caught none at all	Total pairs of traps set
Adult *D. marginalis*					
07/5/06	3 (3)	2 (2)	1	4	10
14/5/06	5 (3)	1 (0)	1	3	10
29/5/06	6 (6)	0 (0)	0	4	10
06/6/06	2 (2)	0 (0)	0	8	10
Totals	16 (14)	3 (2)	2	19	**40**
***Dytiscus* larvae**					
07/5/06	2 (2)	0 (0)	0	8	10
14/5/06	3 (3)	0 (0)	0	7	10
29/5/06	6 (5)	0 (0)	0	4	10
06/6/06	8 (6)	0 (0)	0	2	10
Totals	19 (16)	0 (0)	0	21	**40**

In just over 50% of cases at least one trap at each sampling location caught at least one adult *D. marginalis*. On each occasion that pairs of baited and unbaited traps were set, the baited traps attracted greater total numbers of *D. marginalis* adults than did unbaited traps. It is noticeable that unbaited traps appeared more successful at the beginning of the sampling period than at the end. This may be connected with adults being more mobile at a time when they may be looking for mating opportunities.

In slightly under 50% of instances where paired traps were set, at least one of the pair caught at least one larva. Roughly speaking, paired traps were equally successful in terms of catching larvae at sampling locations as they were at

catching adult *D marginalis* beetles, but there was a difference in the effectiveness of baited as opposed to unbaited traps. In only three instances (out of a possible forty) did an unbaited trap catch at least one larva and in each case the corresponding baited trap of the pair caught more.

The raw data on which Tables 3.4, 3.5 and 3.6 are based are included in Appendix C3.

3.4.3 Statistical analyses

The data for *D. dimidiatus* do not lend themselves readily to standard statistical analysis. Chi-squared tests, for example, cannot be performed since only 3 beetles in total were trapped during the time that paired baited and unbaited traps were run. Although it is noted that all three individuals were caught in baited traps, there are simply not enough observations for Chi-squared to be performed reliably according to standard statistical texts [e.g. Clarke & Cooke, (1998)].

3.4.3.1 Hypothesis: Baited traps catch significantly more beetles than unbaited

Sufficient *D. marginalis* beetles were caught to enable Chi-squared tests to be performed. Tests for 'Goodness of Fit' were conducted to investigate whether there was a statistical difference between the numbers of beetles caught in baited versus unbaited traps. Pooling all the data, there are a total number of 37 adult beetles that were trapped between 6 May and 10 June 2006 (31 in baited traps, 6 in unbaited traps). For the purpose of the test, I assumed that unbaited traps were as likely to catch beetles as baited traps, so expected frequencies for each type of trap were calculated by dividing the total number of beetles caught by 2 (i.e. expected frequencies were equal to 18.5 in baited and unbaited traps). The null hypothesis that was assumed was that there was no difference between the effectiveness of traps and, therefore no statistically significant difference between numbers caught in baited and unbaited traps. In this instance $\chi^2 = 15.6$ (1 d of f) so P = <0.001 and the null hypothesis was rejected.

A possible objection to pooling the data in the manner above is that some 'trap happy' individuals might have been caught more than once since the data was accumulated over four separate trapping events. In some cases sufficient beetles were caught in a single trapping event so that Chi-squared tests could be performed. This was in those instances where numbers caught on a particular date exceeded 10, so that expected frequencies would be at least 5 – the minimum number for the test to be a valid one [see Clarke and Cooke (1998)].

Thus for adult *D. marginalis* collected on 7 May 2006 the numbers trapped in baited traps was 13 compared with 2 in unbaited (χ^2 = 6.7, 1 d of f, P = <0.0098). However, the difference in the numbers of adults caught by the two methods on 14 May 2006 was not statistically significant (χ^2 = 1.4, 1 d of f, P = >0.05). For similar tests performed on larval data, the results were as follows: For pooled data (6 May to 10 June 2006) χ^2 = 22.8 (1 d of f, P = <0.001); while for the trapping event on 27 May 2006 (χ^2 = 9.6, 1 d of f, P = <0.005) and for 10 June 2006 (χ^2 = 7.8, 1 d of f, P = <0.01). In all but one case where Chi-squared tests could be conducted there were significantly more *Dytiscus* specimens caught in baited as opposed to unbaited traps.

3.4.3.2 Hypothesis: Males and females display a significant difference in the way they behave towards traps

More male *D. marginalis* beetles were caught between 6 May and 10 June 2006) than females (24 males compared with 13 females). If it is assumed that equal numbers of males and females should be caught, it is possible to calculate expected frequencies of capture of for each sex (37/2 = 18.5). A Chi-squared goodness of fit test conducted on this basis does not suggest the numbers in the traps departed significantly from a 1:1 sex ratio (χ^2 = 2.7, 1 d of f, P = >0.05).

To test whether there was any significant variation in the way the males behaved towards baited or unbaited traps compared with females in adult *D. marginalis* a test of independence of variables was conducted based on the contingency table shown in Table 3.6 below.

Table 3.7: 2x2 Contingency table comparing observed and expected frequencies of male and female D. marginalis in baited and unbaited traps.

		Baited	Unbaited	**Totals**
Observed	Male	20	4	**24**
	Female	11	2	**13**
Totals		**31**	**6**	**37**
Expected	Male	20.1	3.9	**24**
	Female	10.9	2.1	**13**
Totals		**31**	**6**	**37**

The expected frequency in each instance was calculated according to the standard formulae for such contingency tables when the null hypothesis is that the two variables tested (in this case sex and trap type) are independent of one another. The expected frequency in the cell within the table that is in row i and column j is equal to:

$$\frac{\text{(Sum of values in row i) X (sum of values in column j)}}{\text{N}}$$

Where N is the Total number of observations

In the above case, for example, the expected catch of males in baited traps was (31 x 24)/37 = 20.1. In this instance, a Chi-squared test of independence could not be performed because some of the values in the contingency table were less than 5. Fisher's Exact Test is an alternative statistical method recommended in these circumstances [see McDonald (2009)]. The value of the test statistic obtained was 1 for a two-tailed test (DF = 1, P = >0.05) meaning that the null hypothesis could not be rejected indicating that the variables are independent and the sexes are not differentially attracted to one type of trap any more than to another.

3.4.3.3 Hypothesis: Trapping catches more individuals of *Dytiscus* sp. than does netting

From Table 3.5 it may be seen that during May to June 2006 a total of 31 adult D. marginalis and 39 larvae were caught in baited traps. Considerably fewer beetles were caught in unbaited traps (6 adults and 6 larvae) or by netting (3 adults and 9 larvae). To test whether these were statistically significant differences Chi-squared tests were performed comparing the results from netting with those from trapping. The null hypothesis was adopted that there was no significant difference in the numbers caught between netting and trapping and, consequentially, the expected catches in each trap could be calculated by apportioning the total catch equally between the two methods as was done in the analysis in 3.4.3.1.

Comparing the pooled data for baited traps and netting, Chi- Squared values were obtained as follows: *D. marginalis* adults $\chi^2 = 21.4$ (1 d of f, P = <0.001); Larvae $\chi^2 = 17.5$ (1 d of f, P = <0.001). For unbaited traps the results were: *D. marginalis* adults $\chi^2 = 0.4$ (1 d of f, P = 0.51); Larvae $\chi^2 = 0.3$ (1 d of f, P = 0.61).

So far as baited traps were concerned, the null hypotheses could be rejected for both adult *D. marginalis* and *Dytiscus* larvae such that it may be concluded that significantly more animals were caught in the baited traps than by netting. The comparison of netting versus unbaited traps did not find significant differences in the two methods.

3.5 Discussion

Results from a literature review reported in sections 3.1 and 3.2 suggested that trapping might be a more viable method than netting to catch sufficient individual *Dytiscus* beetles and larvae for the purposes of the study. This finding was supported by most authors who had set out specifically to compare capture methods for large water beetles [e.g. Denton (1996), Hilsenhoff (1987,

1991), Aiken & Roughley (1985)]. One study that did not find trapping to be clearly advantageous in terms of catching larger dytiscids was that by Nilsson & Söderberg (1996), however, comparison of methods was not a primary purpose of their work.

Denton's (1996) finding that unbaited traps were better than netting to catch large dytiscids was not replicated in my study, however, the fieldwork I specially undertook to test trapping against netting provided strong evidence that baited traps were more effective than either unbaited traps or netting.

Klečka & Boukal (2011) argued that time taken to collect and process each sample ought to be a consideration when comparing approaches to aquatic macro-invertebrate surveying. However, even when this was taken into account the rates of capture per hour of effort still suggested that trapping would be superior to netting. The hourly capture observed during my 2006 fieldwork was lower for netting compared with trapping. To set, collect in and sort material from 40 baited traps took me an estimated 4 hours and yielded 73 *Dytiscus* specimens (or 18.3 specimens per hour of effort). I found it possible to collect about 10 net samples per hour from ditches at Shapwick. Therefore, in 3 hours I collected 30 sweep net samples containing 11 specimens (or 3.7 specimens per hour of effort). The highest hourly capture rate for *Dytiscus* specimens calculated for contract surveys (see Table 3.3) barely exceeded 8 specimens per hour.

The findings reported above indicated that trapping using baited traps would be the best method to choose to yield the highest numbers of *Dytiscus* specimens per hour of sampling effort. For this reason, I used it in all subsequent fieldwork as the primary means to collect *Dytiscus*. In Chapter 8, the results from further fieldwork in 2007 and 2008 are assessed to see if the reliance on trapping as a method was justified.

Chapter 4: Identification of *Dytiscus* larvae

Introduction

In Chapter 1 it was noted that identification to species of the larvae of UK *Dytiscus* can pose problems, particularly in the field. Having established that trapping offered a satisfactory way to capture larvae for study purposes (see Chapter 3), the option existed to kill every specimen caught and subject all of them to microscopic examination for the purpose of keying them out using morphological features employed in standard identification keys such as Klausnitzer (1991). However, I did not consider this appropriate given the possibility that large numbers of *Dytiscus dimidiatus* might be taken in traps. At the outset of the study nothing was known about the size of the populations of *D. dimidiatus* that occur at the study sites and permission to use several of the sites was granted by site managers on the understanding that every effort would be made to return animals back alive to the locations from whence they were caught.

One important advantage of DNA techniques is that identification can be made with confidence from material extracted from appendages removed at minimal risk to the individual [Beebee & Rowe (2004)]. For reasons explained in Chapter 2 (in section 2.5.1), I decided to use DNA extracted from larval legs in order to assign a larva to species.

Despite the decision to release live animals and the adoption of trapping techniques that aimed to minimise the risk to trapped animals there was a level of trap mortality. This meant that dead larvae were available that could be examined morphologically, providing a means to investigate how closely species identifications obtained using genetic techniques agreed with those made using more traditional biometric approaches.

During the course of fieldwork between 2006 and 2011, a total of 229 dytiscid larvae were caught. The majority of these were trapped in the years 2007 and 2008. 85 *Dytiscus* larvae were released after a hind leg was removed, leaving

material from 144 specimens available for biometric examination. The material examined included not only whole larvae but also specimens that were damaged, possibly due to attempted predation, as well as ones that consisted of partially digested and even fragmentary remains.

Starting with molecular ecological (DNA) techniques and then turning to morphology and biometrics, this chapter focuses on identification of the larvae caught. These two approaches to identification of *Dytiscus* larvae are compared in the discussion.

Results and discussion

4.1 Molecular ecology

4.1.1 Preliminary DNA extraction experiment

The basic methodologies used to attempt to extract and isolate DNA from adult and larval beetle legs were described in detail in Chapter 2 (section 2.5.1).

At the beginning of the molecular ecological investigations an experiment was conducted to establish the quantities of DNA that could be extracted from larval *Dytiscus* legs compared with those which could be obtained from adult flight muscle. The purpose of this experiment was to check that it was feasible to obtain sufficient DNA from beetle legs for subsequent amplification by the Polymerase Chain Reaction (PCR).

The preliminary extractions were undertaken using a DNeasy Blood and Tissue Kit manufactured by Qiagen (http//www.qiagen.com/). The basic protocol used was as described in section 2.5.1.2.

Tissues from the flight muscles of four adult male *Dytiscus* beetles were dissected out (two samples from *D. dimidiatus*, two from *D. marginalis*) and prepared as per the Kit instructions for animal tissue. Specimens of each species were chosen that had been collected about a year apart and kept in undiluted Industrial Methylated Spirit (IMS) B.P. 66 OP (William Ransome & Son PLC). This was done in order to ensure that the flight muscle extracts were

taken from specimens collected at both the beginning and at the end of the period 2006 -2007 when adults were harvested deliberately for molecular ecological study.

Four larval legs were selected from live larvae captured on two separate occasions in 2007 and subsequently stored also in IMS. To liberate soft tissue from which DNA might be obtained, the legs were placed in 180µl of Kit lysis buffer 'ATL' and manually ground using a glass rod for two minutes. Legs were ground in glass and plastic vessels in order to see if the nature of the container made a noticeable difference to the outcomes. Subsequent incubation with Proteinase K and the steps taken following this to isolate the genomic DNA were according to the protocol described in section 2.5.1.2.

The results of the preliminary extraction experiment are summarised in Table 4.1.

Table 4.1: Summary of results from preliminary DNA extraction experiment. The values for DNA concentration are derived from spectrophotometric analysis of the extracts.

Description of extract source	Concentration of DNA (ng/µl)
Flight muscle *D. marginalis* ♂ caught in 2006	160
Flight muscle *D. marginalis* ♂ caught in 2007	370
Flight muscle *D. dimidiatus* ♂ caught in 2006	320
Flight muscle *D. dimidiatus* ♂ caught in 2007	440
	Average *322.5*
	St Dev. *119.0*
Leg from larva caught in May 2007 ground in glass tube	90
Leg from larva caught in May 2007 ground in plastic tube	130
Leg from larva caught in July 2007 ground in glass tube	110
Leg from larva caught in July 2007 ground in plastic tube	60
	Average *97.5*
	St Dev. *29.9*

According to Saunders (1999), the DNA requirements of commonly used analytical techniques vary between 0.1 ng/µl [in Short Tandem Repeat analysis (STR)] and 20 – 100 ng/µl [in Restriction Fragment Length Polymorphism fingerprinting (RFLP)]. Within this range, arbitrarily primed PCR profiling techniques such as RAPD (see below) require 1 – 20 ng/µl and cycle sequencing 10 ng/µl [Saunders (*Op. cit.)*]. The average yields quoted in Table

4.1 easily met these requirements and these results implied that sufficient DNA could be extracted using the basic experimental protocol adopted.

The flight muscle extracts consistently yielded more DNA than did the leg extracts. This was as expected (see section 2.5.1.1) but the experiment did indicate that enough DNA could be obtained from larval legs for subsequent analysis.

There were not enough replicates to test whether there were statistically significant differences between DNA yields gained from legs ground in glass tubes and those ground in plastic tubes. However, the results did not suggest that grinding the legs in plastic rather than glass caused a problem with regards to insufficient yield. The age of the material did not appear to substantially affect yield, but, again, there were not enough replicates to test the relationship between yield and age.

4.1.2 Yields of DNA from extractions

Subsequent extractions from the bulk of both larval and adult material investigated during this study were conducted at the DNA laboratories of Somerset County Council Scientific Services using kits and techniques routinely employed in these laboratories [Tepnel Biosystems (2006), Agilent (2010)]. The basic methodologies were described in detail in Chapter 2 (sections 2.5.1.3 and 2.5.1.4).

Extraction methods based on binding DNA to magnetic beads were used during the period 2009-10 when genetic material was obtained mainly from adult beetles in order to trial some of the PCR-based techniques that are discussed in section 4.2.3. In 2011, when the study focus switched to extraction from larval material, a different technique was employed that utilised spin columns. In both instances, a record of DNA yields was kept, although this practice was discontinued towards the end of the study because of time constraints.

75 extractions were undertaken during 2009-10 using magnetic beads from the BioKit GMO and Allergen DNA Extraction Kit produced by Tepnel Biosystems (http://www.neogeneurope.com/). The protocol that was followed is described in section 2.5.1.3. DNA was extracted successfully from legs from 72 individuals (29 *D. dimidiatus* adults, 33 *D. marginalis* adults and 10 *Dytiscus* sp. Larvae). In two additional instances extractions were made from the flight muscles of adults as well as from the legs and, in one case, two legs from the same individual were processed.

From June 2011 onwards DNA extraction was carried out using the kit in the Agilent DNA Fish ID Ensemble (part number 5500-0100) utilising spin column technology (http://www.genomics.agilent.com/). A full description of the extraction procedure was given in Chapter 2 (section 2.5.1.4). Legs from 220 larvae were processed. However, spectrophotometric assays of DNA were conducted for only the first 64 larval legs processed in this way. In 15 (23%) of cases, the DNA concentration was too low to be measured by the instrument.

Table 4.2 below summarises the results of the assays conducted on the DNA extracts obtained using the two methods. Assuming that amounts of between 1 ng/µl and 20 ng/µl of DNA were ideally required for subsequent analyses (see discussion in section 4.2.1) then it can be seen that where the assays were able to detect measurable amounts of DNA, even the minimum amounts recorded were within this range.

With regards to the relative effectiveness of the two extraction methods compared in Table 4.2, the assay results implied that use of the magnetic bead technique tended to yield more genomic DNA than the spin column method. The average amount of DNA measured in extracts from larval legs was over ten times as great for the bead technique as for the method using spin columns. However, the standard deviations calculated for the datasets suggest that the assay data are very variable, so definite statements ought to be avoided regarding the relative effectiveness of the two methods, particularly since the sample sizes are somewhat different (n = 10, n = 49). Assuming that the assay

results are an accurate reflection of the amount of genomic DNA that has been extracted, the differences in extracted DNA concentrations might be due to factors unconnected with the extraction methods themselves, such as the nature and state of the starting material. For example, larval legs were not weighed or measured prior to extraction so that the amount of DNA extracted could not be expressed in terms of the amount of tissue available at the start of the procedure.

Table 4.2: DNA yields extracted from specimen legs using different extraction methods. The values for DNA concentration are derived from spectrophotometric analysis of the extracts.

Type of specimen	Number (n)	Concentrations of DNA (ng/µl)			
		Maximum	Minimum	Average	Standard Dev
GMO and Allergen DNA Kit - Magnetic Beads - (Tepnel Biosystems 2006)					
D. dimidiatus adult	29	201	23	85.3	47.3
D. marginalis adult	33	276	13	77.5	49.5
Larvae	10	376	10	239.4	150.8
Agilent DNA Fish ID Ensemble - Spin Columns - (Agilent 2010)					
Larvae (positive assays only)	49	111	5	29.2	20.8

Agilent Technologies state that the spin column protocol that was used here "*typically yields samples with a* [DNA] *concentration ranging from 5 ng/µl to 500 ng/µl*" [Formosa *et al.* (2010)]. This observation was made in connection with preparations of fish samples for RFLP analysis, so it cannot be taken as a statement of the yield that might be anticipated when the procedure is applied to extracting genomic DNA from beetle legs. Nevertheless, where amounts of DNA were obtained that were measurable using spectrophotometry, the average yields from larvae was within the range quoted (albeit towards the lower end of that range). Of concern, however, was the observation that, where spectrophotometric assays were performed on samples, no tangible amounts of DNA could be measured in nearly one quarter of the extracts obtained using the Biokit protocol. If the assay results are taken as reliable, this implied either that the method had failed to isolate DNA in an appreciable number of the extractions attempted or that the yield of DNA was very low and, possibly, too low for some of the analytical techniques based on PCR. This issue is

addressed again in section 4.2.5 below where the results from sequencing experiments are reported and discussed.

4.1.3 PCR-based genetic techniques

The Polymerase Chain Reaction (PCR) and some of the molecular ecology techniques based upon it were described in Chapter 2. As explained there, three such techniques were used in this study to attempt to distinguish *D.dimidiatus* larvae from *D. marginalis*:

- Randomly Amplified Polymorphic DNA (RAPD) analysis;
- Use of species-specific primers based on known sequences in the mitochondrial CO1 genes in *D.dimidiatus* and *D. marginalis*;
- DNA sequencing of PCR products obtained from amplification of part of the CO1 gene.

The results obtained using each technique are reported and analysed in the next three sections.

4.1.4 RAPD analysis

The theoretical basis of how it was intended that RAPD would be used in this study to distinguish *D.dimidiatus* and *D. marginalis* larvae was explained in section 2.5.2.1). Details of the primers, PCR master mixes and PCR conditions were given in the same section.

The template DNA used in RAPD here included that which was obtained from flight muscle extracts from two male *D. dimidiatus* and two male *D. marginalis* during initial experiments to test the feasibility of extracting adequate amounts of DNA for analysis (see Table 4.1). In the first RAPD experiments these flight muscle extracts were diluted so as to give notional 30-50 ng/µl DNA concentrations based on the assay results given in Table 4.1. However, no bands of amplified DNA were detected in any of the lanes in the gels run from these PCRs.

To see if DNA could be detected in any of the extracts from both adult and larval material obtained in the initial extraction trial, gels were run for a short

period using undiluted extracts in the lanes. Bands were observed in all the lanes in the electropherogram. This indicated that the extracts did indeed contain DNA, but that they contained less intact, high molecular weight DNA than the spectrophotometric results in Table 4.1 indicated. The gel obtained using undiluted, unamplified extract is reproduced in Figure 4.1 below.

Figure 4.1: Photograph of gel electrophoresis results obtained from running undiluted extracts

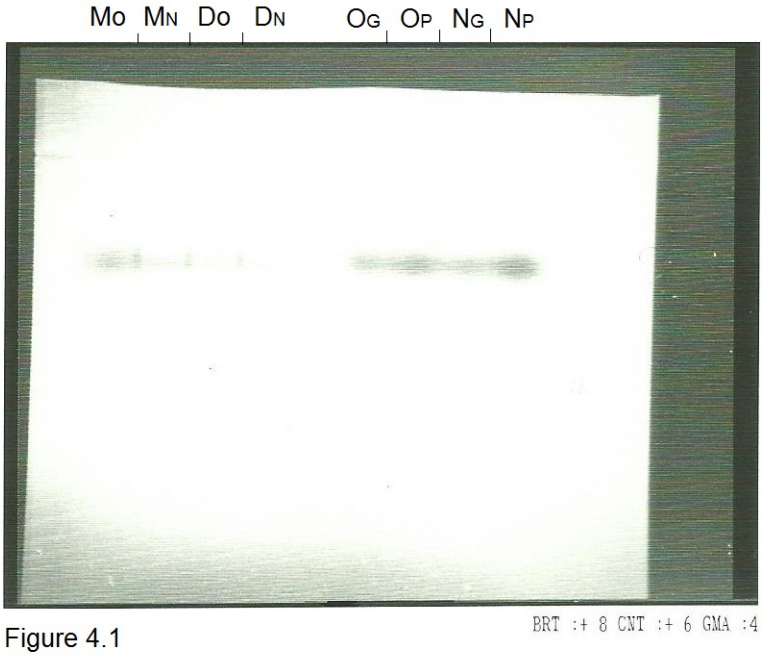


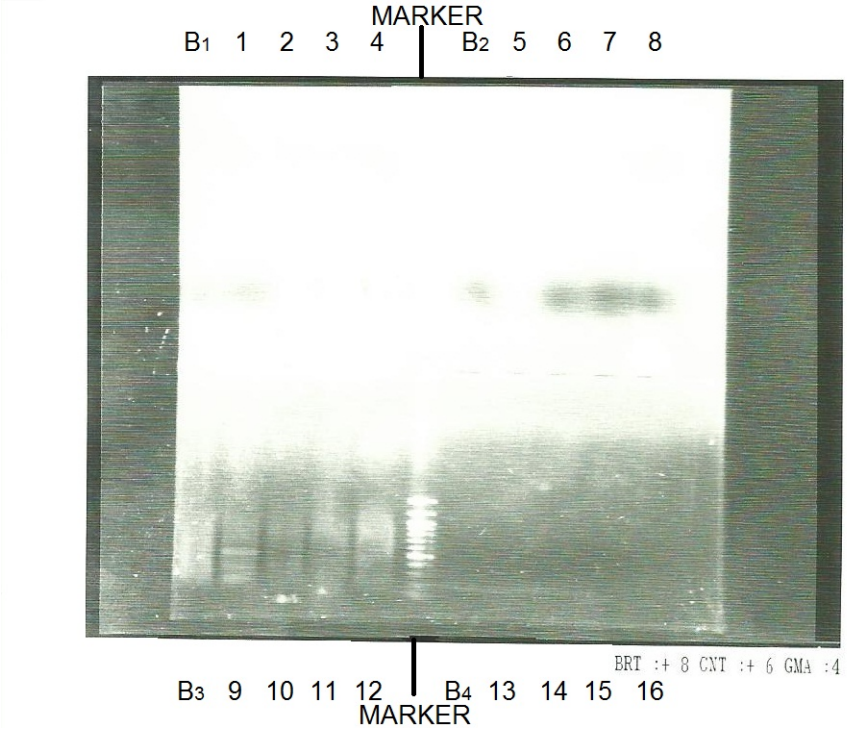
Figure 4.1

Lane	Origin of Extract
M_O	Flight muscle from *D marginalis* ♂ collected in 2006
M_N	Flight muscle from *D marginalis* ♂ collected in 2007
D_O	Flight muscle from *D dimidiatus* ♂ collected in 2006
D_N	Flight muscle from *D dimidiatus* ♂ collected in 2007
O_G	Larval leg collected May 2007 ground in glass container
O_P	Larval leg collected May 2007 ground in plastic container
N_G	Larval leg collected July 2007 ground in glass container
N_P	Larval leg collected July 2007 ground in plastic container

It is noticeable in Figure 4.1 that the bands are brighter in the lanes containing larval extracts than in the lanes with the flight muscle extracts, suggesting a greater concentration of DNA in the larval extracts compared with those from adult beetles. This observation seems to contradict the results of the spectrophotometric assays given in Table 4.1. Given the evidence that the extracts contained at least some viable DNA, a possible explanation for the

non-detection of bands of amplified product in the first electrophoresis was that the starting concentrations of DNA in the template had been too low since the extracts had first been diluted. Therefore, I conducted the next PCRs with neat extract to provide the template DNA. Figure 4.2 shows a digital photograph of the gel gained from running out the PCR products from attempted amplification of the DNA from flight muscle extracts using primers OPD_9 , OPD_{10} , OPD_{19} and OPD_{20} (sequences are given in section 2.5.2.1). The material run in the 'control' lanes comprised PCR mix (with the relevant primer) to which no DNA extract had been added but which had been subjected to the same thermal cycles as the samples run in the other lanes.

Figure 4.2: Photograph of gel electrophoresis results obtained from running products from attempted amplification of flight muscle extracts



Lane		Lane	
B1	Control OPD_9	B3	Control OPD_{19}
1	DM 2006 + OPD_9	9	DM 2006 + OPD_{19}
2	DM 2007 + OPD_9	10	DM 2007 + OPD_{19}
3	DD 2006 + OPD_9	11	DD 2006 + OPD_{19}
4	DD 2007 + OPD_9	12	DD 2007 + OPD_{19}
B2	Control OPD_{10}	B4	Control OPD_{20}
5	DM 2006 + OPD_{10}	13	DM 2006 + OPD_{20}
6	DM 2007 + OPD_{10}	14	DM 2007 + OPD_{20}
7	DD 2006 + OPD_{10}	15	DD 2006 + OPD_{20}
8	DD 2007 + OPD_{10}	16	DD 2007 + OPD_{20}
DM =	*D. marginalis* extract	DD =	*D.dimidiatus* extract

The digital photograph of the gel in Figure 4.2 does not show any particularly sharp, bright bands indicative of amplification of a distinct fragment of DNA from the CO1 gene. A faint light band is possibly discernible in lane 9 in the lower half of the photograph which is one of the lanes run with PCR product from *D. marginalis* material amplified with OPD_{19} primer. There is no indication of fluorescence in the lanes with OPD_{20} primer.

The results from the initial tests were inconclusive. However, further RAPD analyses were not carried out, because information became available regarding the CO1 gene sequence in *Dytiscus* species that led to a new line of enquiry.

4.1.5 Selective amplification of sections of CO1 Gene

One way in which species-specific primers might be used in larval identification was explained in Chapter 2 (section 2.5.2.1). An account was given there regarding CO1 sequence information that was obtained and how it was used to design pairs of forward and reverse primers ('DMF1/R1', 'DMF2/R2', 'DDF1/R1' and 'DDF2/R2') to be used in PCRs.

The objective of preliminary experiments was to discover whether PCR conditions could be identified (i.e. a combination of primers, salt concentration and annealing temperature) that would consistently yield amplified DNA products of predictable size for one *Dytiscus* species but not the other using the DM and DD primer pairs. Template DNA was selected from the extracts from adult *Dytiscus* beetles summarised in Table 4.2 above and listed fully in Appendix D2.

As explained in Chapter 2 (section 2.5.2.2), PCR products were separated by capillary electrophoresis on micro-chips rather than on agarose gels.

In order to discover the optimal conditions for PCR of the *Dytiscus* CO1 sequences, tests were conducted of the effects of magnesium concentration

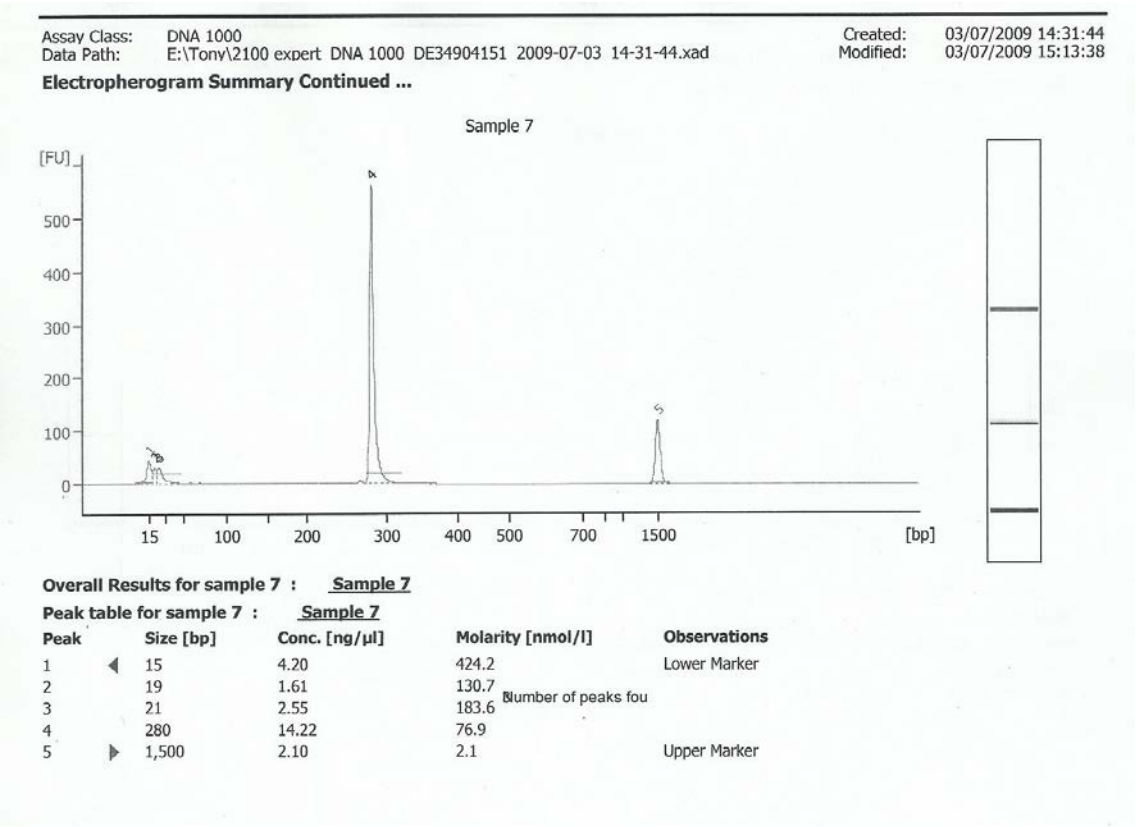
and of different annealing temperatures. These experiments are described in sub-sections 4.1.5.1 and 4.1.5.2.

4.1.5.1 Effect of Magnesium ion concentration

As described in Chapter 2, PCRs were conducted with concentrations of $MgCl_2$ varying between 1.0mM and 6.0mM. The primer pair DDF1/R1 was selected to facilitate amplification of template DNA from an adult *D. dimidiatus* beetle. PCRs were conducted in duplicate with annealing temperatures of 52ºC and 55 ºC.

For both annealing temperatures, a product close to the expected size (275bp) was detected only in PCRs with an $MgCl_2$ concentration of 3.0 mM or greater. A typical positive electropherogram from the experiment is reproduced in Figure 4.3 below.

Figure 4.3: Electropherogram of PCR with *D. dimidiatus* template DNA and $MgCl_2$ concentration 3.0 mM (T Anneal = 55°C)



4.1.5.2 Effect of annealing temperature

In a series of PCR experiments utilising the DM and DD primer pairs, the annealing temperature was varied within the range 50ºC to 65 ºC while the PCR master mix was kept the same, including a $MgCl_2$ concentration of 3.0mM. Template DNA from both species was used. The sources of the template DNA are given in Table D2 in Appendix D2. Where possible, the experimental treatments were duplicated in order to account for chance amplification failure.

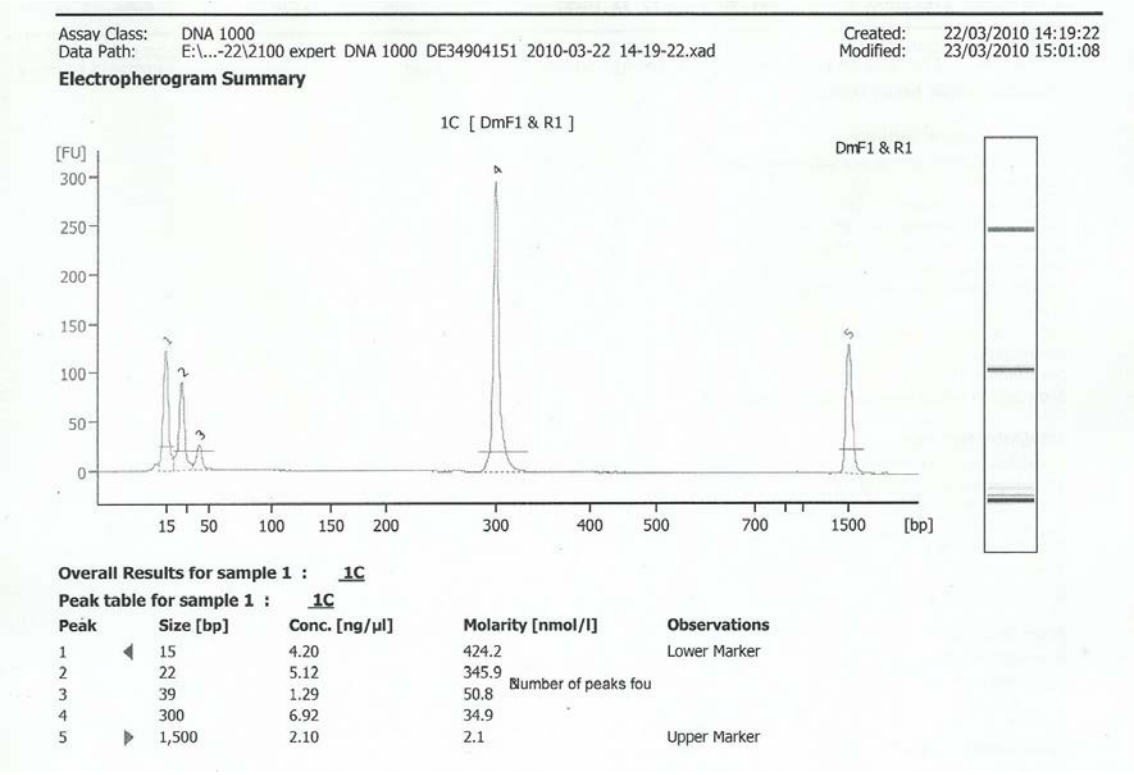
Electropherograms are reproduced below (in Figures 4.4 and 4.5) that show some of the results obtained for each primer pair at each value of T Anneal tested.

I assumed that, where peaks occured in the electropherograms, these represented amplification events. Based on the primer design and knowledge of the CO1 sequence, the size of the fragment that it was expected would be amplified could be calculated. It was noticeable that the size of amplified product as measured by the 2100 Bioanalyser (and shown on the electropherograms above) departed from these expected product sizes. A summary of the variation in product size is presented in Table 4.3 below.

Table 4.3 shows that there was as much as a 20 bp difference between the expected product size and the mean ‘actual’ size as measured by the Bioanalyser software. This apparent discrepancy in product sizes - between those expected and those measured - was taken to be due to error in the measurement rather than any indication that the observed peaks were caused by any DNA source other than amplified fragments from successful PCR of the relevant CO1 segment.

Figure 4.4: Electropherograms of PCRs of *D. marginalis* template DNA conducted with forward and reverse primer (DMF1/R1) at two values of T Anneal (52°C and 55°C).

4.4a DMF1/R1 at T anneal 52°C



4.4a DMF1/R1 at T anneal 55°C

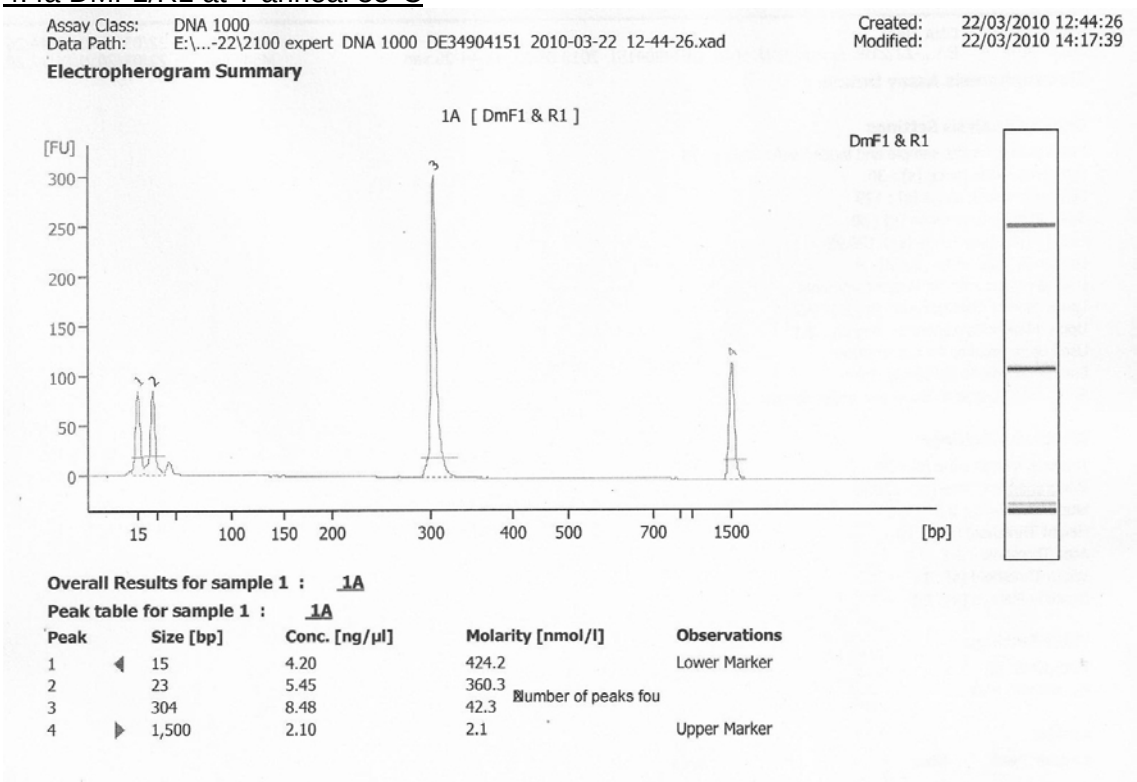
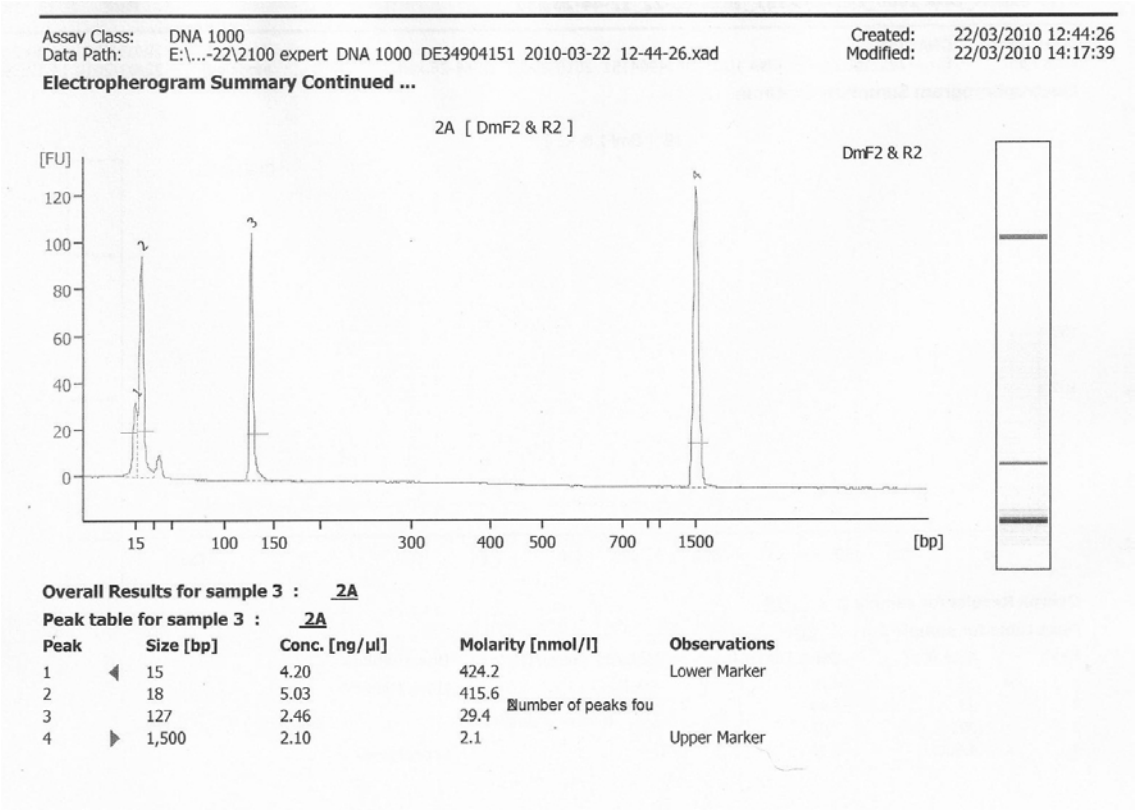


Figure 4.5: Electropherograms of PCRs of *D. marginalis* template DNA conducted with forward and reverse primer (DMF2/R2) at two values of T Anneal (52°C and 55°C).

4.5a DMF2/R2 at T anneal 52°C



4.5b DMF2/R2 at T anneal 55°C

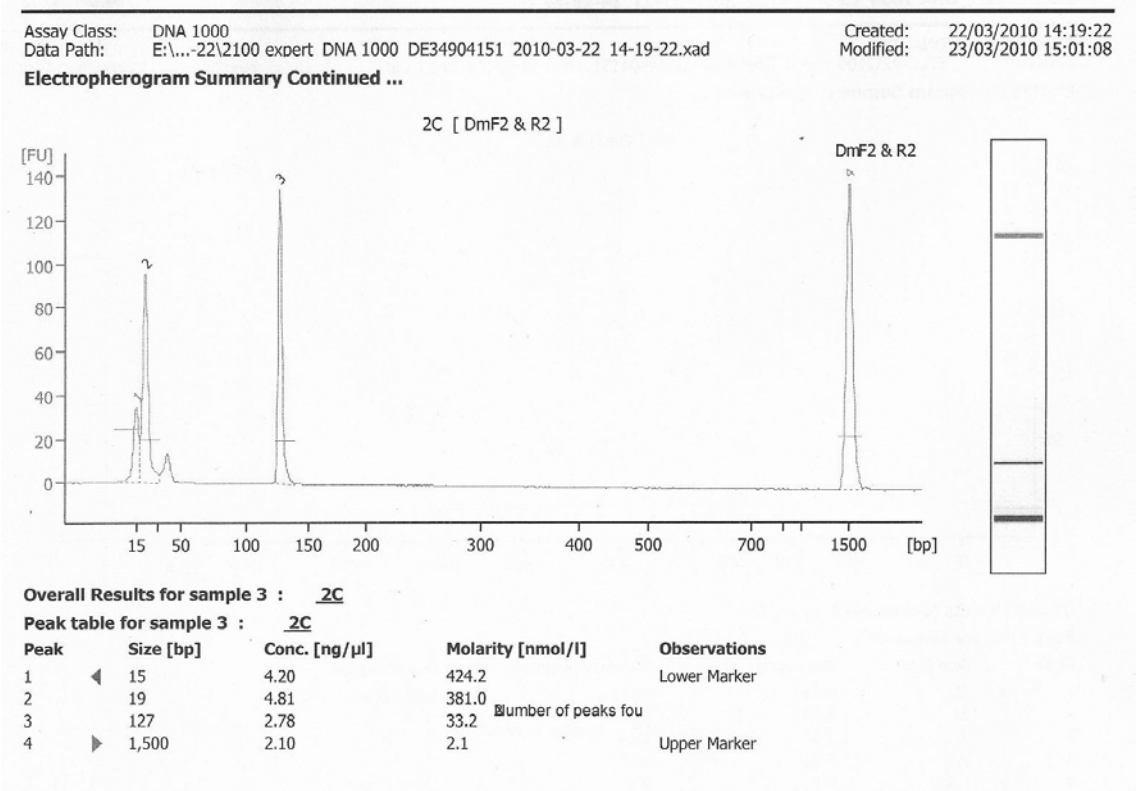


Table 4.3: Variation in PCR product size as measured by 2100 Bioanalyser: Column A = Expected Product Size (bp); Column B = Maximum size measured; Column C = Minimum size measured; Column D = Mean of all measured sizes; Column D = Standard Deviation of the means quoted in C; Column E = Number of replicates.

		A Exp.	**B** Max.	**C** Min.	**D** Mean	**E** SD	**F** n
	Primer Pair						
D.marginalis template DNA	DMF1/R1	279	304	292	297.6	3.4	14
	DMF2/R2	113	133	119	128.5	3.5	13
	DDF1/R1	275	288	275	283.0	4.8	11
	DDF2/R2	113	122	113	118.8	2.7	13
D.dimidiatus template DNA	DMF1/R1	279	303	286	296.1	6.6	11
	DMF2/R2	113	133	122	128.2	4.1	10
	DDF1/R1	275	295	284	289.9	4.1	8
	DDF2/R2	113	121	115	119.4	1.9	9
All DNA	DMF1/R1	279	304	286	296.9	5.0	25
	DMF2/R2	113	133	119	128.3	3.7	23
	DDF1/R1	275	295	275	285.9	5.6	19
	DDF2/R2	113	122	113	119.0	2.4	22

The analytical specifications for the Agilent 2100 Bioanalyser (Agilent 2007) indicate that the 'Typical sizing resolution' is ±5% for DNA fragments in the size range 100 – 500 bp and 'Sizing accuracy' is ±10%. This suggests that measured sizes could deviate by as much as ±15% from the true fragment size. All of the mean average measured sizes were higher than the expected fragment size, but none were greater than +15% different. The largest apparent discrepancy between measured and expected values was +18% (the equivalent of +20 bp) for some of the fragments that occurred in PCRs involving the DMF2/R2 combination.

Based on the analysis presented above I concluded that the peaks in the electropherograms represent actual amplification events. The occurrence of peaks in the electropherograms is summarised in Table 4.6 below.

Table 4.4: Summary of results from PCRs conducted to test effect on amplification of varying annealing temperatures. Note that a single X or ✓ denotes a result from single replicate. In some cases (e.g. DMF1/R1 at 52 ºC) there were multiple replicates.

T anneal (ºC)		50	52	53	55	60	65
	Primer Pair						
D.marginalis template DNA	DMF1/R1	✓- - -	✓✓✓✓	✓- - -	✓✓✓✓	✓✓- -	✓✓- -
	DMF2/R2	✓- - -	✓✓✓✓	✓- - -	✓✓✓✓	✓X- -	✓✓- -
	DDF1/R1	✓- - -	✓✓XX	✓- - -	✓✓✓X	✓✓- -	✓✓- -
	DDF2/R2	✓- - -	✓✓✓✓	✓- - -	✓✓✓✓	✓✓- -	✓X- -
D.dimidiatus template DNA	DMF1/R1	✓✓- -	✓✓- -	✓✓- -	✓✓- -	✓X- -	✓✓- -
	DMF2/R2	✓✓- -	✓✓- -	✓✓- -	✓✓- -	✓✓- -	XX - -
	DDF1/R1	✓X- -	✓✓- -	✓✓- -	✓✓- -	✓X- -	XX - -
	DDF2/R2	✓✓- -	✓✓- -	✓✓- -	✓✓- -	✓X - -	XX - -
Key:	✓ = Peak in electropherogram				X = No peak		

From Table 4.4, it can be seen that the only primer pair/annealing temperature combinations that consistently amplified template DNA from one species but not the other were DMF2/R2 at T anneal = 65ºC and DDF1/R1 also at T anneal = 65ºC. In both cases, amplification of *D. marginalis* DNA occurred while *D. dimidiatus* template DNA was not amplified although the DD series were designed as *dimidiatus*-specific.

Ideally, for the purposes of the study, it would have been possible to identify one primer pair and combination of conditions that would amplify *D. dimidiatus* DNA but not *D. marginalis* DNA and another primer pair and set of conditions with the opposite specificity. Amplification of DNA from a larva could then be attempted using both approaches and, if amplified DNA was observed in one set of PCRs but not the other it would be possible to assign the larva definitely to one species and discount the possibility of it belonging to the other.

The apparent lack of specificity of the chosen primers for *D. dimidiatus* that is indicated by the results in Table 4.4 did not allow definite identification of *D. dimidiatus* larvae. If there was good evidence that only *D. dimidiatus* and/or *D. marginalis* occurred in a particular location, then the species identity of a larva might be inferred as being *D.*

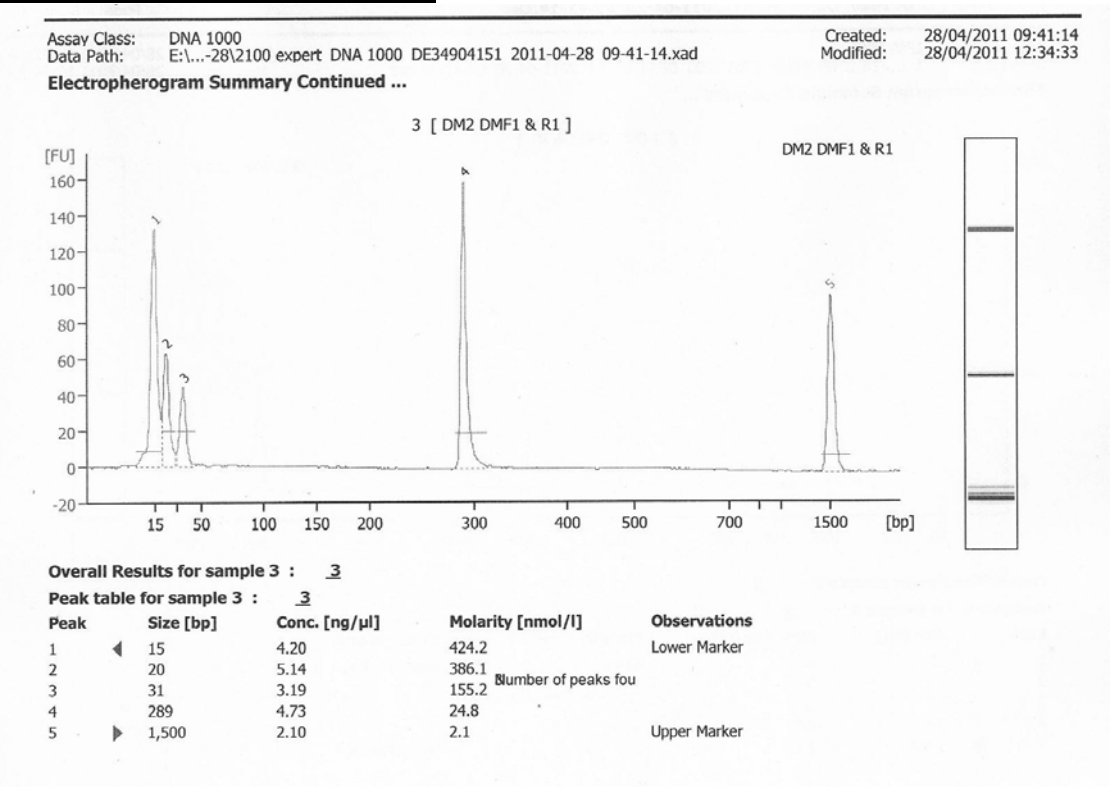
dimidiatus if amplification of DNA failed to occur in PCRs conducted at T Anneal = 65ºC using primers DMF2/R2 or DDF1/R1. However, amplification might fail for reasons unconnected with species identity. Possible causes of amplification failure in PCRs can be low concentration or poor quality of DNA in the larval extract, presence of PCR inhibitors in the template and primer-dimer formation [Saunders & Parkes (1999)]. For these reasons inferences about species identity based on failure of amplification would need to be treated with caution. Nevertheless, I considered there was good reason to proceed with further investigation of the efficacy of primers DMF2/R2 or DDF1/R1 when used in PCRs with T Anneal = 65ºC.

The results presented in Tables 4.3 and 4.4 were for PCRs of material obtained from two beetles only (one specimen of *D. dimidiatus* and one specimen of *D. marginalis*). For development of a useful diagnostic tool it was necessary to investigate if the observation held that, at higher values of T Anneal, the primer pairs identified above facilitated amplification from one species but not the other. To this end, DNA extracts from ten adult *D. dimidiatus* (labelled 'DD1' to 'DD10') and from ten adult *D. marginalis* ('DM1' to 'DM10') were used as templates for further investigation. The DNA was extracted either using spin columns (as described in section 2.5.1.2) or magnetic beads (as in 2.5.1.3). Information concerning the origins and nature of the extracts selected is provided in Appendix D2.

I compared amplification at the two extremes of annealing temperature previously tested (i.e. 50 ºC and 65ºC). In the first instance the efficacy of DMF2/R2 was tested as a possible primer pair to distinguish between the two species. Tests were conducted with DMF1/R1 providing a comparison. All PCRs were conducted in duplicate and details of the PCR mixes used are given in Chapter 2.The electropherograms relating to some of these tests are reproduced below in Figures 4.6 and 4.7. Only one example is shown of each duplicated test.

Figure 4.6: Electropherograms of PCRs conducted at T Anneal = 65°C showing positive results for both *D. marginalis* template DNA ('DM2') and *D. dimidiatus* template DNA ('DD1') using primer pair DMF1/R1.

4.6b: DM2 with DMF1/R1 at 65°C



4.6b: DD1 with DMF1/R1 at 65°C

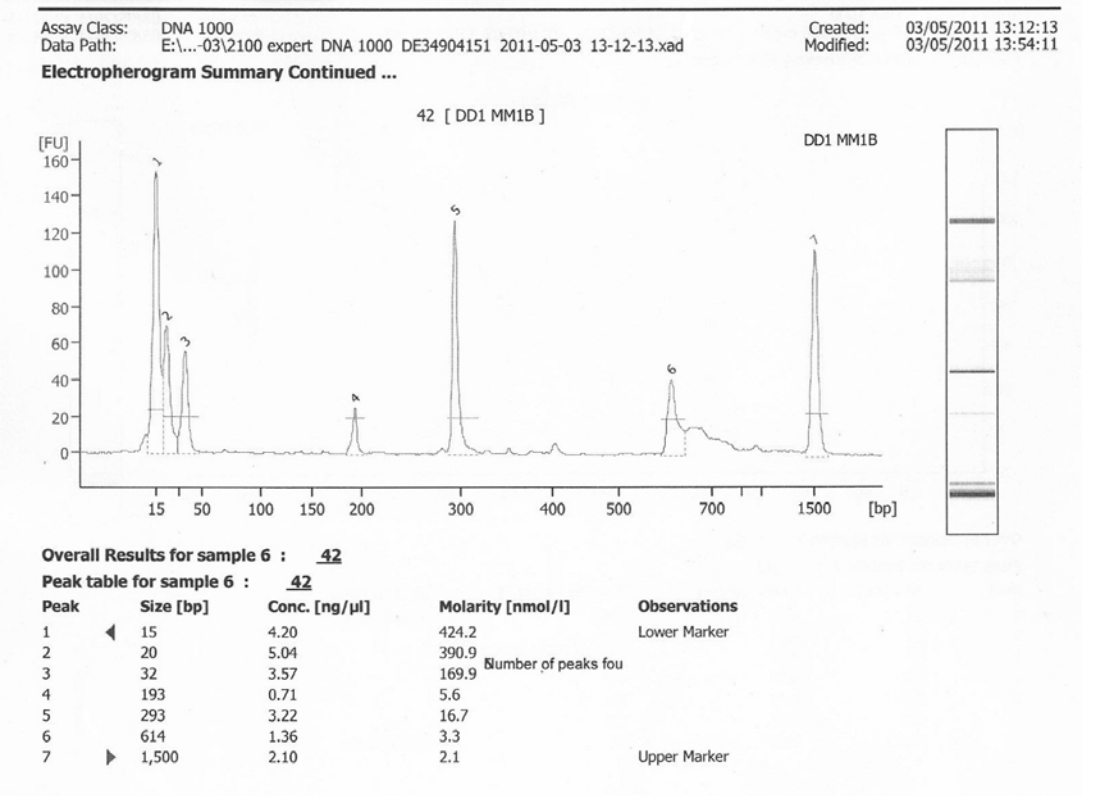
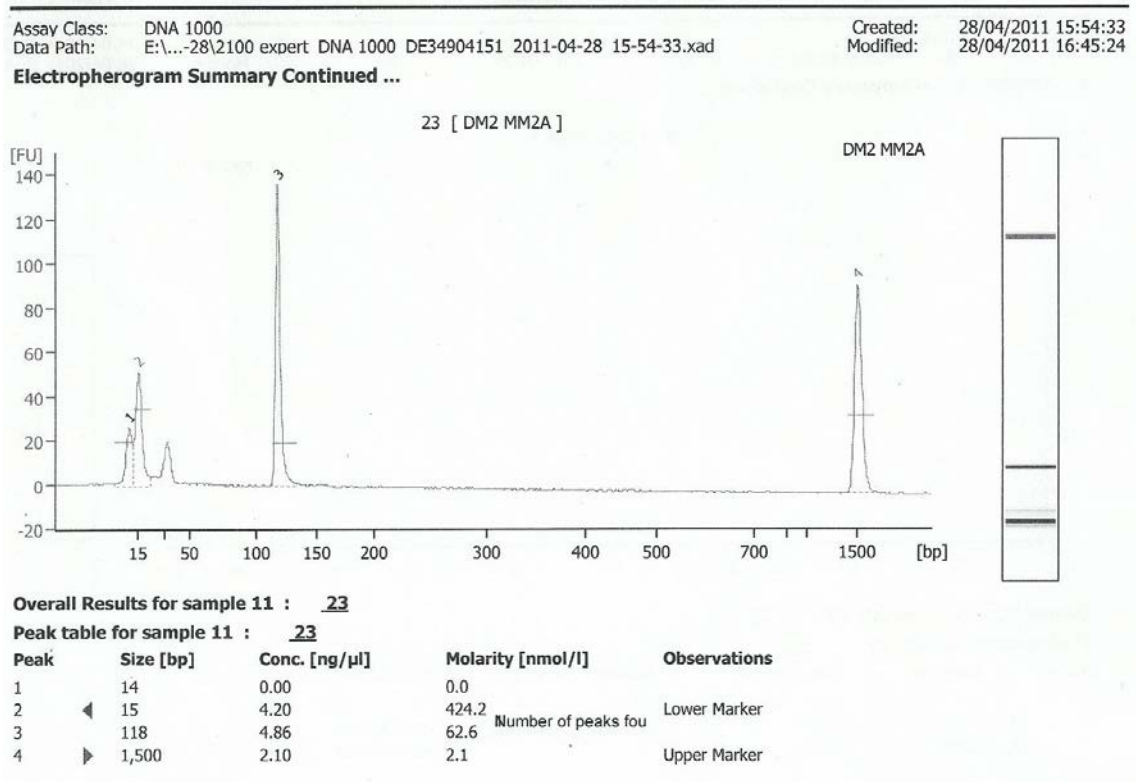
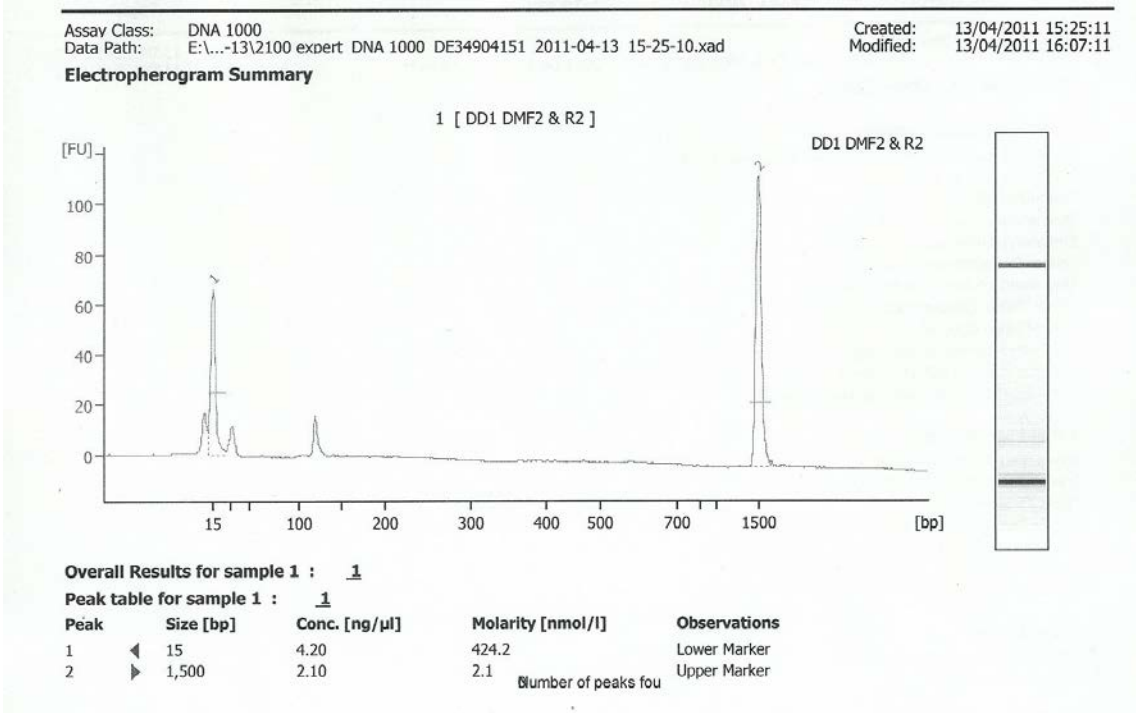


Figure 4.7: Electropherograms of PCRs conducted at T Anneal = 65°C showing positive results for both *D. marginalis* template DNA ('DM2') and *D. dimidiatus* template DNA ('DD1') using primer pair DMF2/R2.

4.7a: DM2 with DMF2/R2 at 65°C



4.6a DD1 with DMF2/R2 at 65°C



The results of the PCRs experiments illustrated in the preceding pages are summarised in Table 4.5 below.

Table 4.5: Summary of results from PCRs conducted to test effect on amplification of varying annealing temperatures. DM1-10 = *D. marginalis* extracts; DD 1-10 = *D. dimidiatus* extracts

T anneal (ºC)	50 ºC				65ºC	
	Template	DMF1/R1	DMF2/R2		DMF1/R1	DMF2/R2
D.marginalis template DNA	DM1	✓ ✓	✓ X		✓ ✓	✓ X
	DM2	✓ ✓	✓ ✓		✓ ✓	✓ ✓
	DM3	✓ ✓	✓ ✓		✓ X	✓ ✓
	DM4	X X	✓ ✓		✓ X	✓ ✓
	DM5	X X	✓ ✓		✓ X	✓ X
	DM6	✓ ✓	✓ ✓		✓ X	X X
	DM7	✓ ✓	✓ X		X X	X X
	DM8	✓ ✓	✓ ✓		X X	X X
	DM9	X X	X X		X X	X X
	DM10	✓ ✓	✓ ✓		X X	X X
D.dimidiatus template DNA	DD1	X X	✓ ✓		✓ X	✓ X
	DD2	X X	X X		✓ ✓	X X
	DD3	X X	✓ X		✓ ✓	X X
	DD4	X X	✓ X		✓ X	X X
	DD5	X X	✓ X		X X	X X
	DD6	✓ X	X X		X X	X X
	DD7	X X	X X		X X	X X
	DD8	X X	X X		X X	X X
	DD9	✓ X	X X		X X	X X
	DD10	X X	X X		X X	X X
Key: ✓ = Peak in electropherogram X = No peak						

The results contained in Table 4.5 did not suggest that a reliable system could be made for distinguishing the larvae of *D. dimidiatus* and *D. marginalis* based on use of the two primer pairs tested even when PCRs were conducted at the higher values of T Anneal which enhance specificity.

Firstly, there were a relatively large number of replicates where amplification was achieved in one PCR volume but not in the duplicate volume. For example, for DMF2/R2 at T Anneal 65ºC amplification was noted in one duplicate only in 3 out of 20 replicates (i.e. 15%). In other respects the DMF2/R2 pair at T Anneal 65ºC looked like a good

candidate as a suitable combination of primer and conditions since, except for one instance, it did not facilitate amplification of *D. dimidiatus* template DNA. However, the combination also failed to amplify DNA from *D. marginalis* in 50% of replicates. It seemed unlikely that this was due to issues connected with the quality or quantity of DNA in the *D. marginalis* extracts since DMF2/R2 did amplify DNA fragments at T Anneal 50ºC from four out of five extracts that 'failed' at T Anneal 65 ºC.

The conclusion drawn from the suite of experiments reported in this section was that a consistent and reliable diagnostic tool for identification of *Dytiscus* larvae could not be derived using the four primer pairs tested. It might have been possible to design different primers and investigate their efficacy, but I decided instead to look at a different molecular ecological approach to larval identification using DNA sequencing.

4.1.6 DNA sequencing

In section 4.1.2 it was reported that 220 larval legs were processed between June 2011 and November 2011 to extract DNA using the kit in the Agilent DNA Fish ID Ensemble (part number 5500-0100). The DNA extracts were stored at -20ºC to be used to identify the larvae to species level by molecular ecological means. Because identification techniques based on RAPD or on species-specific primers could not be made to work reliably in this study, sequencing was undertaken instead based on amplified, short sections of the CO1 gene with known differences between the two species. The theory behind the design of primers to amplify these sections was explained in detail in Chapter 2 (section 2.5.3) as was the double PCR protocol used to obtain samples of amplified and purified DNA suitable for sequencing.

Sequencing of Adult material - Before larval DNA was processed for sequencing, DNA from adult beetles taken on the Somerset Levels and Moors was isolated and sent for sequencing so that it might be compared with the Swedish sequences. This was done in order to make sure that the primer design based on the Swedish sequences was likely to work with beetles from

the Levels and Moors and to assess the level of possible intraspecific variation in the relevant portion of the CO1 gene. DNA extracts from two separate *D. dimidiatus* adults and two separate *D. marginalis* adults from Somerset were sent for sequencing with the forward primer:
F = GAGGAAAAAACGAAACTTTTGGTTC.

Figure 4.8 illustrates the partial CO1 sequences of the Somerset samples and the corresponding sequences from the Swedish material. An 'N' or gap (-) in the sequence data denotes a base that was not properly determined during sequencing.

Figure 4.8 shows that the partial CO1 sequences from two *D. marginalis* adults collected from the Somerset Levels and Moors were conserved and matched the sequence provided by Bergsten for *D. marginalis* from a locality in Sweden (Figure 4.53). Similarly, partial CO1 sequences from two adult *D. dimidiatus* from Somerset were identical to each other and to a Swedish sequence from the same species [Johannes Bergsten (*pers. comm.*)]. The *D. marginalis* and *D. dimidiatus* sequences differed consistently at certain bases as highlighted in the figures.

From these results, I inferred that CO1 sequence information can be used to distinguish reliably *D. dimidiatus* larvae caught in the Somerset Levels and Moors from *D. marginalis* larvae.

Figure 4.8: Sequences of 158 bp sections of CO1 genes in specimens of *Dytiscus marginalis* and *D. dimidiatus* from Somerset Levels and Moors & Sweden. The bases highlighted in red are consistent in *D. marginalis* but are different in *D. dimidiatus*. The bases highlighted in blue are consistent in *D. dimidiatus* but are different in *D. marginalis*. Underlined bases indicate the reverse primer binding site.

4.8a: *D. marginalis* (specimen 'DM2') from Shapwick Heath.

ATTAGCATGGTCTATTAGGATTTGTTGTATGAGCACATCATATATTTACTG
TAGGAATAGATGTAGACACACGGGCATATTTTACTTCTGCTACTATAATTA
TTGCCGTAC

4.8b: *D. marginalis* (specimen 'DM3') from Shapwick Heath.

ATTAGCATTGGTCTATTAGGATTTGTTGTATGAGCACATCATATATTTACTG
TAGGAATAGATGTAGACACACGGGCATATTTTACTTCTGCTACTATAATTA
TTGCCGTAC

4.8c: *D. marginalis* from Oland, Sweden.

ATTAGCAATTGGTCTATTAGGATTTGTTGTATGAGCACATCATATATTTACTG
TAGGAATAGATGTAGACACACGGGCATATTTTACTTCTGCTACTATAATTA
TTGCTGTAC

4.8d: *D. dimidiatus* (specimen 'DD1') from Shapwick Heath.

ACTAGCA-TTGG-TTATTAGGGNTGTTGTATGAGCACATCATATATTTACTG
TAGGGATAGATGTAGACACACGAGCATATTTTACTTCTGCTACTATAATTA
TTGCCGTAC

4.8e: *D. dimidiatus* (specimen 'DD2') from Shapwick Heath.

ACTANNNTTGGTTTATTAGGGTTTGTTGTATGAGCACATCATATATTTACTG
TAGGGATAGATGTAGACACACGAGCATATTTTACTTCTGCTACTATAATTA
TTGCCGTAC

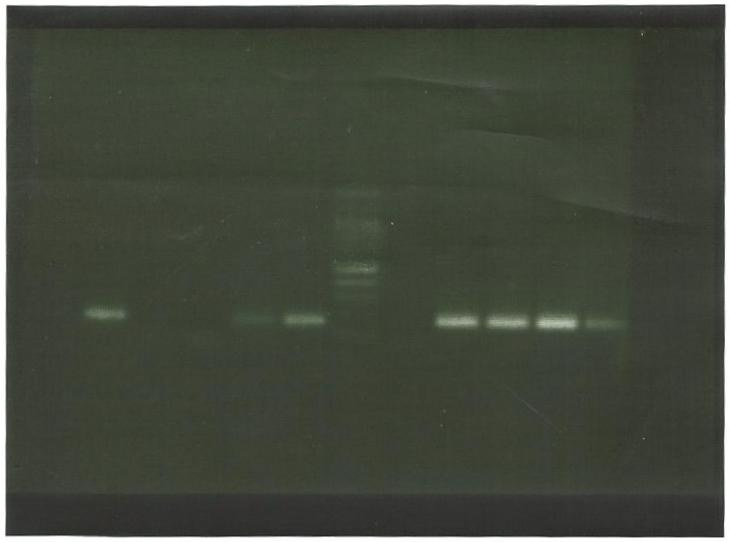
4.8f: *D. dimidiatus* from Oland, Sweden.

ACTAGCAATTGGTTTATTAGGGTTTGTTGTATGAGCACATCATATATTTACTG
TAGGGATAGATGTAGACACACGAGCATATTTTACTTCTGCTACTATAATTA
TTGCCGTAC

Preparation of larval material for sequencing – Samples for sequencing were prepared from larval extracts using the double PCR and purification protocols described in Chapter 2 (section 2.5.3). First round PCR product not used in the subsequent second round PCR was retained stored at -20ºC pending receipt of the sequencing results.

Concern was expressed in section 4.2.2 that the extraction technique used to obtain template DNA from the larvae might have failed to isolate sufficient DNA for amplification and sequencing. This was investigated using the stored first round PCR products. Ten first round PCR products were run out on an agarose gel – five from PCRs where the spectrophotometric assay of template DNA had indicated high concentrations of DNA in the template, five from PCRs carried out using template DNA in low concentration according to assay results. The gel is shown in Figure 4.9 below.

Figure 4.9: Photograph of gel obtained from electrophoresis of ten first round PCR products from *Dytiscus* larvae.



The strongest bands in the gel in Figure 4.9 were for three PCR products that the spectrophotometric assay results implied were amplified from extracts containing little or no template DNA. Whatever the cause of this discrepancy, the results of the gel suggest that little reliance should be placed on the spectrophotometric assays as predictors of PCR success.

Sequencing Results - Purified products from the double PCR of larval material were sent for sequencing in two tranches and the results were likewise received in two tranches.

First tranche - From the first tranche of 68 larval leg extracts, 61 yielded positive identifications (i.e. the larva was either *D.dimidiatus* or *D. marginalis* based on the occurrence of at least two unique 5 – 6 bp segments in the CO1 sequence – see section 2.5.3).

However, 15 of the 68 samples sent for sequencing failed at the first attempt. I assumed that the problem lay in contamination of the negative samples with PCR inhibiting substances originating in the extracts. Fresh samples were prepared from the 15 extracts where sequencing had not worked and from 3 in which positive results had been obtained. These new samples were made up following exactly the same protocols as before except for the first round PCRs being prepared with 1 µl of extract per 20 µl PCR volume as opposed to 4 µl. This was done in order to dilute any contaminant from the extract.

Sequences were obtained again for all 3 of the new samples made from extracts that had previously produced positive results. Eight of the 15 samples that had not worked before now yielded sequences. This suggested that at least some of the samples that initially failed to be sequenced did so because of issues connected with purity of the sample rather than insufficient DNA.

Second tranche - In the second tranche only 15 out of 152 samples did not yield a species identification. The primary reason for failure to make a positive identification from second tranche material was poor sequences which gave ambiguous or spurious results. This contrasts with the first tranche, where failure was related in all cases to no sequences being generated (see above).

Some examples of sequencing results are illustrated in Appendix D3.

Summary - Of the 220 DNA samples from larval material tested, sequencing data was obtained that allowed 198 (90%) to be identified unequivocally either as *D. dimidiatus* or *D. marginalis* using the criteria described above. Of these 198 samples, 91.4% (181) were identified as *D.marginalis* larvae and 8.6% (17.) *D. dimidiatus* larvae. Appendix D3 gives the complete results of the analysis.

Table 4.6 below summarises information regarding the origin and nature of material identified as belonging to *D. dimidiatus* larvae. Although there are records for *D. dimidiatus* in the Somerset as far back as 1897 [Duff (1993)], these are the first known records of larvae. The records in Table 4.6 provide definite proof of breeding at three sites which is potentially valuable information for conservationists interested in the management of ares of the Levels and Moors to maintain the full range of species listed in the Ramsar citation.

Table 4.6: Details regarding larval material from sources identified as *Dytiscus dimidiatus*.

No.	Date caught	Site	Trap no.	Taken (T) or Released (R)	Condition if taken
1	8/4/2007	Shapwick Heath	18	R	-
2	5/5/2007	Shapwick Heath	2	T	Whole
3	20/5/2007	Shapwick Heath	8	R	-
4	20/5/2007	Shapwick Heath	10	R	-
5	20/5/2007	Shapwick Heath	14	R	-
6	17/6/2007	Shapwick Heath	8	R	-
7	17/6/2007	Shapwick Heath	11	R	-
8	10/5/2008	Westhay Heath	9	R	-
9	10/5/2008	Westhay Heath	10	T	Fragments
10	2/6/2008	Westhay Heath	8	T	Whole
11	8/6/2008	Shapwick Heath	9	T	Whole
12	27/6/2008	Tealham Moor	17	T	Whole
13	29/6/2008	Westhay Heath	1	R	-
14	29/6/2008	Westhay Heath	3	R	-
15	29/6/2008	Westhay Heath	10	T	Whole
16	17/7/2008	Westhay Heath	2	R	-
17	17/7/2008	Westhay Heath	9	R	-

4.1.7 Conclusions from molecular ecological work

From initial experiments it was concluded that sufficient DNA of adequate quality could be extracted from both adult and larval legs from *Dytiscus* spp., capable of amplification by Polymerase Chain Reaction (PCR) for subsequent analysis.

Tests to investigate whether material from *D. dimidiatus* could be distinguished from that originating from *D. marginalis* by RAPD (Randomly Amplified Polymorphic DNA) techniques were inconclusive. Evidence was obtained, however, suggesting that at least one of the 10-mer oligonucleotides tested might be capable of use as part of a battery of primers to identify the species apart from one another.

However, the study concentrated later on the CO1 locus widely used for 'barcoding' identification of invertebrates when sequence information for the two *Dytiscus* species became available. The diagnostic potential was investigated of using species-specific pairs of 25+-mer oligonucleotides as primers in PCRs to amplify sections of the CO1 gene. Although no consistent results were obtained, the experiments suggested that the primer combination 'DMF2/R2' might amplify *D.marginalis* but not *D. dimidiatus* DNA at higher annealing temperatures (i.e. >60ºC) and $MgCl_2$ concentrations of at least 3.0 mM in the PCR mix. Such a finding is consistent with the observation that, where species are closely related, amplification specificity can often only be achieved at relatively high annealing temperatures [Beebee & Rowe (2004)]. No PCR conditions were discovered that reliably amplified *D. dimidiatus* DNA but not *D. marginalis* genetic material. Due to the constraints of time and the amounts of template DNA available, investigations had to be curtailed before an accurate test could be developed based on differential amplification by PCR.

Sequencing of a fragment of the CO1 gene amplified from larval material was however very successful and did allow individual larva to be assigned to a species (*D. dimidiatus* or *D. marginalis*) in 90% of cases where DNA was successfully extracted from legs. On the basis of this molecular ecological

approach to species identification 8.6% of the total larvae collected were identified as being *D. dimidiatus*.

4.2 Larval morphology and biometrics

In this section the results are reported of using morphological observations and biometrics to identify *Dytiscus* larvae. How the features to be measured and observed were selected was described in Chapter 2 along with an account of the methods by which observational data and measurements were obtained (section 2.6).

Not taking into account legs removed from larvae which were then released, 144 dytiscid larval specimens were collected in the field. On close examination some of the smaller larvae collected were identified not as *Dytiscus*, but rather as individuals of other taxa (5 Colymbetine larvae and 1 probable *Acilius sulcatus*). This left 138 examples of *Dytiscus* material available for morphological examination and measurement.

4.2.1 Morphology

As reported in Chapter 2 (section 2.6), where the material allowed, note was taken of the shape of the frontoclypeus, the position of hairs on the front tarsi and the presence or absence of swimming hairs on abdominal segment 7 (A7).

In all cases where an intact head could be observed, the frontoclypeus was definitely convex in shape. No larvae were recorded with distally-located swimming hairs on the front tarsi.

10 larvae were seen to lack swimming hairs on Abdominal segment 7 (A7), indicating that they were first instar (L_1) larvae.

4.2.2 Biometrics

138 specimens of *Dytiscus* species were examined. A full set of measurements and observations were obtained for 113 specimens (82%). The specimens

from which a full set of measurements could not be made varied from one lacking urogomphi only through to material comprising only heads. The complete dataset is included in Appendix D5. It is summarised below in Table 4.7.

Table 4.7: Results of Larval Biometry. Summary of data in the form of measurements (in mm) and calculations from 138 specimens of *Dytiscus* larval material from Somerset Levels and Moors. SD = Standard Deviation
n = Number of observations/calculations

	Measurement	**Mean**	**SD**	**n**
A	Length of antenna	4.64	0.655	122
B	Length of head capsule	5.32	0.911	126
C	Width of head capsule	6.12	1.050	126
D	Width of neck	3.25	0.623	126
E	Length of thoracic segment 1 (T1)	5.92	1.237	125
F	Length of thoracic segments 2 & 3 (T2 – T3)	4.75	1.541	122
G	Length of abdominal segments 1, 2 & 3 (A1 – A3)	7.20	2.199	120
H	Length of abdominal segments 4, 5 & 6 (A4 – A6)	9.61	2.676	119
I	Length of abdominal segment 7 (A7)	3.91	0.859	120
J	Length of abdominal segment 8 (A8)	6.20	1.097	124
K	Length of urogomphus	3.90	0.726	123
L	Length of tarsal claw of hind leg	0.82	0.100	124
M	Length of tarsus of hind leg	2.40	0.295	124
N	Length of tibia of hind leg	3.61	0.462	124
O	Length of femur of hind leg	4.56	0.639	124
P	Length of coxa of hind leg	3.60	0.606	123
	Calculation	**Mean**	**SD**	**n**
C1	Body Length (B+E+F+G+H+I+J)	37.84	8.188	119
C2	Leg length (M+N+O+P)	14.18	1.849	123
C3	Length of Head / Width of Head (B/C)	0.87	0.068	126
C4	Width of Head / Width of Neck (C/D)	1.90	0.118	126
C5	Length Hind Coxa / Length Hind Tarsus (P/M)	1.50	0.191	123
C6	Length A8 / Length of Urogomphus (J/K)	1.62	0.215	122

4.2.3 Identification based on morphology and biometrics

Sufficient observations and measurements could be made in 119 cases (87.5% of total specimens) to allow the specimen to be keyed out using the key in Klausnitzer (1991) (see Appendix D6) or that of Rozkošnȳ (1980). The following account explains the results obtained mainly from the use of Klausnitzer's key. Where reference is made to individual specimens, these are numbered according to the number assigned to the specimen in the list provided in Appendix D5.

I assumed that certain species – *D. circumcinctus, D. latissimus* and *D. lapponicus* – could not occur among the larvae even though the species are theoretically capable of being identified using the keys. This assumption was based on the lack of historical records for these species in the Somerset Levels and Moors [Duff (1993)] (see Chapter 1). For this reason, the only outcomes considered possible were identification as *D. circumflexus*, *D. dimidiatus*, *D. marginalis* and *D. semisulcatus*.

An important diagnostic feature of *D. semisulcatus* larvae is the relatively thick larval neck in all instars, which approaches three quarters or more of the width of the head capsule [Klausnitzer (1991), Rozkošnȳ (1980)]. This would equate to a measured head width / neck width (C/D) ratio of 1.33 or less. The lowest value for C/D ratio obtained was 1.66 for specimen 9, while the average value for the measure was 1.90 (see Table 4.7). This was taken to be strong evidence that none of the larval specimens collected were of *D. semisulcatus*. This conclusion agreed also with the observation that no larvae were seen with distally located swimming hairs on the front tarsus and none had anything other than a strongly convex frontoclypeus.

None of the late instar larvae examined possessed more than one of the diagnostic features of *D. dimidiatus* that are outlined in Chapter 2 (section 2.6.2). The bodies of 5 larvae were measured as being over 51 mm long which would suggest they were *D. dimidiatus* individuals if Rozkošnȳ (1980) is followed. However, all of these individuals had legs somewhat shorter than would be expected (i.e. ≤19.5 mm) even when the claws are included in the measurement. In only two instances (for specimens 4 and 29) did measured leg length exceed 17 mm for any larva. Of the 10 early (L_1) instar larvae examined, 4 exceeded 23 mm in body length, which would mean they would be identified as *D. dimidiatus* larvae using Klausnitzer's key.

On the basis of meeting at least two out of four diagnostic criteria from measurements (length of abdominal segment 8 ≤ 5.5mm, length of hind femur ≤4.5mm, P/M = c.1.6, length of urogomphus c. 3.5 mm) 8 larvae could be keyed

out as *D. circumflexus* if it is assumed they were all final instars. However, it was considered at least as likely that these larvae were second instar (L_2) larvae of *D. marginalis* or *D. dimidiatus* that Klausnitzer (1991) does not enable to be identified. This interpretation seems to be the best explanation if it is considered that in all but one case, the length of the head did not exceed 6.0 mm which would be expected if the individuals were final instar *D. circumflexus*.

Based on the key in Klausnitzer (1991) and the one due to Rozkošnȳ (1980), most larvae keyed out as closest to *D. marginalis*. If one assumes that all late instar larvae over 51 mm and all L_1 larvae over 23 mm were *D. dimidiatus,* then 9 (7.5%) of those larvae that could be keyed out were this species. Further, if it is taken that all the 8 larvae keyed out as possibly *D. circumflexus* were all this species, then at the very least 102 larvae (85.7% of the larvae capable of being assigned to species) were *D. marginalis* so far as could be told from the keys.

4.2.4 Comparison of morphological and molecular ecological approaches to larval identification

DNA sequencing allowed 198 (90% of all larval material processed for DNA) to be identified either as *D. dimidiatus* or *D. marginalis*. Of these 198 samples, 181 (91.4%) were identified as *D.marginalis* larvae and 17 (8.6%) as *D. dimidiatus* larvae. As noted in the previous section, the morphological analyses suggested that about 7.5% of larvae might tentatively be assigned as *D. dimidiatus*.

Although there was reasonable agreement between molecular and morphological methods in terms of numbers and percentages of larvae identified as *D. dimidiatus*, the important question was whether the two methods agreed in particular cases.

There were 96 cases in which the identifications using DNA and those using the keys could be compared directly since there were identifications for the larvae available using both methods. Ten L_1 larvae were caught during the study but sequencing data was obtained only for six of these. The identifications of these

six larvae using the key in Klausnitzer (1991) are contrasted with those obtained from DNA sequencing in Table 4.8 below.

Table 4.8: A comparison of Identifications of L_1 larvae reached using different techniques. N.B. Specimens 13 and 96 exceeded 23mm in total body length [the supposed maximum length for *D. marginalis* according to Klausnitzer (1991)] but were less than 27mm, so were identified only tentatively as *D. dimidiatus*.

Specimen Number	Identification using DNA	Identification using key
10	*D. marginalis*	*D. marginalis*
13	*D. marginalis*	***D. dimidiatus?***
26	*D. marginalis*	*D. marginalis*
77	***D. dimidiatus***	*D. marginalis*
96	*D. marginalis*	***D. dimidiatus?***
116	*D. marginalis*	Not possible due to fragmentary nature

As reported in 4.2.3, none of the later instar larvae (i.e. L_2 or L_3) keyed out definitively as *D. dimidiatus*. Five larvae had bodies that exceeded 51 mm in length - which would have caused them to be keyed out using Rozkošnȳ (1980) as possibly *D. dimidiatus* given that they were larger than the maximum size attributed to *D. marginalis*. Four further L_2/L_3 larvae had head capsules that were greater than 7.3 mm in width which would have indicated possible *D. dimidiatus* specimens according to Klausnitzer (1991), assuming, along with couplet 3 of the key, that the head capsules of *D. marginalis* are always narrower than this. Table 4.9 summarises the conclusions reached concerning the probable species identity of these nine individuals using DNA sequences.

Table 4.9: A comparison of Identifications of L_2/L_3 larvae reached using different techniques.

Specimen Number	Identification using DNA	**Identification using key** [R = Rozkošnȳ (1980), K = Klausnitzer (1991)]
3	Not sequenced	***D. dimidiatus?*** R
4	Not sequenced	***D. dimidiatus?*** R & K
43	*D. marginalis*	***D. dimidiatus?*** R
72	*D. marginalis*	***D. dimidiatus?*** R
75	***D. dimidiatus***	***D. dimidiatus?*** K
108	Inconclusive	***D. dimidiatus?*** R & K
118	*D. marginalis*	***D. dimidiatus?*** K
128	*D. marginalis*	***D. dimidiatus?*** K
133	***D. dimidiatus***	***D. dimidiatus?*** K

It can be seen from Table 4.9 that two specimens (75 and 133) tentatively keyed out as *D. dimidiatus* on the basis of their head capsules exceeding 7.3 mm in width were identified as this species by DNA sequencing data. However, another two specimens keyed out in the same way (118 and 128) were shown to be *D. marginalis*. Where sequencing data were available they indicated that specimens tentatively identified as *D. dimidiatus* due to their body length being greater than the supposed maximum for *D. marginalis* were actually the latter species.

4.2.5 Multivariate statistical analyses

I considered the possibility that, whilst the Klausnitzer and Rozkošnȳ keys might not enable the *D. dimidiatus* larvae caught during the study to be distinguished from *D. marginalis* larvae, it might be possible still to separate the species by relying upon a suite of measurements. In order to investigate whether it might be feasible to use biometric criteria to separate out the larvae, ordination techniques were employed on the measurements that are listed (A to P inclusive) in Table 4.7 above.

The multivariate technique employed to analyse them was Principal Components Analysis (PCA) which is described in section 2.7.2.1. A variance-covariance matrix was used in the ordination rather than a correlation matrix since the former is better suited to analysing datasets comprised of similar quantitative measurements [Henderson & Seaby (2008)]. PCA using a covariance-variance matrix was preferred to that using correlation because the latter type of PCA gives equal weight to all variables which is unnecessary when all the measurements have been made in the same way as is the case here [Henderson & Seaby (*Op. cit.*)].

Other ordination techniques were investigated using the CAP software [e.g. DECORANA, Multidimensional Scaling (MDS)], but PCA was selected above other methods available because the PCA plots that were generated produced the clearest separation of the data into a few groupings.

Since PCA is sensitive to sparse variables (i.e. individuals with many measurements lacking) certain specimens were removed from the dataset to be analysed [Henderson & Seaby (*Op. cit.*)]. Initially, all L_1 larvae were left in the dataset, but specimens were taken out for which there was more than one measurement missing. Thus all the specimens consisting of heads only and those with badly fragmented bodies were left out of the analysis. This meant that data concerning 22 out of 138 *Dytiscus* specimens were not used in the initial PCA.

Before the dataset from the remaining 116 larvae was used in the PCA it was investigated for normality, skew and kurtosis as described in section 2.7.1. The results are given in Tables 4.10 and 4 11 below.

Table 4.10: Shapiro-Wilk Normality Tests conducted on biometry results.
Measurement data are from 116 specimens of *Dytiscus* larvae. SD = Standard Deviation, W = Shapiro-Wilk Test Statistic, P = Probability of Null Hypothesis (The distribution of the variable does not deviate from normality). All measurements were made in mm.

	Measurement	Mean	SD	W	P
A	Length of antenna	4.7	0.6	0.98	<0.05
B	Length of head capsule	5.4	0.8	0.87	<0.01
C	Width of head capsule	6.2	0.9	0.86	<0.01
D	Width of neck	3.3	0.6	0.74	<0.01
E	Length of segment T1	6.1	1.1	0.78	<0.01
F	Length of segments T2 – T3	4.8	1.5	0.98	0.10
G	Length of segments A1 – A3	7.3	2.2	0.99	0.85
H	Length of segments A4 – A6	9.7	2.7	0.99	0.30
I	Length of segment A7	3.9	0.8	0.95	<0.01
J	Length of segment A8	6.3	1.0	0.87	<0.01
K	Length of urogomphus	3.9	0.7	0.96	<0.01
L	Length of tarsal claw	0.8	0.1	0.95	<0.01
M	Length of tarsus	2.4	0.3	0.86	<0.01
N	Length of tibia	3.6	0.4	0.82	<0.01
O	Length of femur	4.6	0.6	0.79	<0.01
P	Length of coxa	3.6	0.6	0.91	<0.01

Table 4.11: Medians and Coefficients of Skewness and Kurtosis of measurement data. Data from 116 specimens of *Dytiscus* larvae.

	Measurement	Median	Skewness	Kurtosis
A	Length of antenna	4.7	-0.3	0.7
B	Length of head capsule	5.5	-1.6	3.7
C	Width of head capsule	6.4	-2.1	4.9
D	Width of neck	3.4	-2.0	4.4
E	Length of segment T1	6.3	-2.0	4.3
F	Length of segments T2 – T3	4.7	0.5	0.6
G	Length of segments A1 – A3	7.2	0.0	-0.3
H	Length of segments A4 – A6	9.7	-0.3	0.0
I	Length of segment A7	4.0	-0.9	0.9
J	Length of segment A8	6.5	-1.4	2.0
K	Length of urogomphus	4.0	-0.8	0.7
L	Length of tarsal claw	0.8	-0.6	1.4
M	Length of tarsus	2.5	-1.7	4.1
N	Length of tibia	3.7	-1.8	4.6
O	Length of femur	4.8	-1.9	4.3
P	Length of coxa	3.7	-1.3	2.3

From Table 4.10 it can be seen that the majority of the variables (13 out of 16) deviated significantly from a normal distribution. Table 4.11 shows that there was an appreciable skew to the data for most of the variables. Generally this was negative skew indicating a heavier left tail to the distribution, which might be expected from a dataset that includes measurements from L_1 larvae together with ones from later stage larvae.

In all but two instances the variables displayed a leptokurtic tendency, which means the kurtosis coefficients were positive, indicating that a larger number of individuals clustered around the mean for these variables than would be expected in a normal distribution.

Because of the observed skew in the raw data, transformation of the data was attempted to see if this would reduce skewness. Since zero values occurred in a few instances, log transformations could not be calculated, so the data was square root transformed. The transformed data was re-checked for skew and it was apparent that the transformation had not made an appreciable difference to skew and had in some cases actually increased skewness. I decided to proceed to conduct PCA on the raw data, keeping in mind, however, that the skew might affect the interpretation of results.

In the initial PCA of measurement data from 116 larvae, each individual was assigned *a priori* to one of two groups on the basis of the presence or absence of swimming hairs on abdominal segment 7 – L_1 Larvae (hairs absent) and L_2/L_3 larvae (hairs present). The result of this ordination is presented in the plot shown in Figure 4.10 below.

The first two principal components in the PCA plot together explained nearly 80% of variability of the data set analysed. The first principal component which explains nearly 70% of the variability was interpreted as being primarily due to measures that contribute to overall body length, such as the length of the first three abdominal segments (L A1 – A3). There is a clear separation in this plot between L_1 and later instar larvae and this is due mainly to body size, the later instar larvae being measurably and distinctly larger than L_1 larvae. The second principal component appears to be strongly influenced by the length of the first thoracic segment and by the dimensions of the head and legs, which are all indicators of overall size of a larva.

The separation of L_1 from other instars was not perfect in the sense that six outliers assigned to the group L_2/L_3 larvae clustered with the eight L_1 larvae. There are a number of possible explanations for the clustering of these outliers with the L_1 larvae. One possibility is that these outliers are freshly metamorphosed L_2 instars that have had not had sufficient time to grow to the size typical of an L_2 instar. Another explanation is that it is simply not possible to separate L_1 and L_2 instars on the basis of the measurements made and the main cluster comprises almost entirely L_3 larvae. Other possibilities are that the absence of hairs has either been missed during some observations or the feature is not diagnostic of all L_1 larvae.

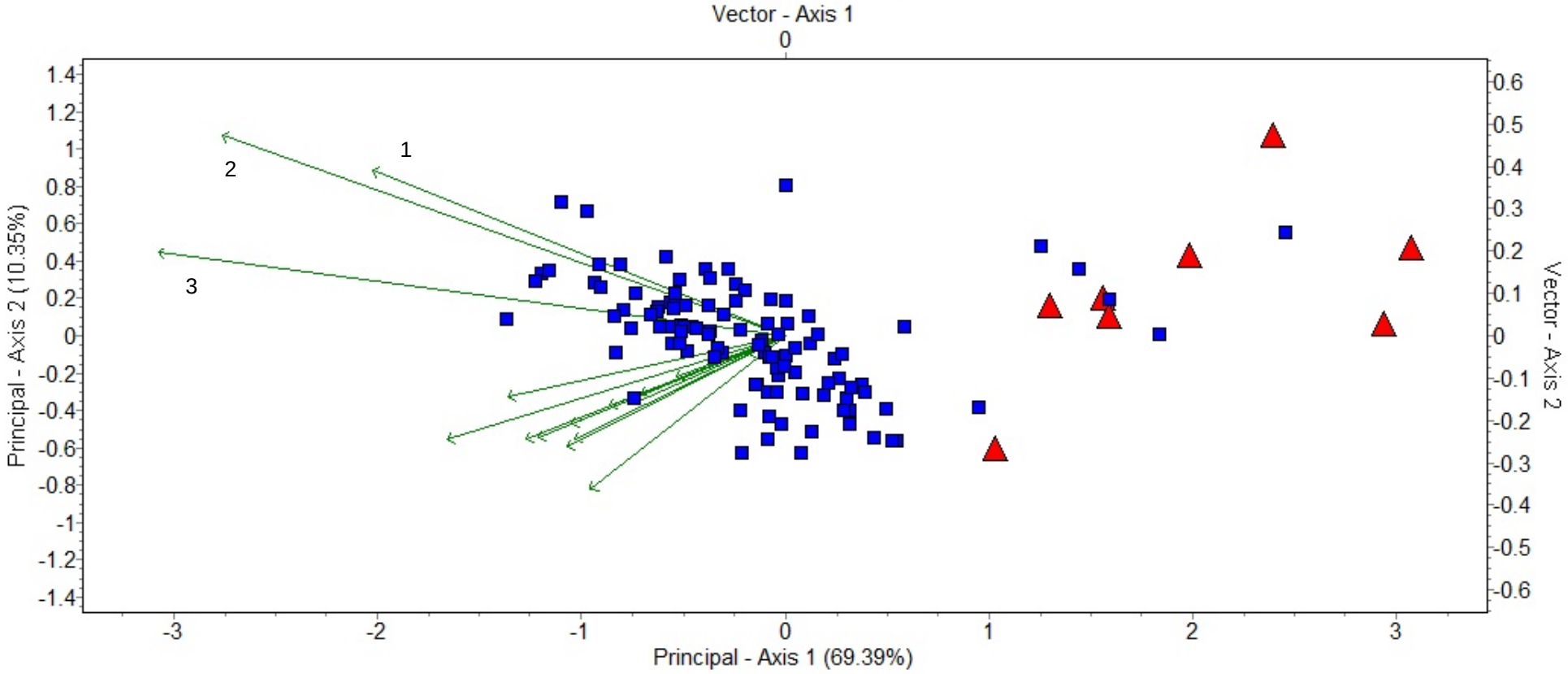
A further PCA was carried out on the data with the eight L_1 larvae removed from the data set leaving 108 specimens in the analysis. This time the larvae were categorised according to the species identity inferred from DNA sequencing data. This was done in order to see if the 4 specimens identified as *D.*

dimidiatus by molecular ecological methods could be readily separated from others on the basis of the biometric measurements taken. The categories assigned *a priori* of the PCA were: '*D. dimidiatus*'; '*D. marginalis*' and 'unidentified'. The last category was for those larvae (19 in number) that it had not been possible to assign to species due to lack of clear DNA sequencing data.

The PCA plot obtained using the dataset with L_1 larvae removed is reproduced in Figure 4.11 below. The first three principal components explained 68.7% of the variability in the data (or nearly the same amount as did the first plot).

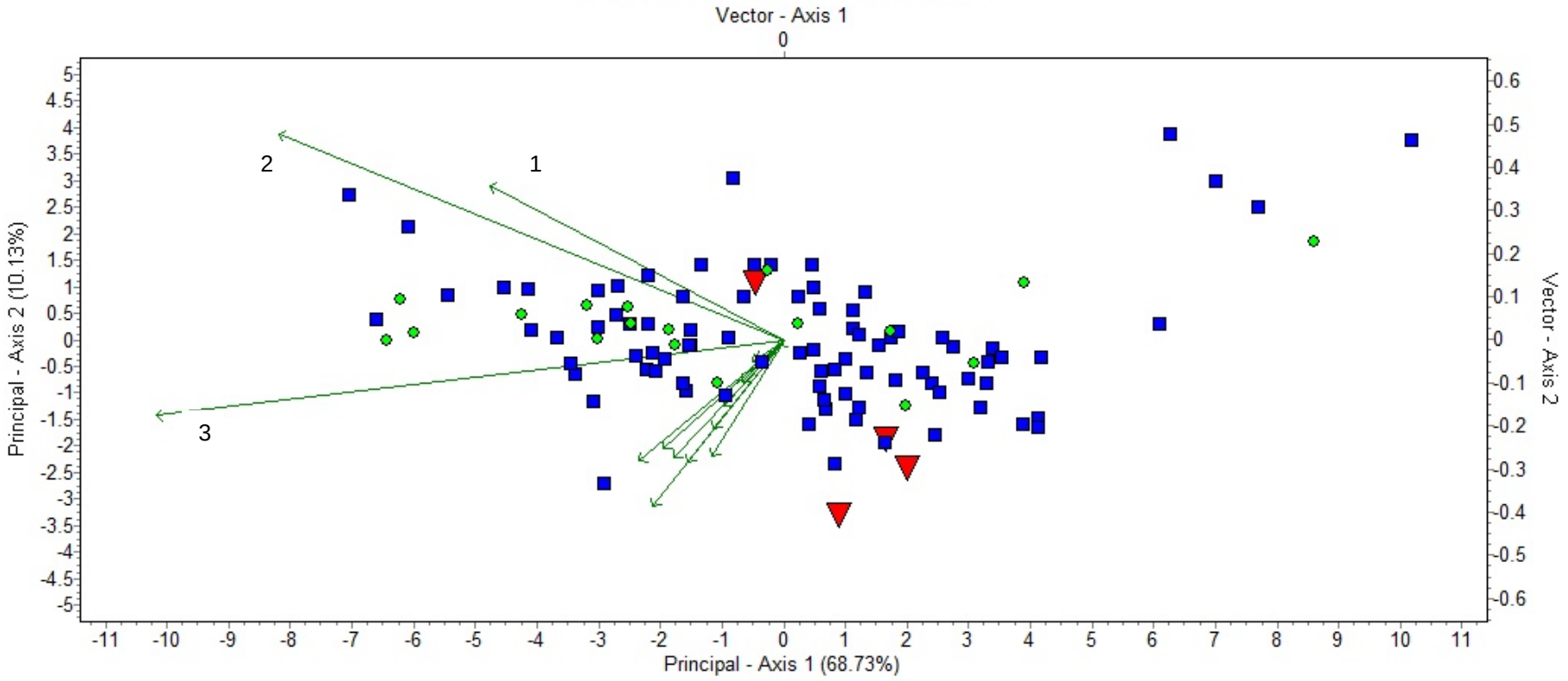
It is important to note that the most influential variables in both plots (variables F, G and H in Tables 4.10 and 4.11) are precisely the ones that displayed the least amount of skew and which were normally distributed. PCAs using skewed data can be unreliable because the skewed data dominates the plot since it is the source of most of the variance [Henderson & Seaby (2008)]. In this instance, skewness did not seem to influence the PCA results unduely, because the first principle components were due in a very large degree to the non-skewed variables. The skewed variables influenced the second principle component in the plot but this component explained only about 10% of the variance compared with nearly 70%. Re-running the plot using the correlation matrix (which is less influenced by magnitudes of variables) rather than the variance-covariance matrix produced very similar results to that shown in Figures 4.10 and 4.11, so I have not included these plots here.

Figure 4.10: PCA Biplot of measurement data from 116 *Dytiscus* larvae. PCA undertaken using the variance-covariance matrix. The three most influential eigenvectors are labelled: 1 = F: Length T2-T3; 2 = G: Length A1 – A3; 3 = H: Length A4 – A6. The other, less influential eigenvectors are represented on the graph by unlabelled green arrows which reflect the variables A – P as listed in Table 2.7 in Chapter 2.



KEY: ▲ L_1 Larvae ■ L_2/L_3 Larvae ← Eigenvector for variable

Figure 4.11: PCA Biplot of measurement data from 108 *Dytiscus* L_2/L_3 larvae. PCA undertaken using the variance-covariance matrix. The three most influential eigenvectors are labelled: 1 = F: Length T2-T3; 2 = G: Length A1 – A3; 3 = H: Length A4 – A6. The other, less influential eigenvectors are represented on the graph by unlabelled green arrows which reflect the variables A – P as listed in Table 2.7 in Chapter 2.



The plot in Figure 4.11 achieved a less clear separation of assigned groups than that produced previously. Three of the four larvae identified as *D. dimidiatus* by DNA sequencing were clustered towards the bottom of the plot. However, the fourth specimen identified as *D. dimidiatus* was separate from these three and closer to the centre of the main cluster. There was no obvious separation between *D. marginalis* group and the unidentified group. In order to test whether the groups assigned to the different larvae on molecular ecological grounds had any statistical significance an Analysis of Similarity Test ('ANOSIM') was performed using the CAP software as described in section 2.7.2.1.

It was mentioned in Chapter 2 that ANOSIM is unreliable in situations where sample size (n) is large and the samples are highly diverse. In this instance the number of samples (i.e. specimens) was moderate (n = 108), the number of variables measured relatively small (16) and there was not excessive diversity in the variables measured (mean for pooled variables = 4.9, standard deviation = 2.3, n = 1726).

The results of the ANOSIM are summarised in Tables 4.12 and 413 below.

Table 4.12: Analysis of Similarity Test (ANOSIM) on larval dataset

Sample Statistic (R)	0.104
P Value	0.127
Level %	12.7
No Randomizations	1000
No >= Obs	127

Table 4.13: Pairwise Tests conducted on assigned groups

		Permutations		Significance			
1st Group	2nd Group	Possible	Done	P Value	Level %	No >= Obs	Sample Statistic
D. dimidiatus (4)	D. marginalis (85)	> 1000000	1000	0.283	28.3	283	0.077
D. dimidiatus (4)	Unidentified (19)	8855	1000	0.287	28.7	287	0.073
D. marginalis (85)	Unidentified (19)	> 1000000	1000	0.082	8.2	82	0.105

The results from the ANOSIM support the view that the assigned groupings were not meaningful, which is to say that, on the basis of the particular

biometrical measurements conducted, there was no statistically significant greater similarity (P = 0.127) between members of the same group than between individuals from outside their group. That there was no significant difference (P = 0.283) between larvae assigned to *D. dimidiatus* and those assigned to *D. marginalis* on the grounds of molecular ecological data encourages the view that it may not be possible to infer the probable species identity of later instar larvae on the basis of biometrics. However, because there were only four larvae in the *D. dimidiatus* group it is difficult to state this with complete confidence. However, the PCA plots did indicate that it would be difficult to derive simple rules (such as those which dichotomous keys depend upon) to separate reliably the *D. dimidiatus* larvae from other species, because some specimens of *D. marginalis* are grouped around what might be termed the '*dimidiatus* cluster', suggesting a degree of similarity which would make separation difficult.

As well as there being no significant difference between *D. dimidiatus* and *D. marginalis* larvae, the results displayed in Table 4.9 show that a significant difference (P = 0.287) could not be detected between either *D. marginalis* or *D. dimidiatus* larvae and those classified as 'unidentified' on molecular ecological criteria.

4.3 Discussion

A number of different molecular ecological techniques were tried to identify *Dytiscus* larvae to species level: RAPD; the use of species-specific primers and DNA sequencing. The first two of the techniques listed could not be made to work reliably enough to enable larvae of *D. dimidiatus* and *D. marginalis* to be separated with a reasonable degree of confidence. Where DNA of requisite quality could be obtained from a larval leg in sufficient quantity, DNA sequencing allowed an identification to be made in approximately nine out of every ten cases.

Approaches to identification were also tried based on biometrics, morphological observation and the use of dichotomous keys. Although these techniques suggested that the large majority of larval specimens obtained were *D. marginalis*, a result that agreed with the sequencing data, they gave no indication that larvae positively identified as *D. dimidiatus* were morphologically different from *D. marginalis* so far as could be told from the suite of observations and measurements taken.

On the basis of morphological observation and biometrics it would seem possible to readily distinguish early instar (L_1) *Dytiscus* larvae from later stage larvae. PCAs and subsequent ANOSIM Tests indicated, however, that there were no significant biometrical differences between larvae that ecological molecular methods had identified as *D. dimidiatus* and those that had been identified as *D. marginalis*.

Since the choice of observations and measurements was made partly to enable use of available dichotomous keys, the lack of close agreement between identifications based on morphological data and those from molecular ecological evidence brings into question the efficacy of the keys. It is noted that the keys in both Klausnitzer (1991) and Rozkošnȳ (1980) are very similar and both rely upon the same papers written in the 1920s. That no range of figures is quoted for certain measurements suggests that the keys may be based on observations from a small number of larvae reared in aquarium conditions. Since no indication is given in the keys regarding the range of values within which measurements would be expected to fall, it is difficult to judge whether a particular larva that does not conform to the key might be a smaller or larger than normal individual of one species or an example of a completely different one.

One recent published description of *Dytiscus carolinus* larvae [White *et al.* (2000)] was based on specimens reared to the third instar in the laboratory, but this study – in contrast to Klausnitzer and Rozkošnȳ - quoted mean values for

anatomical measurements along with standard deviations and ranges to indicate how mature specimens might vary in terms of the measurements.

White *et al.* (2000) sought to find ways to distinguish *D. carolinus* larvae from the larvae of other *Dytiscus* species occurring in the US state of Georgia and focussed not on biometrics but on morphological differences (e.g. secondary segmentation or lack of it in the proximal labial segment, form of ocelli). Other authors, most notably Nilsson (1988), have developed identification methods for *Dytiscus* larvae based on what are perceived to be "primary" pores and setae on the legs of larvae. Nilsson's 1982 key to the larvae of European *Dytiscus* spp. is based partly on such characters. The diagnostic features that these authors rely upon to distinguish species would be difficult to observe in the field and almost certainly require that specimens be taken and killed for examination.

Not only are some of the supposedly diagnostic characters mentioned above rather difficult to observe and measure, but there is also some evidence of confusion in the literature about whether the characters are consistent or not within the species. Thus, for example, White *et al.* (2000) differ from Roughley (1990) and Wilson (1923) regarding secondary segmentation of the labial mouthparts of larvae of *Dytiscus verticalis* – a character that is supposed to separate this species from *D. carolinus*.

In this study the identifications of larvae from DNA sequencing appeared to be more reliable than those obtained using available keys based on morphology and biometrics. It may be possible to identify anatomical features which form a sound basis for distinguishing the larvae of the *Dytiscus* species that occur in the Somerset Levels and Moors, but this has not been done in this study. However, so far as the purposes of this study are concerned, it has been possible to positively identify some *D. dimidiatus* larvae captured during the course of fieldwork so that a comparison may be attempted of their habitat requirements with those of *D. marginalis*.

Chapter 5: Annual Activity Cycle in 2007

Introduction

In this chapter I assess the evidence for the hypothesis that the coexistence of *Dytiscus marginalis* and *D. dimidiatus* in ditches in the Somerset Levels and Moors might involve temporal niche separation.

In order to study the annual cycle of *Dytiscus* beetles in the Somerset Levels and Moors I decided to record the frequency over the course of a year with which adults and larvae were caught in bottle traps set at intervals along ditches. The underlying assumption was that frequency of capture would reflect directly the actual numbers of beetles present in the watercourses studied rather than just being a measure of activity of individuals drawn from a relatively static population. Because the numbers caught in traps might be influenced by variations in environmental conditions and not simply by the numbers present, I measured certain environmental parameters at trap locations over the course of the year, including maximum and minimum water temperatures. I did this with a view to seeing whether frequency of capture could be related to these environmental parameters.

5.1 Methods

5.1.1 Trapping protocol

During 2006 several ditches at Shapwick Heath National Nature Reserve (NNR) were investigated as sites suitable for permanent traps the following year. I selected ditches that looked typical of those in the part of the Reserve selected for study purposes. These were situated away from areas where public access was encouraged to reduce the possibility of interference with traps. Ditches were selected that were not scheduled for cleaning out during 2006 or the following year so that the study ditches would not be subjected to wholescale disturbance. Water samples were collected and analysed in the laboratories of Somerset Scientific Services to check that the water chemistry was not unusual or extreme by comparison with ditches in this part of the Levels and Moors.

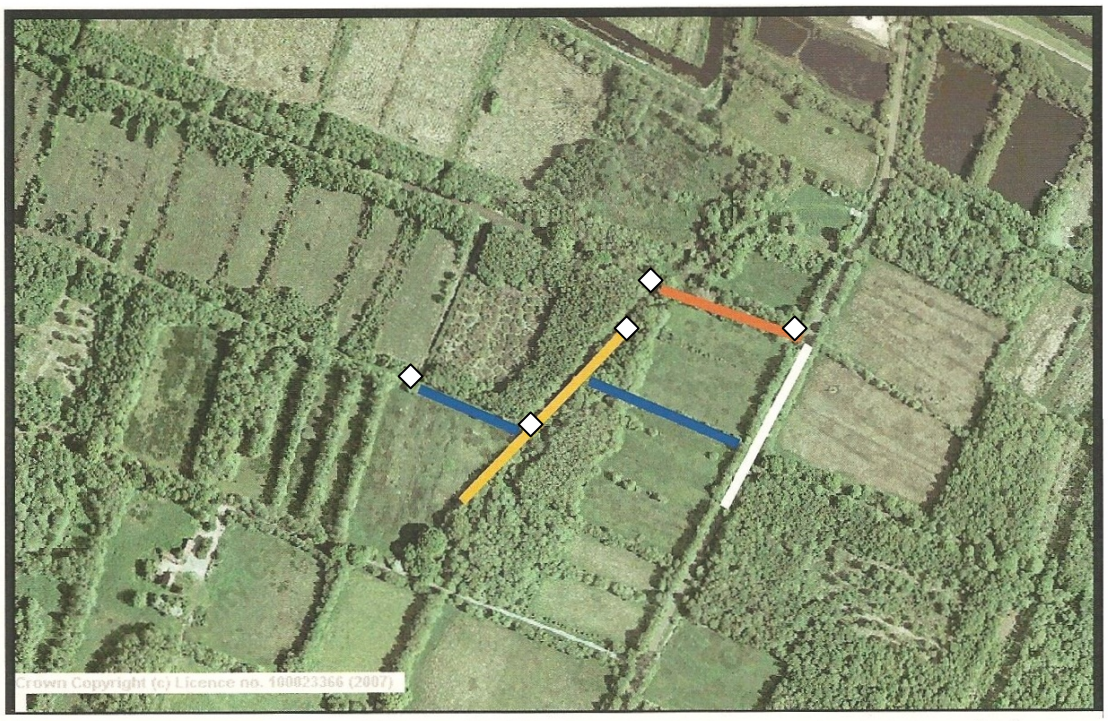
In 2007, trapping stations were established at twenty locations along the three connected ditches in the NNR that were selected for study. The trapping stations were set up approximately 30 metres apart and were marked with numbered stakes on the nearside bank. Baited traps were set as described in Chapter 2 at each trapping station at fortnightly intervals from 29 January 2007 to 20 January 2008 (a total of 25 separate sampling events). Traps were run for approximately 24 hours at a time, generally being set in late morning or early afternoon one day and collected in at the same time the following day. The order in which the traps were set and then subsequently collected in was reversed each fortnight to try to even out the total amount of trapping time at a particular station over the seasons and over the year. The location of the ditches sampled at Shapwick Heath is indicated in Figure 5.1.

5.1.2 Habitats in study site

Photographs of trap locations are in Appendix E1. As can be seen from the photographs and from Figure 5.1, stations 1 to 15 inclusive were located on sections of ditch running through areas of wet woodland. As a consequence these stations were quite heavily shaded for much of the year. In contrast, stations 16 to 20 inclusive were located in more open, grassed habitat.

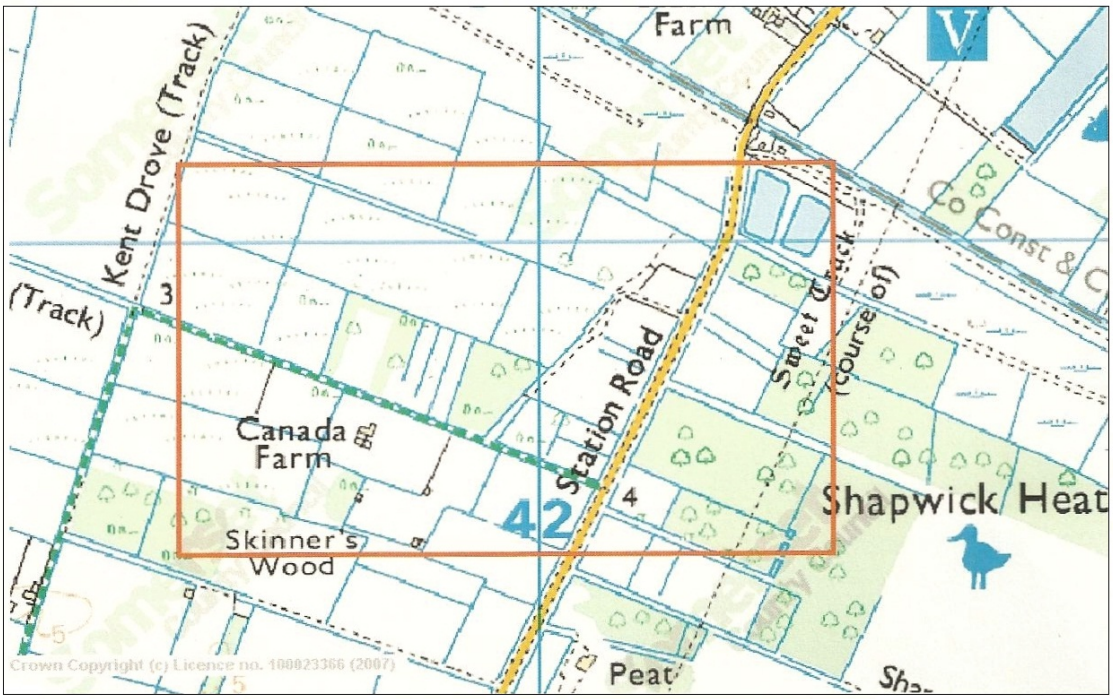
The wet woodland canopy-forming species comprised mainly Alder (*Alnus glutinosa*) with Birch (*Betula* sp.) and Oak (*Quercus* sp.) frequent and Willows (Salix sp.) occurring occasionally. The understorey was sparse with Bramble (*Rubus fruticosus* agg.) and Bracken (*Pteridium aquilinum*) often dominating. In terms of National Vegetation Classification (NVC), the woodland was probably closest to W6 *Alnus glutinosa – Urtica dioica* woodland [Rodwell (1991)].In the more open areas the habitat on the nearside bank (i.e. the ditch bank nearest the trap) was unimproved pasture as mentioned in the SSSI citation for Shapwick Heath (see Appendix B2). On the ‘farside’ bank of the ditch there occurred far more Bog Myrtle (*Myrica gale*) and dense clumps of Purple Moor-grass (*Molinia caerula*), characteristic of the ‘wet heathy grassland’ plant communities described in the SSSI citation.

Figure 5.1: Trapping Stations at Shapwick Heath 2006 – 7. The sections of ditch marked with diamond symbols at either end where those sampled in 2007.



Above: Aerial photograph of Shapwick Heath with ditches marked along which sampling occurred in 2006. (Red = 6-7/5/06, Orange = 13-14/5/06, White = 28-29/5/06, Blue = 5/6/06) (Photo: Somerset County Council)

Below: Approximate location of aerial photograph (Map: Ordnance Survey)



5.1.3 Environmental parameters

On three separate occasions during the year (8 May, 17 June and 9 September) the physical aspects of the environment in the vicinity of each trap station were recorded along with the vegetation as described in sections 5.1.3 and 5.1.4. The section of ditch considered in each case was that from 2m 'upstream' of the trap to 2m 'downstream'. Two metres was taken as a convenient distance since it was the length of the standard ranging pole used here for fieldwork (see Chapter 2).

5.1.3.1. Physical characteristics of the ditches

At each trapping station a number of physical features were assessed that could have a bearing on whether or not the section of ditch might support *Dytiscus* spp. Ditch dimensions (width and water depth) were assessed because of evidence that these can affect the richness of aquatic invertebrate communities in ditches in the Levels and Moors [e.g. Drake *et al.* (1984)]. Bankside gradient and degree of poaching were chosen also as parameters as it has been suggested both might affect the choice of pupation sites by beetles (Pat Hill-Cottingham *pers. comm*.).

Width of Waterbody - At each station the width of the waterbody was measured using the ranging pole. Because the amount of water in the ditch fluctuated over time, the width of the waterbody as defined here was capable of changing between recording events.

Water Depth - The depth of water was also measured using a ranging pole which was held at arms length while standing as close as possible to the edge of the water. The pole was lowered into the water until it met resistance from substrate at the bottom of the ditch.

Poaching – Both banks of the ditch for 2 metres either side of the trapping station were checked for signs of trampling ('poaching') at the

ditch margins. This feature was recorded either as 'Yes' or 'No' for poaching.

Gradient – The gradient of the bank as it met the water was recorded both for the bank nearest the trap ('nearside') and for that on the opposite side ('farside'). The banks were scored as follows: 4 = Abrupt (near vertical); 3 = Steep (more than 1 in 3); 2 = Sloping (less than 1 in 3) and 1 = Shallow.

5.1.3.2. Vegetation of ditches and ditch margins

Observations of the vegetation in and on the margins of the ditches are set out below.

Shade – The percentage area of water shaded out by trees and shrubs was estimated for the section of ditch 2m 'upstream' to 2m 'downstream' of the trap station.

Floating vegetation –The percentage area of water covered by *Glyceria* mats was estimated for the 4m section of ditch centred on the trap station. An estimate was made of the percentage water area covered by Duckweed but no attempt was made to distinguish the percentage contribution of any of the five species present in the area (Wolseley *et al*. 1984). Over a season, *Glyceria* mats may become semi-submerged beneath layers of Duckweed, thus it was possible for the combined Duckweed/Glyceria cover to exceed 100%. In addition, at each of the three visits when environmental parameters were noted a record was made of shade casting plant species, marginal plants, floating plants, emergents and submerged plants.

5.1.3.3. Physical and chemical factors

At each fortnightly trapping event maximum and minimum temperature thermometers were placed as stated in Chapter 2. The maximum and minimum water temperatures over the 24 hours that the traps were

running were recorded at trap locations at each of the site visits. The trap stations used for this purpose were numbers 1, 5, 10, 15 and 20.

pH, electrical conductivity and dissolved oxygen readings were taken on three occasions during the year as described previously in section 2.3.3.

5.2 Results

5.2.1 Preliminary Fieldwork in 2006

The results of water chemistry measurements made in 2006 are given in Table E2 in Appendix E2.

5.2.1.1 pH

The average pH measured from water samples taken from ditches at Shapwick Heath was 6.5 (St Dev = 0.4, n = 5). A Shapiro-Wilk Test for normality indicated that the values obtained did not deviate significantly from a normal distribution (W = 0.8, P = 0.2). This compared with averages of pH 7.3 (St Dev = 0.4, n = 2) measured from samples taken from Catcott North and pH 6.5 (St Dev = 0.1, n = 3) from Westhay Moor ditch water samples.

pH values taken during an invertebrate survey of 55 Levels and Moors ditches during 1981 ranged between 6.4 to 9.9 [Armitage (1981) cited in Wolseley *et al.* (1984)]. The values obtained from Shapwick Heath, Catcott North and Westhay Moor did not indicate that ditches on these sites were unusual in respect of pH. Sites with ditch water pHs towards the lower end of the range of values measured by Armitage were invariably located on peat as were the three study sites mentioned.

5.2.1.2 Electrical conductivity

Conductivities in the range 303 µS to 818 µS were measured in the field and from water samples analysed in the laboratory from 10 samples from

ditches on Shapwick Heath. The average conductivity measurement was 508.5 µS (St Dev = 185.2, n = 10). These values did not indicate excessive pollution or any significant saline influence, however, they did deviate from a normal distribution (Shapiro-Wilk Test W = 0.8, P < 0.01). The conductivity in most ditches (6 out of 10) fell within the range 404 – 430 µS but a few values were substantially higher (712 – 818 µS).

Electrical conductivity values measured in ditch water from Westhay Moor tended to be lower than those at Shapwick Heath (Average = 300.0, St Dev = 15.8, n = 6), while those from Catcott North were higher (Average = 836.8, St Dev = 79.0, n =4).

A Two-tailed t Test conducted to compare the means of values from Shapwick Heath and Catcott North found a statistically significant difference (t = -4.7, D of F = 11, P < 0.01). The same test applied to Shapwick Heath and Westhay Moor also revealed a significant difference in means (t = 3.5, D of F = 9, P < 0.01). In terms of conductivity, the water chemistry differed at all three sites.

5.2.1.3 Dissolved Oxygen

Six readings of dissolved Oxygen were taken at Shapwick Heath, the average value being 1.3 mg/l (St Dev = 0.9). This compared with Catcott North (Average = 0.12 mg/l, St Dev = 0.04, n = 2) and Westhay Moor (Average = 1.0 mg/l, St Dev = 0.3, n = 3). Values for dissolved Oxygen measured at Shapwick Heath were normally distributed (Shapiro-Wilk W = 0.9, P = 0.5).

Two-tailed t Tests found a statistically significant difference between the means of values from Shapwick Heath and Catcott North (t = 3.1, D of F = 5, P < 0.05) but none between Shapwick Heath and Westhay Moor (t = 0.6, D of F = 6, P > 0.05).

5.2.2 Frequencies of *Dytiscus* spp. at Shapwick Heath during 2007

Table 5.1 contains the field data collected. Figures 5.2 to 5.4 inclusive show the numbers trapped respectively of *Dytiscus marginalis*, *D. dimidiatus* and of *Dytiscus* larvae at fortnightly intervals over the period January 2007 to January 2008. The average minimum and average maximum temperatures recorded when traps were collected are plotted on the same graphs.

Table 5.1: 2007/8 Field data (D. m = *D. marginalis*; D .d = *D. dimidiatus*)

Date	D. m Fem	D. m Male	D.m Total	D. d Fem	D. d Male	D. d Total	D. larva
29-Jan	1	0	1	0	1	1	0
11-Feb	0	3	3	1	0	1	0
25-Feb	1	2	3	0	4	4	0
11-Mar	1	9	10	2	4	6	0
25-Mar	0	3	3	0	1	1	0
08-Apr	4	17	21	4	8	12	1
22-Apr	7	9	16	1	9	10	5
05-May	17	22	39	7	10	17	13
20-May	1	8	9	5	1	6	27
03-Jun	6	5	11	2	4	6	30
17-Jun	7	12	19	8	7	15	32
02-Jul	5	7	12	0	1	1	15
16-Jul	2	1	3	3	3	6	4
29-Jul	5	2	7	2	4	6	1
10-Aug	6	7	13	4	5	9	0
26-Aug	7	5	12	4	2	6	0
09-Sep	7	3	10	1	4	5	0
22-Sep	9	20	29	4	13	17	0
07-Oct	1	2	3	1	2	3	0
28-Oct	0	0	0	1	1	2	0
11-Nov	0	0	0	2	0	2	0
25-Nov	0	2	2	1	5	6	0
15-Dec	0	2	2	1	0	1	0
30-Dec	0	1	1	0	0	0	0
20-Jan	0	1	1	0	2	2	0

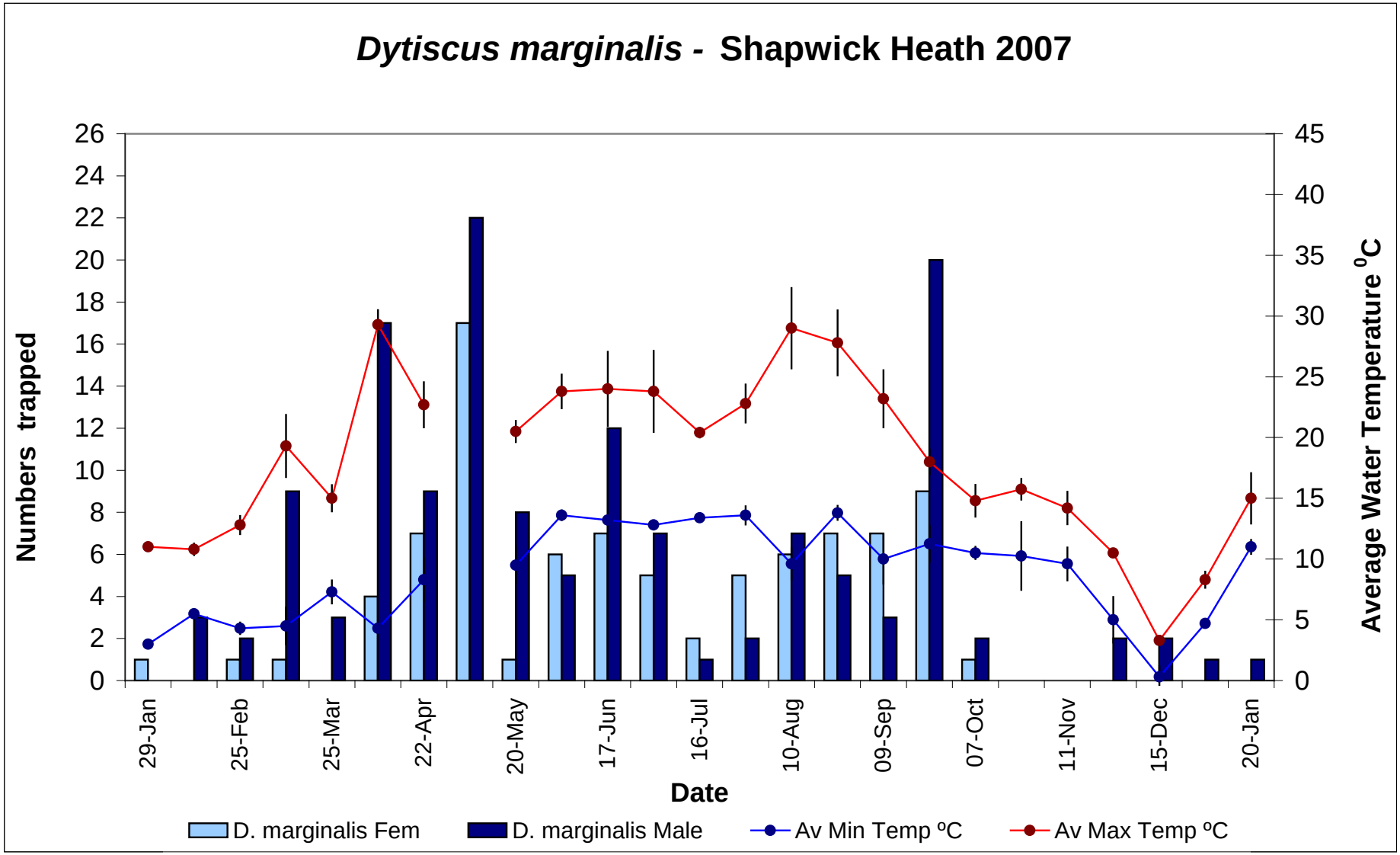


Figure 5.2: Seasonal activity pattern of *D.marginalis*. Shapwick Heath 2007

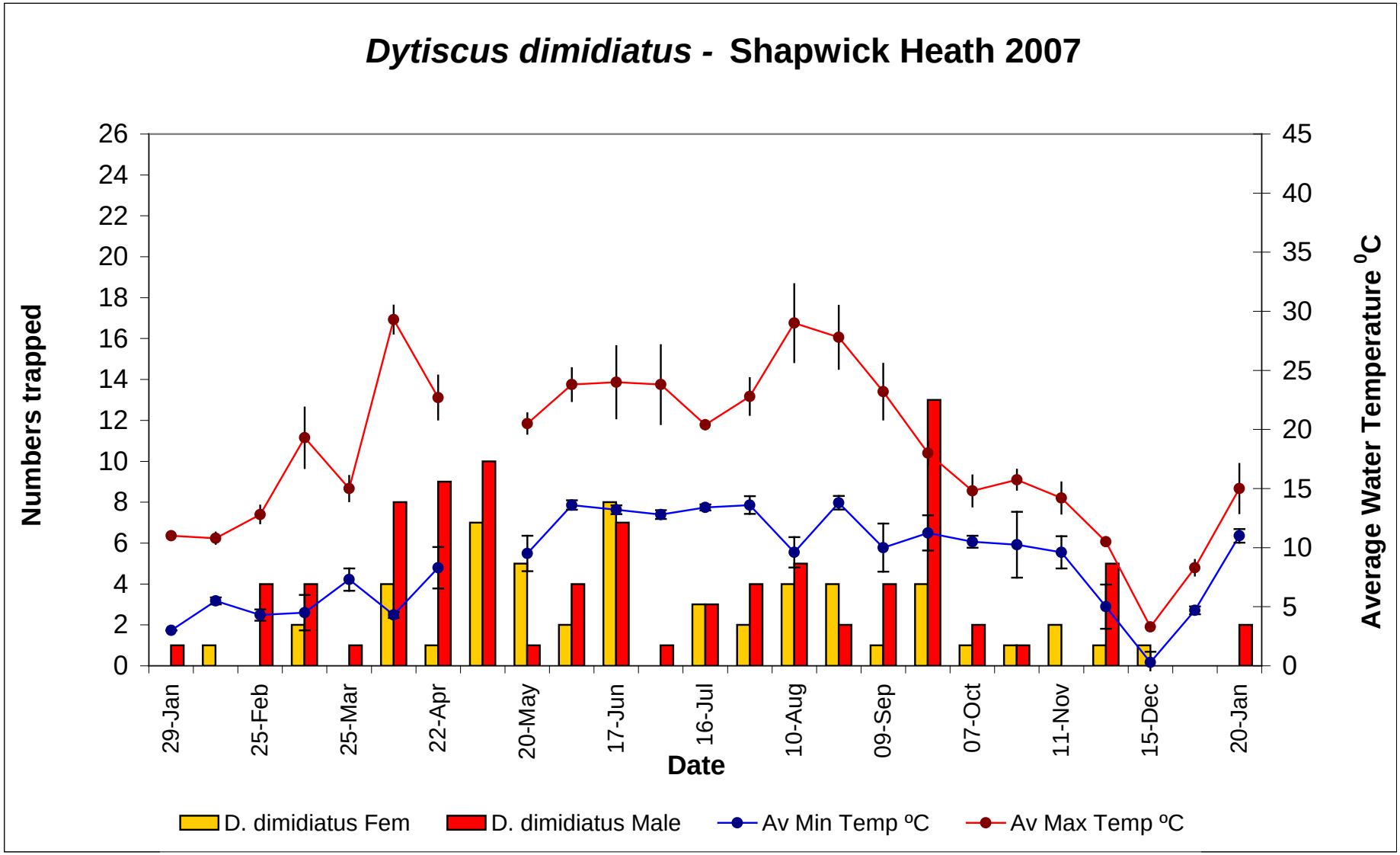


Figure 5.3: Seasonal activity pattern of *D.dimidiatus*. Shapwick Heath 2007

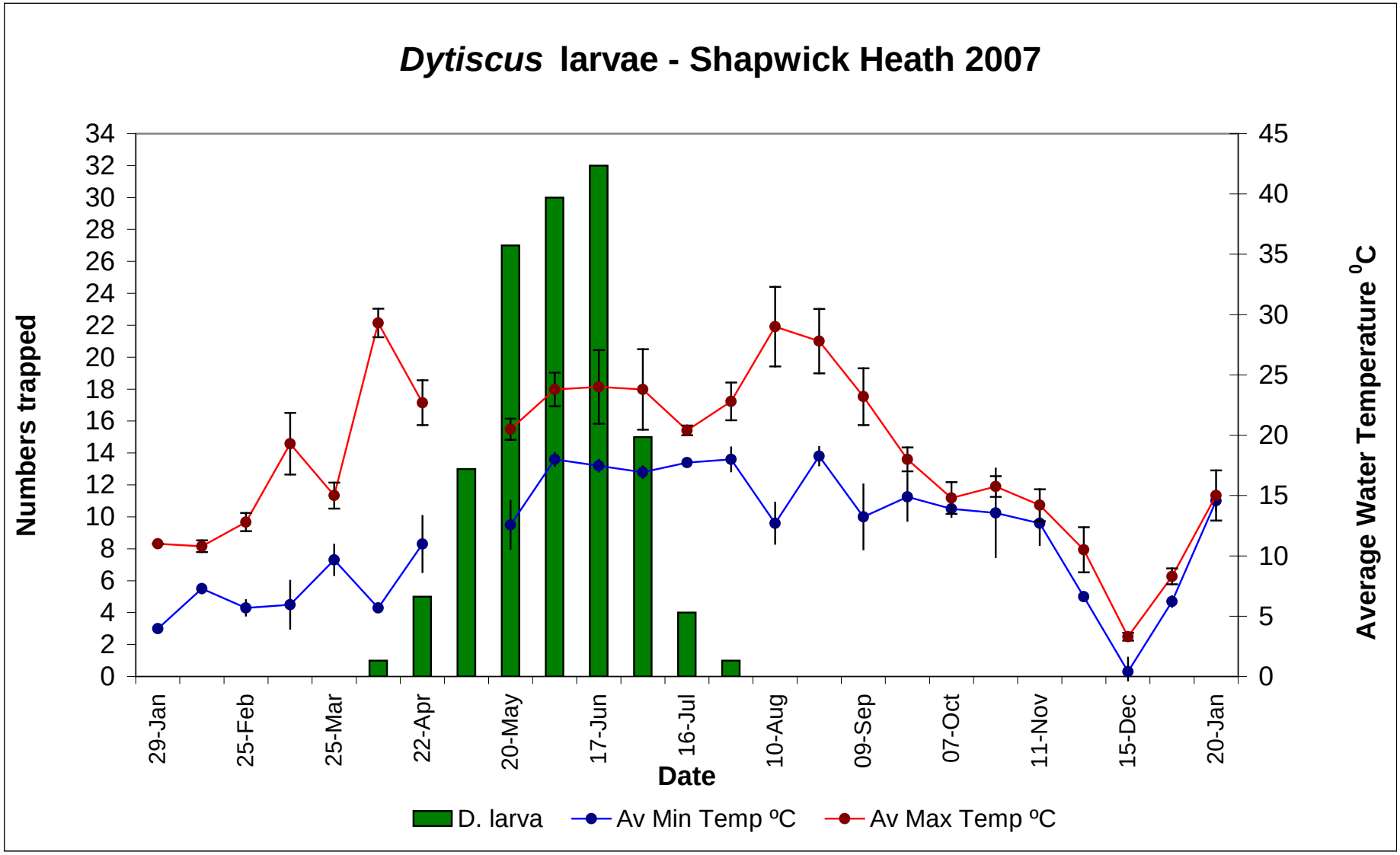


Figure 5.4: Seasonal activity pattern of *Dytiscus* larvae. Shapwick Heath 2007

5.2.2.1 Adults

A total of 230 *D. marginalis* beetles were trapped during the year at Shapwick Heath comprising 143 males and 87 females. 145 *D. dimidiatus* adults were caught over the same period (91 males, 54 females). The ratio of males to females caught was very similar in both species (i.e. about 1:0.6). The peak numbers of adults were caught on 20 May in both species. A second peak in numbers caught was observed in both species on 22 September.

5.2.2.2 Larvae

128 larvae were caught at Shapwick Heath between 29 January 2007 and 20 January 2008. The pattern of catches shown in figure 5.4 indicated that *Dytiscus* species were univoltine at Shapwick Heath with a single peak of activity between 20 May and 17 June indicating a single generation. It was mainly late instar (L_2 and L_3) larvae that were attracted to the baited traps (see Chapter 4). Just seven of the larvae caught were identified as *D. dimidiatus* from DNA analysis (Table 4.6). The only L_1 larva positively identified as *D. dimidiatus* was collected on 5 May 2007. All other L_1 larvae (9 in total) that were collected over the course of fieldwork between 2006 and 2008 were trapped or netted later in the season. Five of these were positively identified as *D. marginalis*. Three of the remaining *D. dimidiatus* larvae (i.e. half the L_2/L_3 larvae positively identified as being of that species) were caught on 20 May which, together with the above, might suggest that the larvae of this species develop earlier than those *D. marginalis.* There are too few records of *D. dimidiatus* in reality, however, to make definitive statements on the subject as is explained in section 5.2.3.2 below.

5.2.3 Inter-specific and intra-specific comparisons

The graphs of adult catch frequencies (Figures 5.2 and 5.3) suggested a bimodal distribution of results with two distinct peaks (one at 20 May 2007 and one at 22 September 2007). Therefore, I analysed data from these two periods

separately (a total of nine data points taken from eight weeks either side of each peak).

5.2.3.1 Hypothesis 1: Adults of *D. marginalis* and *D. dimidiatus* differed in their pattern of annual activity

The ratio of adult *D.marginalis* to adult *D. dimidiatus* captures during 2007/8 was 230:145 (or 1:0.63). If this ratio was maintained evenly throughout the year then the number of *D.dimidiatus* adults expected at any trapping event would be the number of *D. marginalis* adults multiplied by 0.63. Using this means to obtain expected frequencies, χ^2 =: 9.05 (d of f = 6, P = 0.17) for early 2007 and χ^2 = 0 76 (d of f = 3, P = 0.86) for later in the year. In both cases the null hypothesis (no difference between the species) could not be rejected.

5.2.3.2 Hypothesis 2: Larvae of *D. marginalis* and *D. dimidiatus* differed in their pattern of annual activity

Only seven (5.5%) out of 128 larvae caught during 2007/8 were identified positively as *D. dimidiatus* using the molecular ecological techniques reported in Chapter 4. Eleven larvae (8.6%) caught over the period could not be identified by analysis of DNA. On the basis that 7 out of 117 larvae that could be identified were *D. dimidiatus*, then the proportion of *D.marginalis* larvae to *D. dimidiatus* larvae was 1:0.06 and since the maximum number of larvae caught at one visit was 32, no expected value for the number of *D. dimidiatus* larvae would exceed 5. For this reason the hypothesis could not be tested.

5.2.3.3 Hypothesis 3: There was a difference in activity patterns between males and females of adult *D. marginalis*

The ratio of males:females of *D.marginalis* caught over the period was 1:0.61. If this ratio was maintained throughout the year then the number of males expected to be caught on a particular

trapping expedition would be equivalent to the number of females multiplied by 1.6. Using this method to calculate expected frequencies, χ^2 = 21.2 (d of f = 4, P = 0.0003) for early 2007 and χ^2 = 12.3 (d of f = 3, P = 0.006) for later in the year. The null hypothesis of no difference was rejected. This indicated that the males tended to be more active than the females throughout the year, with female captures being more focussed on the peaks in the activity cycle.

5.2.3.4 Hypothesis 4: There was a difference in activity patterns between males and females of adult *D. dimidiatus*

Using the same method to calculate expected frequencies of males from observed female numbers as described in the preceding section: χ^2 = 3.2 (d of f = 2, P = 0.21) for early 2007 and 1 χ^2 = 0.1 (d of f = 2, P = 0.006) for later in the year. From this it was concluded that there was no statistically significant difference in the activity of males and females early in the year but that in the second half of the year a difference could be discerned in terms of a relative increase in the number of males caught compared with females.

5.2.4 Influence of temperature on beetle activity/abundance

Average minimum water temperatures measured did not deviate significantly from a normal distribution according to a Shapiro-Wilk Test (W = 0.9, n = 24, p = 0.08) and nor did the value for average maximum temperatures (W = 1.0, n = 24, P = 0.7). The two variables were strongly, positively correlated (Pearson Correlation Coefficient r = 0.6, t = 3.7, D of F = 22, P < 0.01).

In order to investigate the effect of temperature on beetle activity, I correlated numbers of beetles trapped and water temperature variables. Given that the data for beetle activity were non-continuous, contained many zeroes and it could not be assumed that they fitted a normal distribution, all correlations between temperature and beetle numbers were conducted using Spearman's

Rank Correlation. In each case the null hypothesis was that there was no correlation between temperature and beetle activity.

Since the Chi-squared tests reported above indicated a difference in activity patterns between males and females of both species, I conducted separate correlations for the two sexes rather than pooling all records together. Because there was an apparent bimodality to the numbers of adults trapped (see Figures 5.2 and 5.3), as well as examining the correlation between variables over the whole year, I conducted separate Spearman Rank Correlations for the frequencies of trapped beetles on the two peak dates and eight weeks before and after the peaks (nine data points). The correlations are summarised in Table 5.2 below. Only the results from correlations with average maximum water temperature are given because these were the strongest and also it had been established that maximum and minimum temperatures were strongly positively correlated themselves (see above).

For larvae and for adult beetles over the whole year, irrespective of sex and species, apparently significant coefficients were obtained in all instances involving correlation with average maximum water temperatures. As can be seen from Table 5.2, the correlations were all positive ones, suggesting a linear relationship between activity or abundance of adult beetles and maximum water temperature.

Table 5.2: Spearman Rank Correlations between average maximum water temperatures and numbers of beetles trapped at Shapwick Heath NNR January 2007 to January 2008.
* P ≤ 0.05 **P ≤ 0.01 ***P ≤ 0.001

Correlation	All data (n = 24)	± 8 weeks May Peak (n = 9)	± 8 weeks Sept Peak (n = 9)
Male *D.marginalis* with Av. Maximum Temperature	0.63**	0.59	0.66
Female *D.marginalis* with Av. Maximum Temperature	0.77***	0.64	0.73*
Male *D.dimidiatus* with Av. Maximum Temperature	0.55**	0.51	0.25
Female *D.dimidiatus* with Av. Maximum Temperature	0.60**	0.45	0.58
Larvae with Av. Maximum Temperature	0.57**	N/A	N/A

Since multiple comparisons from the same dataset were attempted, a Sequential Bonferroni Correction was applied [Dunn (1961)]. The technique is

explained in section 2.7.2. As can be seen from Table 5.3, this resulted in some null hypotheses being accepted where before they had been rejected.

Table 5.3: Summary of results of Sequential Bonferroni Correction applied to apparently significant Spearman Rank Correlations from Table 5.2. (15 comparisons, P = Probability of Null Hypothesis. The P Value threshold was calculated sequentially according to the formula α/15, α/14, α/13….etc., where α is the 95% confidence interval = 0.05)

Correlation with Average Maximum Water Temperature	r_s Value	P	P Value threshold
Null hypothesis rejected (Significant correlation)			
Female *D.marginalis* [All data (n =24)]	0.77***	9.3×10^{-6}	0.0033
Male *D.marginalis* [All data (n =24)]	0.63**	0.0010	0.0036
Female *D.dimidiatus* [All data (n =24)]	0.60**	0.0018	0.0038
Larvae [All data (n =24)]	0.57**	0.0033	0.0042
Null hypothesis accepted (No significant correlation)			
Male *D.dimidiatus* [All data (n =24)]	0.55**	0.0050	0.0046
Female *D.dimidiatus* [± 8 weeks Sept Peak (n = 9)]	0.74*	<0.05 >0.01	0.0050

With the Bonferroni Correction there were significant positive correlations between average maximum water temperature and adult and larval catches except in the case of male *D. dimidiatus*. A significant correlation that had been noted before – that of female *D. dimidiatus* with average maximum water temperature recorded in the eight weeks before and after the September peak – was no longer recognised. The Bonferroni Correction is supposed to ensure that 'false positive' results are screened out from multiple comparisons (i.e. apparently significant results that are likely to have arisen by chance are removed). The Correction has been criticised, however, for producing overly conservative interpretations of datasets and for potentially introducing errors through the rejection of real significant outcomes [e.g. Perneger (1998), Moran (2003)].

Whether the Bonferroni Correction is accepted or not it is clear that there was a strong positive correlation over the year in most instances between beetle activity and average maximum water temperature. Such a relationship was not in much evidence, however, at the times of the two peaks in numbers which implies that beetles are not merely most active when temperatures are high, but that the peaks correspond with events in the annual lifecycle of the species.

5.2.5 Influence of temperature and other factors on larval trap mortality

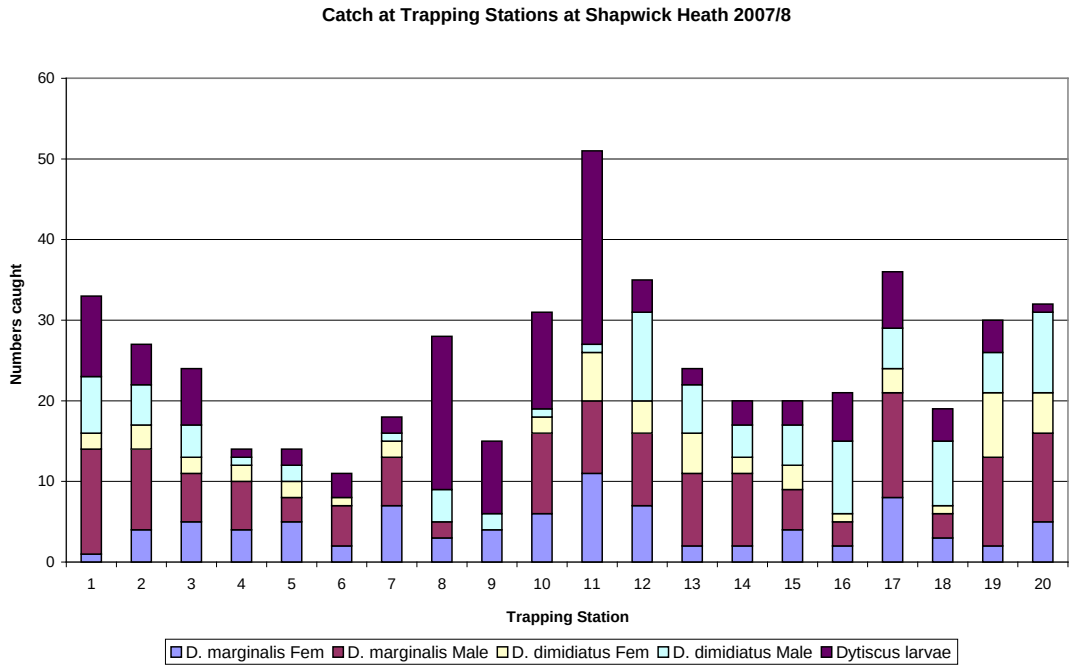
During fieldwork in 2007/8 a record was kept of whether the larvae that were trapped were alive or dead when the trap was collected. Up to 80% of the larval catch in any one 24 hour period could consist of dead specimens. Spearman Rank Correlation was conducted to investigate whether any correlation could be discerned between the proportion of the catch that was found dead and average water temperatures. There was no significant correlation found between proportion of the total larval catch that was dead and average maximum or minimum temperatures. In the case of average maximum water temperature r_s = -0.13 (df = 8, P = > 0.05), for average maximum temperatures r_s = -0.57 (df = 8, P = > 0.05). These results imply that there was no linear relationship between water temperature and larval mortality in traps.

As a general observation, it appeared that relatively large numbers of dead larvae occurred in traps with comparatively high overall numbers of trapped larvae and/or adult *Dytiscus* beetles. To investigate whether there was a relationship between overall numbers of *Dytiscus* specimens and trap mortality of larvae I examined the data for the three trapping events with the highest larval mortalities (22 April 2007 – 80% mortality, 20 May 2007 – 70% mortality and 3 June 2007 – 67% mortality). Pooling the data from these three separate trapping events, I ranked the traps in which at least one larva was caught according to the size of overall catch (adult *Dytiscus* and larvae). The traps that were positive for *Dytiscus* larvae were ranked also in ascending order of percentage mortality of larvae. This generated a positive Spearman's Rank Correlation (r_s = 0.86, n = 23, P = 4.27 x 10^{-7}) a significant result suggesting that mortality was density dependent and might be linked to aggression within the traps.

5.2.6 Comparison of trap efficiency at different trapping stations

Figure 5.5 shows the catch at each trapping station over the period January 2007 to January 2008.

Figure 5.5: Graph of *Dytiscus* specimens caught at trapping Stations



It is clear from Figure 5.5 that the numbers of beetles captured was not evenly distributed among traps.

Assuming an expected frequency of capture over the year at a particular trap site as the annual average across all traps for each category then for *D. marginalis* the expected capture rate per trap was 11.5 adults per year and for *D. dimidiatus* adults it was 7.25. Chi-squared Tests performed indicated that the actual observed results were significantly different from the expected for *D. marginalis* $\chi^2 = 39.4$ (d of f = 19, P = 0.004) and for *D. dimidiatus* $\chi^2 = 45.2$ (d of f = 19, P = 0.001).

For female adults of both species and for adult male *D. dimidiatus,* the total annual catch was such that averages per trap were below 5 and, therefore, Chi-squared tests could not be conducted. Sufficient male *D.marginalis* and *Dytiscus* larvae were caught to enable Chi-squared tests to be performed. In the case of male *D. marginalis* $\chi^2 = 37.8$ (d of f = 19, P = 0.006) and for larvae $\chi^2 = 107.9$ (d of f = 19, P < 0.001).

These results demonstrated that some traps were better than others at catching beetles. However, Figure 5.2 does not indicate that those traps

that were good at catching larvae, for example, were equally good at catching adults and at this early stage in the analysis of the results it is difficult to identify anything possibly 'special' about 'good' or 'bad' traps. However, since an effort was made to standardise the traps, it was assumed that differences in trap success were connected to the trap location in terms of the environment at each location.

5.2.7 Environmental factors and trap success

Measurements of the environmental parameters around trap stations described in sections 5.1.3 and 5.1.4 are tabulated in Appendix E3 and the results are summarised below in Table 5.4.

Table 5.4: Summary of environmental parameters at 20 trapping stations at Shapwick NNR. Observations were made on three occasions during 2007.

	Average	St Dev	Range
Abiotic			
Width of waterbody (m)	1.8	0.3	1.2 – 2.3
Depth of water (cm)	48.5	8.7	37.5 – 62.5
Gradient of nearside bank	2.5	0.9	Combined Scores 3 - 8
Gradient of far-side bank	2.5	1.1	
Degree of poaching	0.6	0.5	0.0 – 1.0
Biotic			
Estimated % shade by trees and shrubs	50.5	30.9	0.0 – 83.3
Estimated % Glyceria cover	9.0	14.7	0.0 – 33.3
Estimated % Duckweed cover	95.0	10.7	56.7 – 100.0

I used Canonical Correspondence Analysis (CCA) to investigate the relationships between catch numbers and environmental parameters around the traps, an appropriate method used when possible explanatory variables are to be included in the ordination alongside the dependent variables (Henderson & Seaby 2008). One advantage of CCA over most ordination methods is that it allows hypothesis testing so that not only can one tell the proportion of the variability that is explained by the independent (i.e. environmental) variables but also whether or not this is statistically significant. In order to test for significance a Monte Carlo randomisation was run as described in Chapter 2 (section 2.7.2) where CCA and other multivariate statistical techniques used in this investigation are explained in more detail.

The dependent variables used in the CCA were the numbers of *Dytiscus* caught in each trap over the year in the following categories: *D.marginalis* adult female; *D.marginalis* adult male; *D.dimidiatus* adult female; *D.dimidiatus* adult male; *Dytiscus* larvae. The figures were broken down into sexes partly to account for any differential behaviour that might have occurred and also in order to create sufficient categories of dependent variable against which could be correlated a reasonable number of independent ones. Since the number of independent variables to be tested must be less than the number of dependent variables two separate CCAs were conducted. The first CCA examined the relationship between the *Dytiscus* caught and the abiotic physical characteristics of the trapping stations. The second CCA looked at catches in relation to biotic factors.

Before CCAs were conducted the independent variables were correlated against each other to investigate the degree to which they were independent. The results are displayed in Table 5.3 below. Seaby and Henderson (2008) have warned that: "*If any combination of the environmental variables is highly correlated, the results obtained by a multiple regression method such as CCA can be unreliable*". Other authors (e.g. Palmer 2012) have pointed out that so long as the correlation is less than perfect (i.e. the correlation coefficient is neither exactly 1.0 nor -1.0) there will be some variation in each variable which is not redundant with the other. Palmer (*Op. cit*.) argued that: "*The existence of intercorrelated variables is not an obstacle for CCA, but it may be an obstacle for interpretation.*"

5.5: Values of Spearman Rank Correlation Coefficient (r_s) for correlation of biotic and abiotic data collected from 20 trapping stations at Shapwick Heath in 2007			
ABIOTIC	Summed Gradient	Depth of Water	Width of waterbody
Summed Gradient	-	-0.49	-0.51
Depth of Water	-0.49	-	0.53
Width of waterbody	-0.51	0.53	-
BIOTIC	% Tree cover	% Glyceria cover	% Duckweed cover
% Tree cover	-	-0.17	-0.21
% Glyceria cover	-0.17	-	-0.22
% Duckweed cover	-0.21	-0.22	-

The correlations reported in Table 5.5 were statistically significant (n=20, D of F = 18, P = < 0.025) but all are moderate to weak in strength.

5.2.7.1. CCA with Physical characteristics as independent variables

The physical (abiotic) characteristics used in the CCA were width of waterbody, depth of water, gradient and degree of poaching. In order to reduce the number of independent variables in the analysis, I combined the 'nearside' and 'far-side' gradient scores to produce one 'summed gradient score' which was a measure (on a scale 2 – 8, shallow to steep) of the steepness of the banks on both sides of the trapping station. In the first analysis I omitted poaching so that there were only three independent variables. In order to ensure that the values of the independent variables were of roughly the same magnitude the depth of the waterbody in cm was divided by ten.

The percentage variance explained by the first canonical axis was relatively low (16.6%) and the first two axes together explained only 22.5% of variance, suggesting no close linear relationship existed between the dependent variables and the particular physical parameters chosen. The Monte Carlo Test (1000 replicates) implied that at least as much variability could be

explained by arranging the independent variables randomly with regards to the dependent ones. P values were obtained as follows for each axis: 1st axis P = 0.21; 2nd axis P = 0.11; 3rd axis P = 0.47. In all three cases the null hypothesis of no relationship could not be rejected.

In a variant of the CCA described above, I substituted average degree of poaching for combined gradient. However, the results from this second CCA were similar to the above. The amount of variation explainable by the first canonical axis rose slightly to 16.9% but the Monte Carlo test (100 replicates) indicated that the probability of any of the three main canonical axes explaining more than could be generated by random chance was not significant ($P \leq 0.20$).

5.2.7.2. CCA with estimates of vegetation coverage as independent variables

The three biotic measures listed in Table 5.2 were used as the basis of the independent variables used in the CCA; estimated percentage shade by trees and shrubs; estimated percentage *Glyceria* cover and estimated percentage cover of Duckweed. The figures were re-scaled by dividing by ten so that, for the most part they were comparable in magnitude to each other and to the dependent variables.

The CCA Plots obtained are reproduced in Figures 5.6a and 5.6b. The plots illustrated are from the same ordination with the results presented to emphasise different features. In Figure 5.6a the plot has been scaled with the species at site centroids which emphasises the differences between samples (trapping locations). The same ordination is shown in Figure 5.6b, but in this case the plot has been scaled with the sites at species centroids which emphasises the differences between species

In both plots, the first canonical axis explained 33.8 % of the dependent variability. From Figures 5.6a and b it can be seen that the first axis was closely aligned to % Duckweed cover. In fact the Biplot score for this measure was 0.988 indicating that the axis is close to comprising almost entirely this one measure. The Monte Carlo test with 1000 replicates indicated that this first axis explains more of the data variability than would be expected by chance (P = 0.005, therefore the null hypothesis of no relationship could be rejected.)

The other two canonical axes explained relatively little more of the variability (just over 5% cumulatively) and in both cases P was greater than 0.30 meaning that the null hypothesis cannot be rejected. Eigenvalues, variances, sample scores, species scores and other figures relating to this CCA are contained in Appendix E4.

Figure 5.6c is a ranked biplot produced by the CCA. It shows how the dependent variables (i.e. the species categories) were ordered in relation to the independent variable 'percentage Duckweed cover'. The direction of the arrow on the vector line indicates increasing magnitude of the environmental vector (i.e. increasing percentage cover of Duckweed). The positions of the adult beetles projected onto the vector were relatively closely clustered around the middle of the vector line indicating that they are weakly influenced by the environmental variable. The dependent variables '*Dytiscus* larvae' and, particularly, '*D. dimidiatus* larvae' were located towards the far end of the vector indicating a strong negative correlation with % Duckweed cover.

Figure 5.6a Canonical Correspondence Analysis (CCA) Triplot of data from 20 trapping stations at Shapwick Heath collected during 2007/8. The arrows represent the environmental variables (Duckweed cover, *Glyceria* cover Shade by trees and shrubs), the blue squares represent the samples (i.e. the measurements made at the 20 trapping locations) and the red triangles represent the species (i.e. the six categories of *Dytiscus* – *D. marginalis* male and female, *D. dimidiatus* male and female, *Dytiscus* larvae and *D. dimidiatus* larvae). The plot has been scaled with the species at site centroids which emphasises the differences between samples (trapping locations).

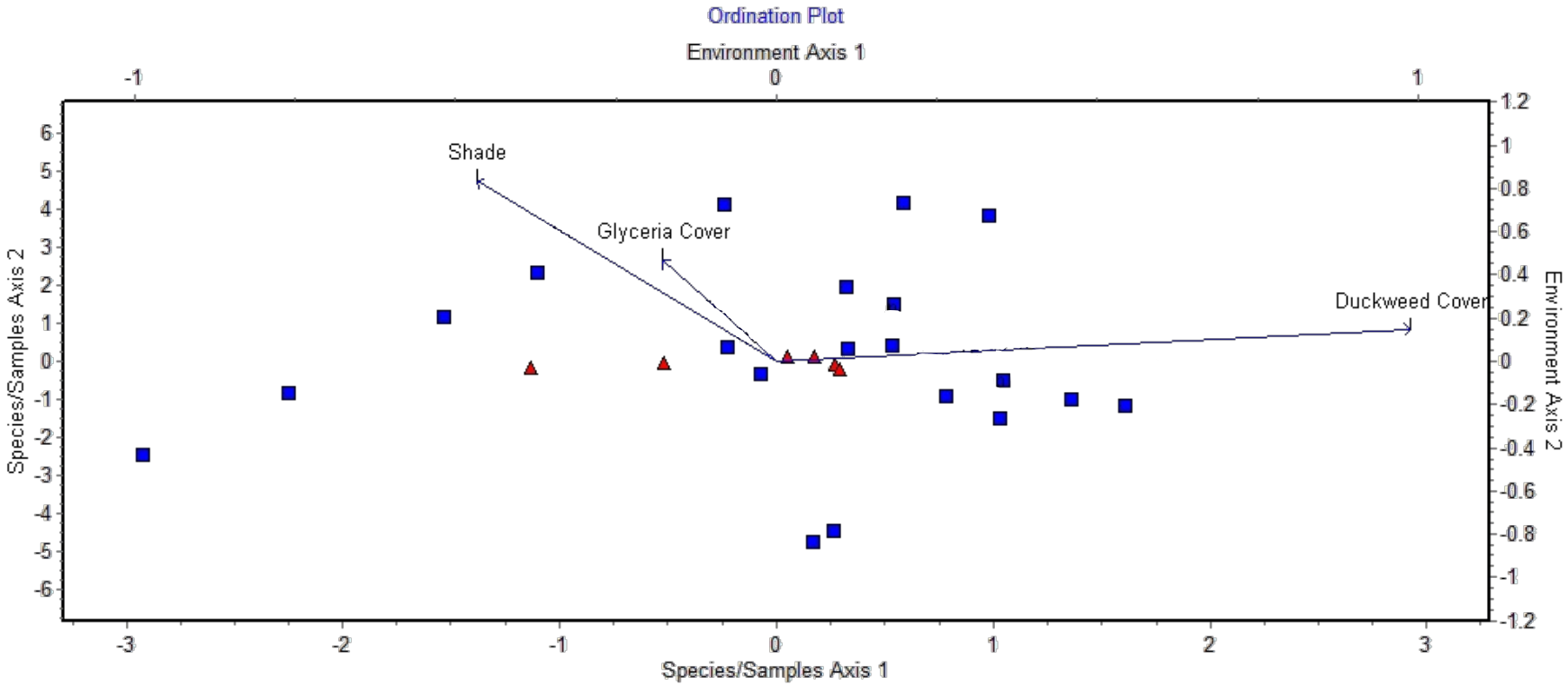
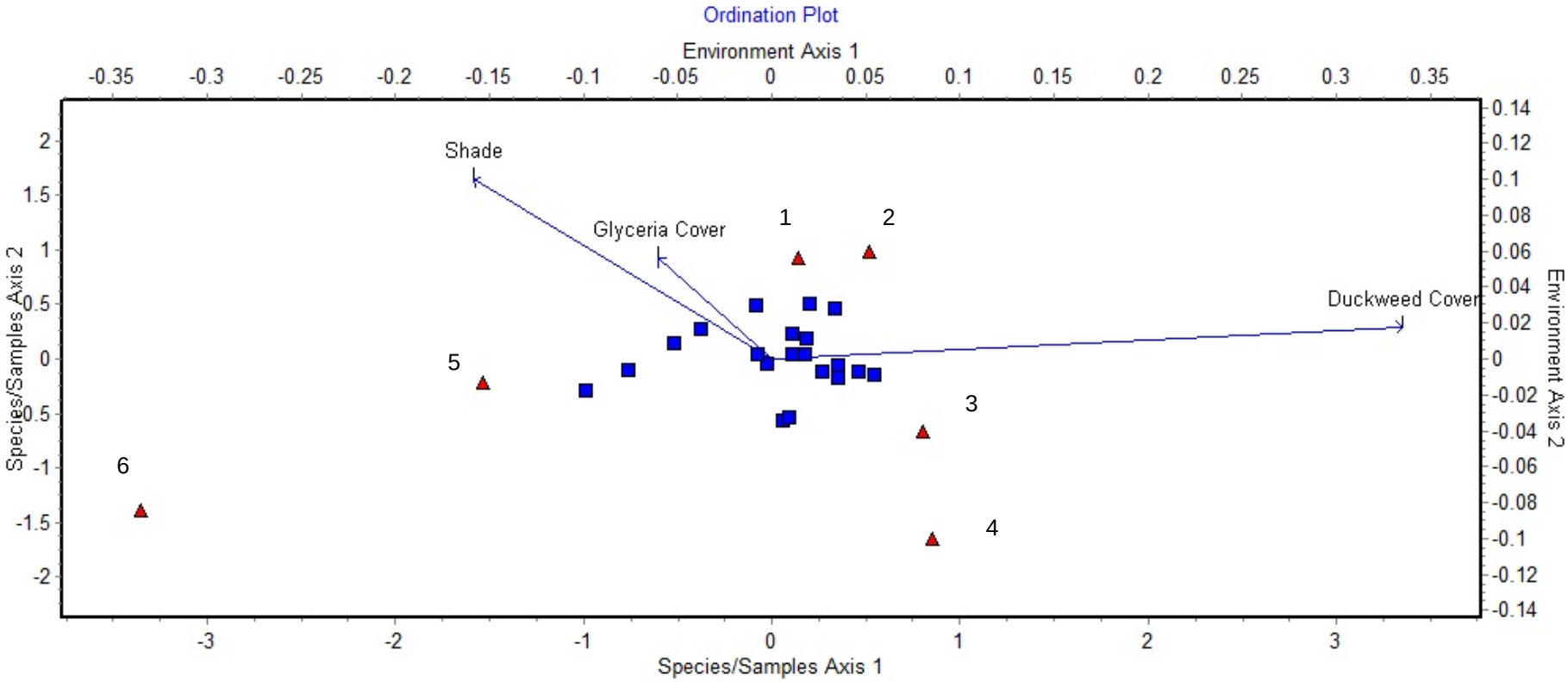


Figure 5.6b Canonical Correspondence Analysis (CCA) Triplot of data from 20 trapping stations at Shapwick Heath collected during 2007/8. This is the same ordination as shown in Figure 5.6a but in this case the plot has been scaled with the sites at species centroids which emphasises the differences between species. (1 = *D. marginalis* female, 2 = *D. marginalis* male, 3 = *D. dimidiatus* female, 4 = *D. dimidiatus* male, 5 = *Dytiscus* larvae (not *D. dimidiatus*) and 6 = *D. dimidiatus larvae.)*



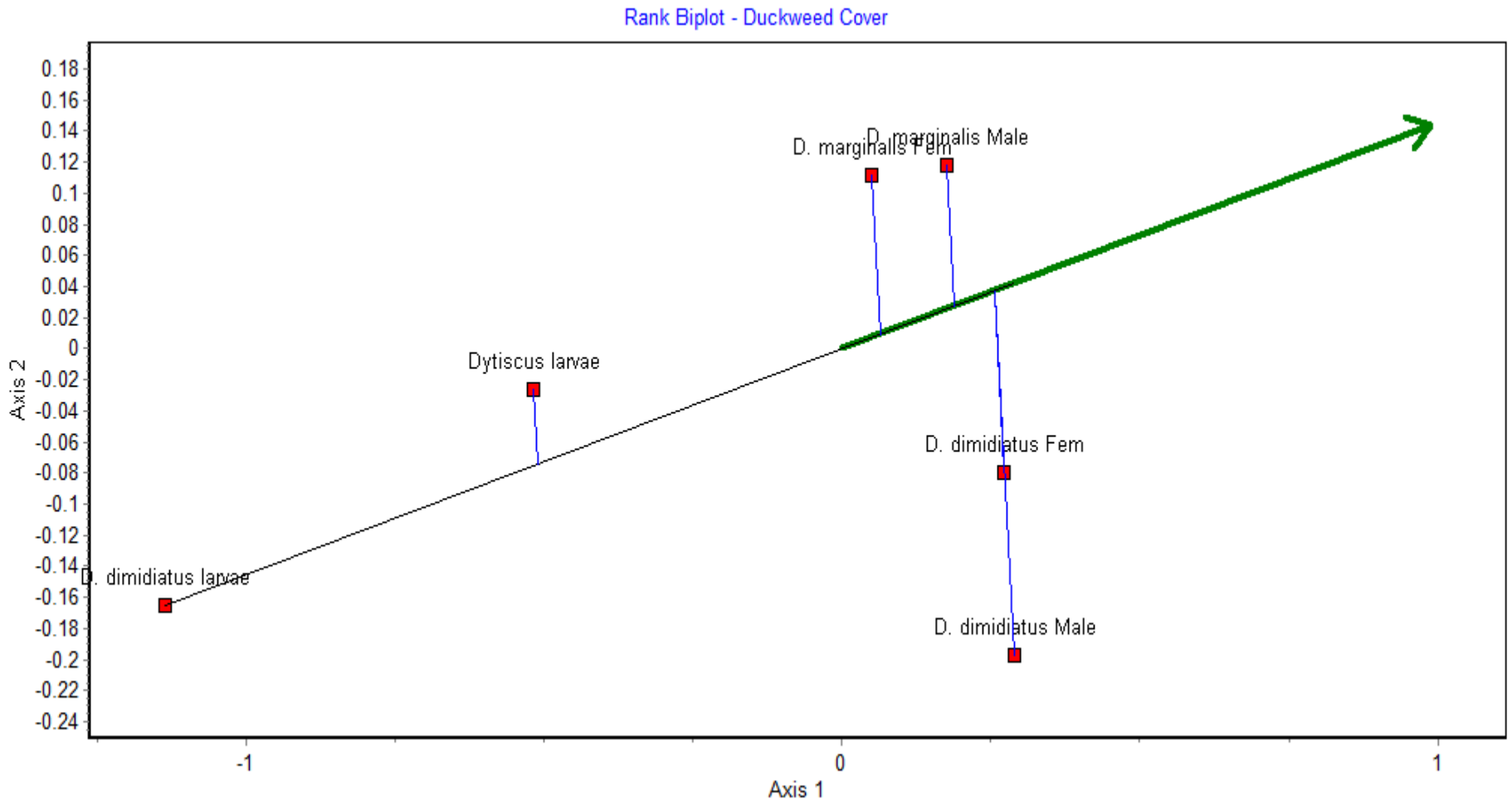


Figure 5.6c: Ranked biplots of *Dytiscus* spp. along the % Duckweed Cover vector

5.3 Discussion

5.3.1 Seasonal activity patterns

In terms of adult beetles no statistically different patterns of annual activity were found between *D. dimidiatus* and *D. marginalis,* suggesting that niches occupied by the adults of these species are not differentiated temporally to any extent resolvable by fortnightly observations. There were insufficient specimens of *D. dimidiatus* larvae distinguished to enable a statistical comparison concerning possible temporal niche separation with larvae positively identified as *D. marginalis* but the limited data did not suggest substantive temporal differentiation at this life stage either.

Two peaks of adult activity (as gauged by numbers trapped) were observed, one in May and the other in September. Larval activity peaked in June. These observations are consistent with both *D. dimidiatus* and *D. marginalis* being univoltine at Shapwick Heath. More males than females of both species were caught and the pattern of activity was different between the sexes. In *D. marginalis* male numbers were consistently high compared with females, the numbers of which fluctuated more between peak activity periods. The difference in male:female numbers trapped was not so great in *D. dimidiatus* in the early part of the year but widened in the eighteen week period during which the September peak activity took place.

The patterns of activity observed for *D. marginalis* at Shapwick Heath in 2007/8 matched those described in standard works on British water beetles [e.g. Foster & Friday (2011)] – adult activity through more or less the whole year but with two peak periods – one in May and another in September. According to Foster and Friday (2011), the second seasonal peak of activity in *D. dimidiatus* is generally during August, although a later peak than this was observed at Shapwick. The precise timing of peaks may vary a bit between years according to weather conditions [Foster & Friday (2011)].

Landin (1976) described two patterns of seasonal abundance that he observed in water beetle communities inhabiting the shoreline of a lake near Stockholm,

Sweden: ‘Pattern (a)’ Maximal abundance during spring, minimal during summer, with sometimes a new, smaller maximum during late summer and autumn and ‘Pattern (b)’ An abrupt peak in abundance during July-August and very low abundance at other times. The activity patterns observed during 2007/8 of the adults of both *D. dimidiatus* and *D. marginalis* seemed to match ‘Pattern (a)’ more closely than they did Pattern (b). Landin (1976) thought Pattern (a) was probably the general one for water beetles in northern temperate latitudes that have a univoltine life cycle, where the beetles breed during early summer and overwinter as adults. He argued that Pattern (a) was typical of species adapted to a closed, relatively stable habitat, whereas Pattern (b) was a response to ephemeral habitats such as temporary pools. Other European workers have described activity patterns which are similar to Pattern (a) in water beetle communities [Brancucci (1980), Dettner (1976), Meyer & Dettner (1981)]. Landin’s studies were largely of hydrophilid species but Aiken and Wilkinson (1985) reported a similar pattern of abundance to Landin’s ‘Pattern (a)’ in *Dytiscus alaskanus* caught in bottle traps in a lake habitat in central Alberta, Canada. They interpreted the fall off in numbers after the first peak as being due in part to a dying off of some of the overwintered beetles but also to a general reduction in activity over the latter half of the summer. Aiken and Wilkinson acknowledged that another possible explanation of their findings was dispersal of the adult population to temporary ponds away from the lake, citing work by Popham (1952) and James (1970) in support of this possibility. Dispersal could be an explanation for the observed drop in adult beetle numbers trapped towards the end of the summer at Shapwick but it is unclear where the beetles would go since the water levels in surrounding ditch systems all tend to drop together over the course of the summer months.

With regards to the late summer peak in adult activity observed in *Dytiscus* species in the UK, this is usually put down to the emergence of fresh adult beetles which have pupated that year [e.g. Sutton (2008)]. The earlier peak in May is less easy to explain. It does not correspond with the main mating period when one might expect the overwintered adults to be maximally active. Most accounts of the life histories of UK *Dytiscus* species (e.g. Sutton 2008) agree

that eggs are laid in March following mating around this time or earlier. Given that *Dytiscus* eggs take three weeks or more to hatch in temperate climes [Inoda *et al.* (2007), Blunck (1912)] and that peak numbers of late instar larvae were observed at Shapwick Heath in June, this suggests that the main mating period was much earlier than May. Mating in the previous autumn followed by egg-laying in early spring of the next year has been reported in some species of *Dytiscus* such as *D. lapponicus* [Balfour-Browne (1925)], *D. alaskanus* [Aiken & Wilkinson (1985)] and *D. sharpi* [Inoda *et al.* (2007)]. These species occur in parts of the world with relatively short summers and this behaviour is probably an adaptation to give larvae the longest possible period of favourable weather over which to develop. There is no evidence to suggest that either *D. marginalis* or *D. dimidiatus* mated during the latter part of the year at Shapwick and, indeed the few instances where adults were observed mating in traps this was in early spring. If the first (May) peak in activity is not connected with mating then perhaps it is correlated more with warm weather and with the exploitation of prey resources. This latter idea is explored in more depth in Chapter 7 on predator-prey relationships.

5.3.2 Differences in male and female activity levels

Aiken and Wilkinson (1985) found that the sex ratio of *D. alaskanus* bottle trapped at their Canadian study site was highly biased towards males but that the sex ratio of beetles raised in the laboratory from eggs collected in the wild was nearly 1:1. They concluded that the male-biased ratios observed in the traps was a reflection of greater male activity compared with females. As evidence of this they pointed to the fact that in their study the sex ratio was most male-biased at the times of year when they would have expected females to have been egg-laying and at their least active in terms of searching for food. At Shapwick Heath during 2007/8 I too observed a bias of males over females in the numbers of *D.dimidiatus* and *D. marginalis* caught in baited traps. The extremes in male:female ratios were not as great as those reported in the Canadian study where ratios of nearly 81:1 male:female were observed at some periods in the year. However, such ratios may be a function of the overall greater numbers of adults trapped by Aiken and Wilkinson due possibly to the

nature of the habitat being sampled and the use of larger-sized bottle traps deployed over a longer period than in this study.

It seems plausible that the bias in *Dytiscus* males over females observed at Shapwick was due to the males being more active than females. Using microsatellite genetic markers Lagisz *et al.* (2010) demonstrated male-biased dispersal patterns in the ground beetle *Pterostichus oblongopunctatus*. The authors postulated that this was due to the males travelling further due to their active searching for females during the reproductive period in early spring. At least some of the difference between males and females observed in *Dytiscus* beetles might be attributable to males being more mobile in search of mates but the bias in male numbers was observed throughout the year, so this cannot be the complete explanation. The presence of females in the trap may be a positive attractant to males as well as or instead of the food bait. It has been suggested that the heavy bias of male beetles caught in activity traps is due to this mechanism, although experiments comparing pitfall traps run with or without female Darkling Beetles (*Eleodes obsolete*) found no differences in the numbers of males caught [Mcintyre (1998)]. As above, one might expect the sex-bias in the *Dytiscus* to last only through the mating season if it were due to strong attraction of males to traps with females. Over-representation of males in trap captures may indicate a general limitation of traps in assessing population size due to bias by variable behaviour.

The possibility has been suggested that differences in activity levels as postulated between males and females of the same species might account for the different ratios of adult *D. marginalis* to adult *D. dimidiatus* caught as reported above (Alan Stewart *pers. comm*.). This idea is explored more fully in the final chapter (in 8.4.1) where ratios of *D. marginalis: D.dimidiatus* caught at Shapwick Heath in 2007 are compared with those from 2008.

5.3.3 Temperature and activity

Positive correlations between activity and average maximum water temperature were demonstrated for adults of both sexes and both species. For adult

females, there was also a positive correlation with average minimum water temperature. Larval activity was positively correlated with average minimum temperature, but no such linear relationship was demonstrated with regards to average maximum water temperature. Any possible difference between larval *D. dimidiatus* and *D. marginalis* in this regard could not be discerned due to the low numbers of *D. dimidiatus* identified. Larval mortality in traps could not be linked in a direct linear fashion to average water temperatures.

5.3.4 Environmental influences on distribution of adults and larvae in ditches

Of all the various environmental parameters that were investigated the only one for which evidence was found of a correlation with *Dytiscus* numbers was percentage duckweed cover. Specifically, there seemed to be a strong negative correlation of larval numbers with average duckweed cover. Since most larvae trapped were late instar larvae and as by the end of the year most of the study ditches were 100% covered in duckweed this correlation could be connected to an effect of duckweed cover on early instar distribution that, in turn, influenced where later instars were concentrated. It has been noted that duckweed blankets hinder oxygen diffusion into water [e.g. Culley & Epps (1973), Morris & Barker (1977)] and also shade out submerged oxygen-generating plants [e.g. Scheffer *et al.* (2003)]. A heavy layer of duckweed would also be something of a hindrance to air-breathing invertebrates that have periodically to break the water surface to replenish oxygen supplies. Adult beetles and large late instar larvae may be able to penetrate the duckweed layer. Smaller early instar *Dytiscus* larvae may perhaps be less able to do this and therefore tend to become concentrated in those parts of ditches that are free from duckweed for at least the early part of the year.

Breeding sites for the Lesser Silver Water Beetle (*Hydrochara caraboides*) in Somerset tend to lack heavy duckweed cover [Boyce (2006) in Hill-Cottingham *et al.* (2006)]. The same author suggested that *D. dimidiatus* adults are often found at *H. caraboides* breeding sites indicating there is some similarity in habitat preferences between the species [Boyce (2004)]. It may be that in both

cases duckweed cover is influencing choice of breeding site. The possibility, however, should not be ignored that another factor such as, for example, extent of tree cover is influencing both duckweed cover and beetle abundance. In the next chapter the effect of tree cover is examined in greater detail.

Chapter 6: The influence of shade and woodland cover on *Dytiscus* spp.

Introduction

This chapter reports on the fieldwork conducted during 2008 to investigate whether adults or larvae of *D. dimidiatus* or *D. marginalis* show any differential affinity for shaded watercourses of for areas of greater tree cover. This was done primarily in order to ascertain whether there is any evidence to support the hypothesis that *D. dimidiatus* prefers ditches "*with a degree of shade*" [Beebee (1991)]. Other observations were made of physical and chemical characteristics of ditches at study sites to see if any correlations could be found between these and the abundance of target species.

6.1 Methods

6.1.1 Trapping protocol

Fieldwork was carried out at the six study sites listed and described in Chapter 2 (section 2.1). At each site three lengths of linear waterbody were chosen with five trapping stations per section (i.e. a total of 15 trapping stations per site). At Shapwick Heath a majority of the 20 trapping stations used in 2007 were retained, but trapping was discontinued at some (including all the ones located in open habitats) and some new stations at that site were added so that all the 2008 stations were on watercourses shaded heavily by trees and shrubs. Examples of watercourses were selected at Westhay Heath and Westhay Moor that also had heavy tree or shrub cover on at least one bank overshading the watercourse. To provide contrasting sites I chose ditches at Catcott North, East Waste and Tadham Moor that were more or less devoid of tree cover. All the watercourses were in locations where public access was discouraged and where ditches were not thought to be scheduled for cleaning out during 2008.

A potential weakness of the arrangement described above was that differences in beetle abundance/activity might be ascribed to the effect of site rather than to degree of shading *per se*. To overcome this criticism, examples of 'shaded' and 'unshaded' ditches could have been sampled at each site. However, this would have doubled the amount of data that would have needed to have been

collected (which would have been difficult to have accommodated in the time I had available). In addition, some of the sites chosen to represent unshaded habitat lacked any truly shaded ditches at all (e.g. East and West Wastes, Tadham Moor) and no examples of unshaded sites were available at Westhay Heath.

Rather than cut back on the number of sites investigated I conducted sampling in such a way that two sites were trapped on a particular day, one a 'shaded' site, the other 'unshaded'. The study sites were paired so that each 'shaded' site was paired with its closest 'unshaded' site in the following way:

- Shapwick Heath with Tadham Moor;
- Westhay Moor with East Waste and
- Westhay Heath with Catcott North.

Not only did this combination minimise time spent travelling between sites, but it also helped reduce differences that might have arisen due to factors such as the prevailing weather conditions on the day of sampling. The order in which the sites were visited was reversed each time and the order in which the traps were set and then subsequently collected in at each site was also reversed at each visit. Pairing of sites meant that statistical comparisons of results could be made using tests of matched pairs (e.g. Wilcoxon test). Table 6.1 summarises the ten site visits made between April and September 2008.

Table 6.1: Dates when traps were set at paired sites in 2008.

Date	Shaded	Unshaded
18 May	Shapwick Heath	Tadham Moor
8 June	Shapwick Heath	Tadham Moor
17 July	Westhay Heath	Catcott North
23 July	Westhay Moor	East Waste
3 August	Shapwick Heath	Tadham Moor
11 August	Westhay Heath	Catcott North
17 August	Westhay Moor	East Waste
14 September	Westhay Heath	Catcott North
19 September	Shapwick Heath	Tadham Moor
28 September	Westhay Moor	East Waste

As in 2007, so far as site topography allowed, the trapping stations were set up approximately 30 metres apart. Baited traps were set as described in Chapter 2 at each study site on at least six separate occasions between 6 April 2008 and 28 September 2008. Traps were left for approximately 24 hours. Minimum/maximum thermometers were deployed at one of the five trapping stations per ditch on each visit.

6.1.2 Watercourses sampled

The locations of the watercourses that were sampled is given in Appendix B1 along with aerial photographs which show the surrounding habitat. Appendix F1 contains photographs of the watercourses sampled during 2008. Measurements were made using GIS of the tree cover at each of the study sites at distances of 100m and 1000m from the ditches.

Essentially, all the watercourses were field boundary ditches with the exception of those at Westhay Heath. The land at Westhay Heath has been modified by peat extraction such that few original boundary ditches remain within the SSSI. The linear features chosen as trapping locations there were drainage ditches dug next to former peat workings.

6.1.3 Collection of data on environmental parameters

The physical characteristics of the ditches at each trapping station – width, water depth, degree of poaching and bank gradient - were assessed towards the end of the survey season in the same way as was done for Shapwick Heath in 2007 (see section 5.1). Estimated percentage duckweed cover, *Glyceria* cover and shade from trees and shrubs were recorded also at each trapping station. At the same time measurements were made of dissolved oxygen, pH and conductivity at the two stations at the extreme ends of each separate ditch section using the methods described in Chapter 2.

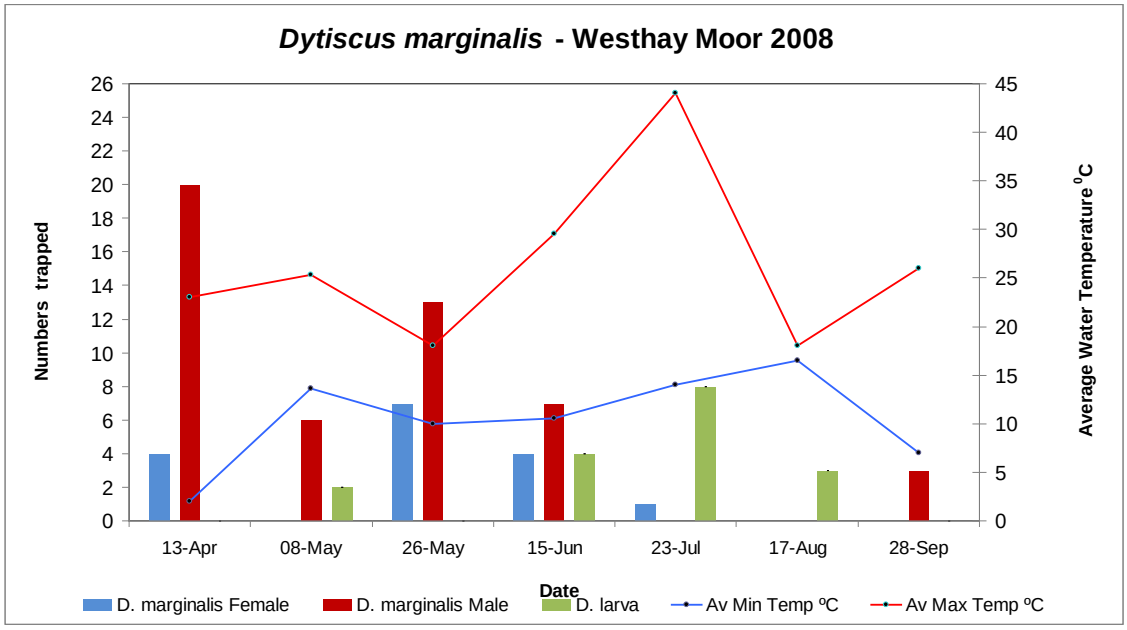
6.2 Results

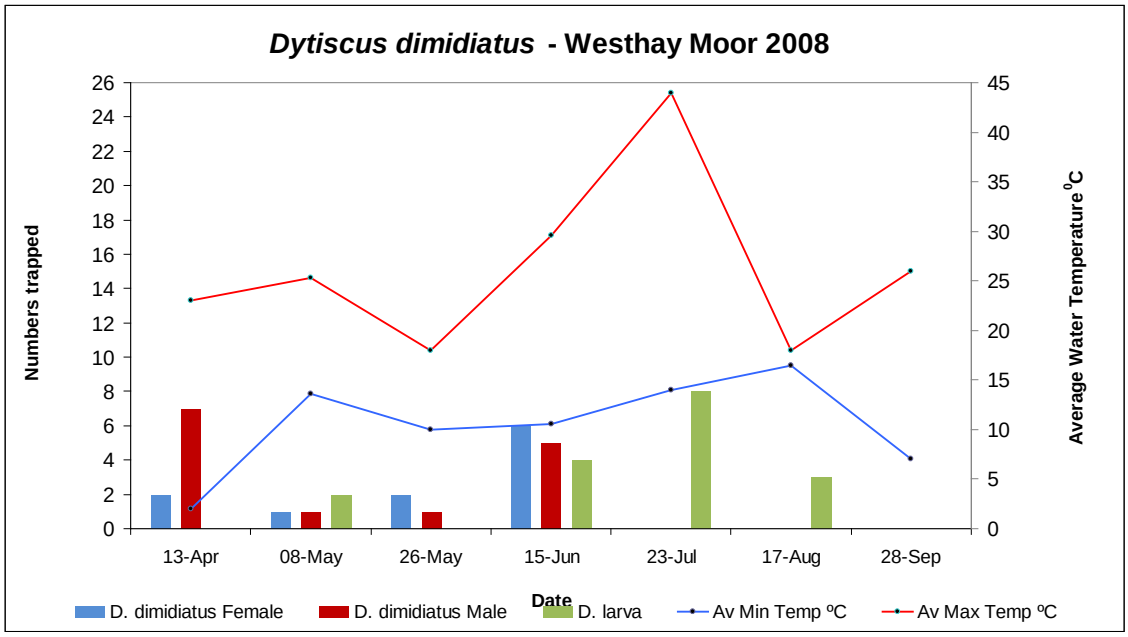
6.2.1 Numbers of beetle captures

Each study site was visited at least six times during 2008. Traps could not be set at two locations on one ditch on East Waste on the last visit of the year because it had become almost completely choked with Sweet-grass (*Glyceria* sp.) and Water Violet (*Hottonia palustris*).

Similar activity patterns were observed in the first part of the year at all study sites to those noted in 2007 at Shapwick Heath. At the sites where more than a few *Dytiscus* were caught there was a spring peak in adult beetle numbers followed by a spike in larval captures in mid- to late summer. However, there were no adult activity maxima in September that corresponded to the autumn peak observed in 2007. Figure 6.1 illustrates a typical pattern observed at Westhay Moor. A complete breakdown of numbers of adults and larvae caught per site is provided in Tables in Appendix F2. The maximum numbers caught at each site are given along with dates. The average numbers of *D. marginalis*, *D. dimidiatus* and *Dytiscus* larvae caught at each study site are summarised in Figure 6.2. The numbers of visits to each site are given in the square brackets within the key to Figure 6.2.

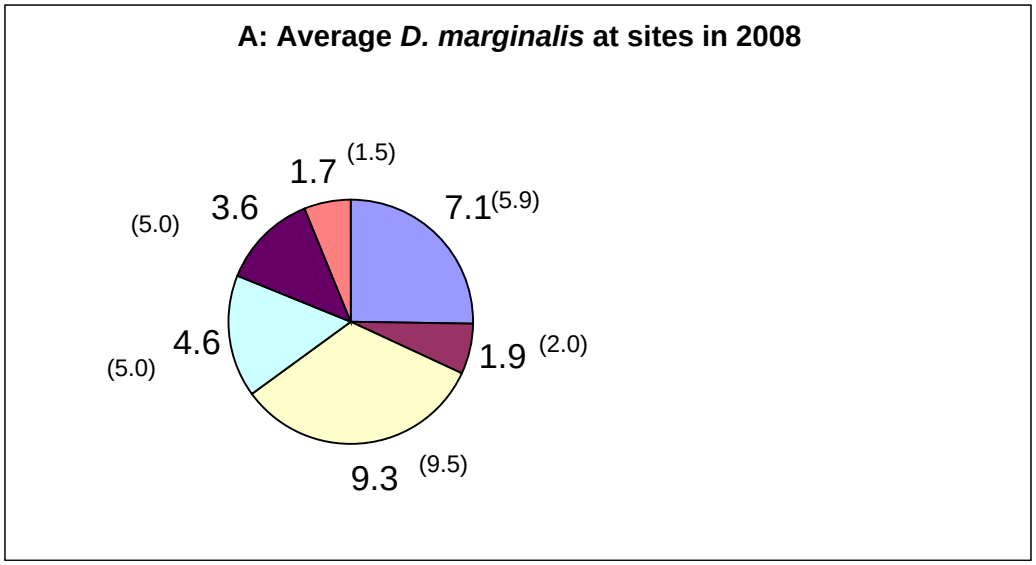
Figure 6.1: Activity pattern for *Dytiscus* spp. at Westhay Moor

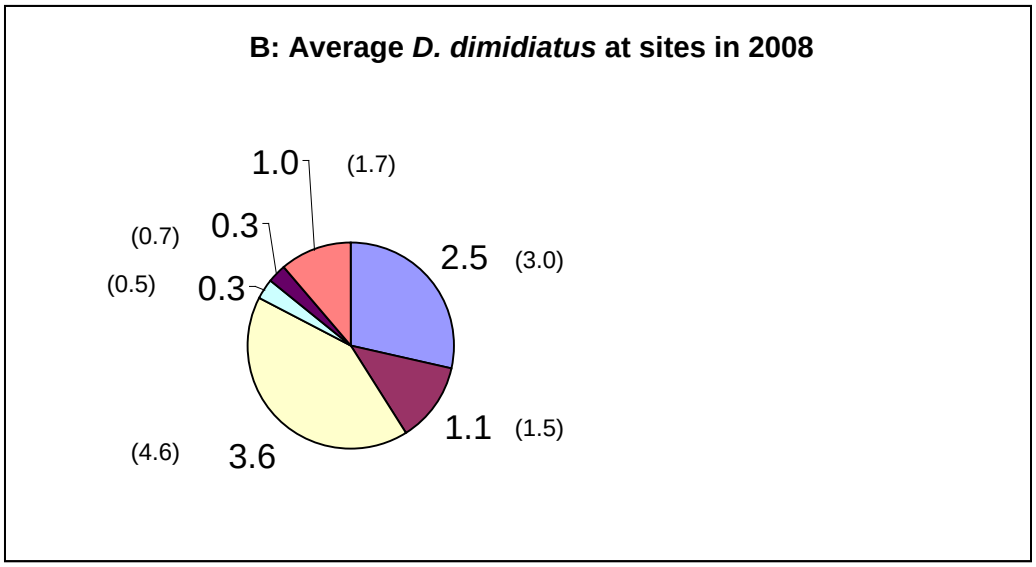


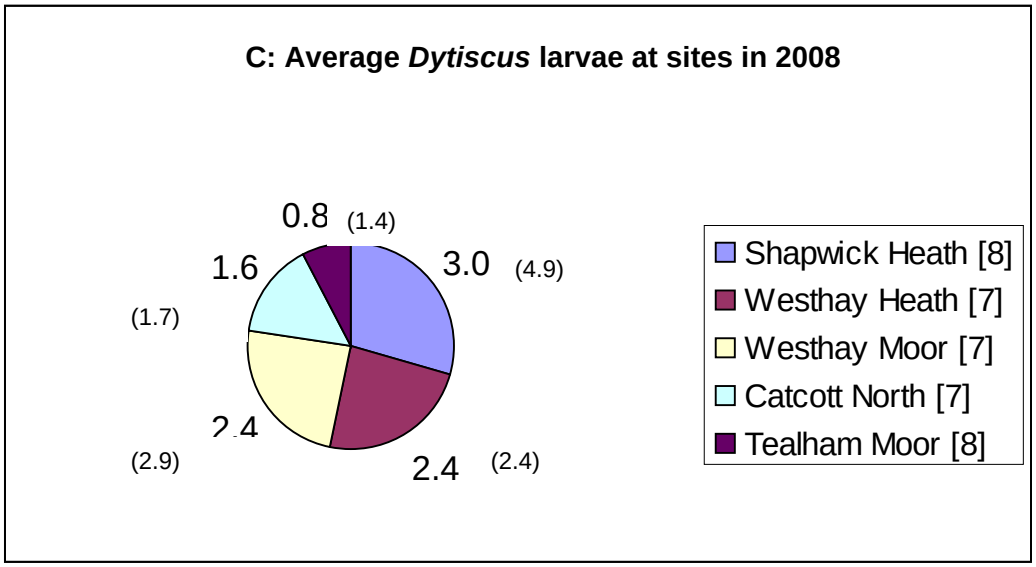


206 adult *D. marginalis* (135 males, 71 females) were trapped at the six sites during 2008. The total number of *D. dimidiatus* adults caught was 63 (comprising 36 males and 27 females). 75 larvae were trapped from five sites, none being recorded from the East Waste ditches. These figures equate to capture rates per trap as: 0.32 for *D. marginalis*; 0.10 for *D. dimidiatus* and 0.12 for larvae. These rates compare with 0.46, 0.29 and 0.26 respectively for trapping at Shapwick Heath the previous season.

Figure 6.2: Beetle captures per site. The figures within round brackets are the standard deviations from the mean of each average.







Adults of both *D. dimidiatus* and *D. marginalis* were found at all six sites.

Table 6.2 compares shaded with unshaded sites in terms of the numbers of *Dytiscus* spp. caught on specific days when paired visits took place.

6.2: *Dytiscus* spp. caught in traps during paired visits to study sites in 2008. DM = D. marginalis; DD = D. dimidiatus; L = Dytiscus larvae

Date	**Shaded**	**DM**	**DD**	**L**	**Unshaded**	**DM**	**DD**	**L**
18 May	SH	6	9	1	TM	6	0	0
8 June	SH	8	2	5	TM	3	0	4
17 July	WH	1	0	5	CN	1	0	3
23 July	WM	1	0	8	EW	0	0	0
3 August	SH	0	0	4	TM	1	0	1
11 August	WH	1	0	0	CN	0	0	4
17 August	WM	0	0	3	EW	0	0	0
14 September	WH	0	2	0	CN	2	0	0
19 September	SH	3	0	0	TM	0	0	0
28 September	WM	3	0	0	EW	1	0	0
	Totals	**23**	**13**	**26**		**14**	**0**	**12**
	Mean	**2.3**	**1.3**	**2.6**		**1.4**	**0**	**1.2**

No *D. dimidiatus* were captured from unshaded sites during the paired visits. Although *D. marginalis* was caught in both shaded and unshaded sites, there were only two occasions on which the numbers caught at the unshaded site exceeded those at the shaded site. On half the visits (i.e. five) more *D. marginalis* were caught at shaded compared with unshaded sites, while on three occasions equal numbers were found at shaded and unshaded sites. With regards to larvae there were more occasions on which greater numbers of larvae were caught at the shaded as opposed to unshaded sites (i.e. six times compared with once).

Where it was possible to do so Wilcoxon Matched-pairs Two-tailed Tests were performed to ascertain whether there were statistically significant differences in the medians of the *Dytiscus* catches at the shaded as opposed to the unshaded study sites using data from paired visits summarised in Table 6.2. No Matched-pairs Test could be performed comparing the counts of *D. dimidiatus* at shaded and unshaded sites because no *D. dimidiatus* were counted at any of the

unshaded sites on the dates when paired visits were undertaken. Where tests could be performed the results are reported in Table 6.3 below.

Table 6.3: Results of Wilcoxon Matched-pairs Two-tailed Test comparing catches from paired visits to shaded and unshaded study sites

	Median Shaded	Median Unshaded	t Statistic	P Value
D. marginalis	1	1	1.27	> 0.10
Dytiscus larvae	2	0	1.35	> 0.10

With regards to adult *D. marginalis*, the null hypothesis cannot be rejected that there was no difference between the median values of counts obtained at shaded shaded sites and unshaded. Therefore, there was no statistically valid evidence that adults of this species prefer shaded to unshaded sites. The same was the case for *Dytiscus* larvae.

Larvae definitely identified as *D. dimidiatus* were found on only ten occasions during 2008. Eight of these captures were at Westhay Heath, with one each at Shapwick Heath and Tadham Moor. These numbers are too small for statistical testing but imply a preference for shade.

One of the three field boundary ditches at Tadham Moor selected for study was de-silted towards the end of the 2008 study period. However, apart from this, none of the ditches were subjected to mechanical disturbance during the study.

6.2.2 Estimates of shade and measurements of tree cover

Table 6.4 summarises the estimated percentage shade cast on each ditch obtained by averaging estimates made at each trapping station. The table contains also the figures for tree cover measured within certain distances of each ditch using GIS-based methods as outlined in Chapter 2.

Table 6.4: Percentage tree cover and shade associated with ditches
A – Direct shade - Average (Av) of values estimated at each of five trapping stations (with Standard Deviation [SD] and Range); **B** – % Tree cover within 100m of ditch as measured using GIS; **C** – % Tree cover within 1000m of ditch as measured using GIS.

Site	Ditch	A			B	C
SHADED		*Av*	*SD*	*Range*		
Shapwick Heath	SHa	89.0	8.2	75 - 95	58.4	28.7
	SHb	88.6	8.4	80 - 98	53.0	29.6
	SHc	75.0	22.9	35 - 90	42.0	29.8
	Average	***84.2***	***15.3***		***51.1***	***29.4***
Westhay Heath	WHa	84.0	19.2	50 - 95	35.1	16.4
	WHb	94.0	2.2	90 - 95	39.9	17.1
	WHc	92.0	4.5	85 - 95	35.0	17.3
	Average	***90.0***	***11.5***		***36.7***	***16.9***
Westhay Moor	WMa	57.0	26.8	15 - 80	27.5	11.2
	WMb	38.0	31.7	5 - 90	27.4	12.6
	WMc	56.0	39.6	0 - 95	27.3	13.2
	Average	***50.3***	***32.0***		***27.4***	***12.3***
UNSHADED		*Av*	*SD*	*Range*		
Catcott North	CNa	0	0	N/A	14.7	12.5
	CNb	10	22.4	0 - 50	11.9	12.5
	CNc	4	8.9	0 - 20	17.6	11.8
	Average	***5***	***13.6***		***14.7***	***12.3***
Tadham Moor	TMa	0	0	N/A	0.9	5.4
	TMb	0	0	N/A	0.6	5.7
	TMc	0	0	N/A	0.7	5.5
	Average	***0***	***0***		***0.7***	***5.5***
East Waste	EWa	0	0	N/A	0.3	4.4
	EWb	0	0	N/A	0.0	4.1
	EWc	0	0	N/A	0.6	4.4
	Average	***0***	***0***		***0.3***	***4.3***

These results demonstrate the categorisation of Westhay Heath, Shapwick Heath and Westhay Moor as ‘shaded’ sites and Catcott North, Tadham Moor and East Waste as relatively ‘unshaded’ sites, although at the landscape level, the tree cover on Westhay Moor approaches that at Catcott North.

6.2.3 Environmental parameters

Data concerning the environmental parameters described in section 6.1.3. were collected over the course of three days during a single week in August, so as to minimise variation due to weather and other factors. Sites were paired as per section 6.1.1 so that on any one day a shaded and an unshaded site were visited. Summaries of the results are given below while tables in Appendix F3 contain the dataset for each ditch.

6.2.3.1 Physical measurements and observations

Table 6.5 below summarises the data collected on width of waterbody, depth of water and bankside gradients.

Table 6.5: Physical parameters –Summary of results

Width of water body (m). Measured on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	2.05	0.5	1.5 – 3.0	0.534
Westhay Moor (30/8/08)	2.47	0.8	1.5 – 4.0	0.767
Westhay Heath (28/8/08)	3.80	1.6	2.0 – 8.0	1.613
Catcott North (28/8/08)	1.60	0.2	1.5 – 2.0	0.207
Tadham Moor (31/8/08)	2.67	0.2	2.5 – 3.0	0.244
East Waste (30/8/08)	3.03	0.4	2.5 – 3.5	0.399
Depth of water (cm). Measured on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	39.33	40	20 - 55	9.976
Westhay Moor (30/8/08)	38.00	40	20 - 55	11.619
Westhay Heath (28/8/08)	66.87	75	15 - 95	25.131
Catcott North (28/8/08)	76.00	75	65 - 110	13.523
Tadham Moor (31/8/08)	43.33	50	10 - 75	25.612
East Waste (30/8/08)	70.00	75	10 - 95	25.912
Summed gradients. Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	6.33	6	5 – 8	0.724
Westhay Moor (30/8/08)	6.53	7	3 – 8	1.552
Westhay Heath (28/8/08)	2.00	2	2 – 2	0.000
Catcott North (28/8/08)	5.13	5	2 – 7	1.408
Tadham Moor (31/8/08)	7.60	8	6 – 8	0.633
East Waste (30/8/08)	5.67	5	4 - 8	1.759

The gradients of the banks on the same side and on the opposite side from the trapping station were ranked according to a numerical scale as

explained in section 5.1. The ranks were summed for each ditch in order to produce a figure that captured the steepness of the banks at the point they entered the water: The higher the number, the greater the combined steepness. Poaching was observed at only two sites – Shapwick Heath and Catcott North - and there only on a small scale. Given the general lack of poaching this parameter was ignored in the analyses and is not reported in Table 6.5.

In order to decide on appropriate statistical tests to investigate the extent of differences between sites, the datasets were analysed to see if they had roughly equal variances around the mean and normal distributions (which are two assumptions that have to be met for ANOVAs to be valid). The results are given in Table 6.6.

Table 6.6: Physical parameters – Variance, skewness and kurtosis

Width of water body (m). Measured on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	4.00	0.29	0.594	-0.806
Westhay Moor (30/8/08)	8.23	0.59	0.128	-0.719
Westhay Heath (28/8/08)	36.40	2.60	1.656	2.308
Catcott North (28/8/08)	0.60	0.04	1.672	0.897
Tadham Moor (31/8/08)	0.83	0.06	0.788	-1.615
East Waste (30/8/08)	2.23	0.16	-0.128	-1.348
Depth of water (cm). Measured on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	1393.33	99.52	-0.661	-0.329
Westhay Moor (30/8/08)	1890.00	135.00	-0.068	-1.009
Westhay Heath (28/8/08)	8841.73	631.55	-1.048	0.021
Catcott North (28/8/08)	2560.00	182.86	0.756	2.404
Tadham Moor (31/8/08)	9183.33	655.95	-0.466	-1.580
East Waste (30/8/08)	9400.00	671.43	-1.848	2.789
Summed gradients. Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	7.33	0.52	0.676	0.948
Westhay Moor (30/8/08)	33.73	2.41	-1.063	0.511
Westhay Heath (28/8/08)	0.00	0.00	N/A	N/A
Catcott North (28/8/08)	27.73	27.73	-0.447	0.182
Tadham Moor (31/8/08)	5.60	0.40	-1.407	1.264
East Waste (30/8/08)	43.33	3.10	0.589	-1.615

From Table 6.6 it can be seen that for each of the physical parameters there was at least one site for which the results displayed considerable variance, skewness or kurtosis. This finding suggests that it would not be appropriate to analyse the differences between sites using ANOVA as a statistical method. In these circumstances the non-parametric equivalent – Kruskal – Wallis is to be preferred, comparing the medians rather than mean values. The results of statistical tests conducted on the data are reported in Table 6.7 below. For each parameter all sites were compared together, shaded sites only (i.e. Shapwick Heath, Westhay Moor and Westhay Heath) and unshaded sites only (i.e. Catcott North, Tadham Moor and East Waste).

Table 6.7: Kruskal-Wallis Tests to analyse variance within physical parameter data obtained from six study sites in August 2008
H = Kruskal-Wallis Test Statistic d of f = Degrees of Freedom
P = Probability of Null Hypothesis s/ns = Significant/Not Significant
*** = P ≤ 0.001

Width of water body (m). Measured on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 90)	51.55	5	5.64×10^{-7}	s***
Shaded sites only (n = 45)	18.14	2	0.000116	s***
Unshaded sites only (n = 45)	34.12	2	6.68×10^{-7}	s***
Depth of water (cm). Measured on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 90)	39.99	5	8.40×10^{-7}	s***
Shaded sites only (n = 45)	12.20	2	0.00224	s**
Unshaded sites only (n = 45)	16.95	2	0.000209	s***
Summed gradients. Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 90)	53.94	5	4.70×10^{-7}	s***
Shaded sites only (n = 45)	31.86	2	4.15×10^{-7}	s***
Unshaded sites only (n = 45)	17.49	2	0.000160	s***

The results from Kruskal-Wallis Tests suggested that all the sites were significantly different one from another in terms of the average measured widths of water bodies and water depths. The average summed gradient estimates also appeared significantly different between sites. These

parameters also varied significantly within the categories of shaded and unshaded sites.

6.2.3.2 Water Chemistry

Table 6.8 gives measurements of dissolved oxygen, conductivity and pH made as described in Chapter 2. The oxygen meter used also recorded water temperature and air pressure in order to check that that dissolved oxygen readings were taken under roughly the same conditions at all ditches. The figures obtained for water temperature and air pressure are not reported here but are summarised in Appendix F3. The average water temperature readings from the conductivity and pH meters used are to be found also in Appendix F.

Table 6.8: Water Chemistry – Summary of results

Dissolved oxygen (mg/l). Measured on one occasion at two trapping stations per ditch (n = 6 at each site except for East Waste where n = 5) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	4.20	2.20	0.7 – 8.6	3.7
Westhay Moor (30/8/08)	1.22	0.90	0.1 – 2.7	0.99
Westhay Heath (28/8/08)	2.05	0.75	0.5 – 6.9	2.5
Catcott North (28/8/08)	2.37	1.85	0.7 – 6.0	2.0
Tadham Moor (31/8/08)	3.80	3.50	1.6 – 6.4	2.2
East Waste (30/8/08)	1.05	0.55	0.4 – 3.6	1.6
Conductivity (µS). Measured on one occasion at two trapping stations per ditch (n = 6 at each site) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	461.17	433.5	387 - 643	99.35
Westhay Moor (30/8/08)	223.83	202.5	194 - 298	41.38
Westhay Heath (28/8/08)	274.17	277	254 - 294	14.44
Catcott North (28/8/08)	532.33	516	440 - 699	94.42
Tadham Moor (31/8/08)	758.83	717	684 - 888	97.76
East Waste (30/8/08)	446.00	446	410 -474	26.48
pH. Measured on one occasion at two trapping stations per ditch (n = 6 at each site) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	7.55	7.5	7.2 – 8.0	0.26
Westhay Moor (30/8/08)	6.83	6.75	6.5 – 7.3	0.31
Westhay Heath (28/8/08)	7.42	7.45	7.1 – 7.7	0.20
Catcott North (28/8/08)	7.30	7.3	7.2 – 7.4	0.09
Tadham Moor (31/8/08)	8.07	8.1	7.8 – 8.2	0.15
East Waste (30/8/08)	7.43	7.5	7.1 – 7.6	0.20

Table 6.9 provides a summary of variance, skewness and kurtosis displayed by the water chemistry data.

Table 6.9: Water Chemistry– Variance, skewness and kurtosis

Dissolved oxygen (mg/l). Measured on one occasion at two trapping stations per ditch (n = 6 at each site except for East Waste where n = 5) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	53.62	13.41	0.538	-2.963
Westhay Moor (30/8/08)	4.88	0.98	0.679	-0.962
Westhay Heath (28/8/08)	31.98	6.40	1.917	3.499
Catcott North (28/8/08)	20.23	4.05	1.408	1.909
Tadham Moor (31/8/08)	23.50	4.70	0.204	-2.629
East Waste (30/8/08)	7.96	1.59	2.347	5.596
Conductivity (µS). Measured on one occasion at two trapping stations per ditch (n = 6 at each site) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	49552.80	9870.57	1.497	2.196
Westhay Moor (30/8/08)	8564.83	1712.97	1.515	1.452
Westhay Heath (28/8/08)	1042.83	208.57	-0.156	-0.676
Catcott North (28/8/08)	44577.30	8915.47	1.214	1.468
Tadham Moor (31/8/08)	47786.80	9557.37	0.753	-1.848
East Waste (30/8/08)	2306.00	461.20	-0.686	1.398
pH. Measured on one occasion at two trapping stations per ditch (n = 6 at each site) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	0.335	0.067	0.830	2.488
Westhay Moor (30/8/08)	0.473	0.095	0.701	-0.930
Westhay Heath (28/8/08)	0.208	0.042	-0.333	0.517
Catcott North (28/8/08)	0.040	0.008	-9.6×10^{-6}	-1.875
Tadham Moor (31/8/08)	0.113	0.023	-1.270	-1.531
East Waste (30/8/08)	0.193	0.039	-1.166	0.419

Given the degrees of variance, skewness and kurtosis shown by the data, non-parametric Kruskal-Wallis Tests were used to determine whether the sites differed significantly in terms of their water chemistry. The results of these tests are summarised in Table 6.10.

Table 6.10: Kruskal-Wallis Tests to analyse variance within water chemistry data obtained from six study sites in August 2008
H = Kruskal-Wallis Test Statistic d of f = Degrees of Freedom
P = Probability of Null Hypothesis s/ns = Significant/Not Significant
*** = P ≤ 0.001 ** = P ≤ 0.01 * = P ≤ 0.05

Dissolved oxygen (mg/l). Measured on one occasion at two trapping stations per ditch (n = 6 at each site except for East Waste where n = 5) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 35)	10.08	5	0.0731	ns
Shaded sites only (n = 18)	2.94	2	0.229	ns
Unshaded sites only (n = 17)	6.84	2	0.0327	s*
Conductivity (µS). Measured on one occasion at two trapping stations per ditch (n = 6 at each site) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 36)	30.27	5	1.34×10^{-5}	s***
Shaded sites only (n = 18)	13.08	2	0.00145	s**
Unshaded sites only (n = 18)	11.91	2	0.00259	s**
pH. Measured on one occasion at two trapping stations per ditch (n = 6 at each site) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 18)	24.6	5	0.00017	s***
Shaded sites only (n = 9)	10.28	2	0.0059	s**
Unshaded sites only (n = 9)	12.44	2	0.0020	s**

Dissolved oxygen was the only chemical parameter for which there was apparently no statistically significant difference between all sites and between shaded sites. A significant difference seemed to exist between unshaded sites in terms of average (median) values of dissolved Oxygen. The results suggested significant differences in median conductivity and pH between all sites compared together as well as between sites in the shaded and unshaded categories.

6.2.3.3 Vegetation

Average estimates of tree and shrub cover at each ditch were given in Table 6.2. The nature of the tree cover at the shaded sites varied. Alder (*Alnus glutinosa*) was a shade-casting tree at 87% of the trapping locations on Shapwick Heath, but at only 13% of those at Westhay Moor and not at all at Westhay Heath. The main shade casting tree recorded at both Westhay Heath (where it occurred at 93% of locations) and Westhay Moor (67%) was Goat Willow (*Salix caprea*). Silver Birch (*Betulus pendula*) and Pedunculate Oak (*Quercus robur*) were also commonly recorded.

Table 6.11 summarises the estimates made of floating vegetation cover and includes the data for both *Glyceria* and duckweed covers. A summary of variance, skewness and kurtosis is found in Table 6.12.

Table 6.11: Floating vegetation cover – Summary of results

Glyceria cover (% area). Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	6.67	0	0 - 40	12.91
Westhay Moor (30/8/08)	6.73	5	0 - 15	5.15
Westhay Heath (28/8/08)	8.33	5	0 - 30	11.60
Catcott North (28/8/08)	38.00	50	10 - 80	25.48
Tadham Moor (31/8/08)	22.33	0	0 - 100	33.96
East Waste (30/8/08)	27.33	10	5 – 100	32.51
Duckweed cover (% area). Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Mean	Median	Min - Max	SD
Shapwick Heath (31/8/08)	73.87	85	5 – 100	30.07
Westhay Moor (30/8/08)	99.73	100	98 - 100	0.70
Westhay Heath (28/8/08)	93.67	95	70 - 100	8.95
Catcott North (28/8/08)	100.00	100	N/A	0.00
Tadham Moor (31/8/08)	80.53	100	15 - 100	30.08
East Waste (30/8/08)	86.67	100	0 - 100	35.19

Table 6.12: Floating vegetation cover – Variance, skewness and kurtosis

Glyceria cover (% area). Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	2333.33	166.67	2.189	3.735
Westhay Moor (30/8/08)	370.93	26.50	0.561	-0.718
Westhay Heath (28/8/08)	1883.33	134.52	1.181	-0.304
Catcott North (28/8/08)	9090.00	649.29	0.108	-1.589
Tadham Moor (31/8/08)	16143.30	1153.10	1.503	1.198
East Waste (30/8/08)	14793.30	1056.67	1.522	1.264
Duckweed cover (% area). Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	Total Variance	Sample Variance	Skewness	Kurtosis
Shapwick Heath (31/8/08)	12659.70	904.27	-0.902	0.0971
Westhay Moor (30/8/08)	6.93	0.50	-2.405	4.349
Westhay Heath (28/8/08)	1123.33	80.24	-1.688	2.473
Catcott North (28/8/08)	0.00	0.00	N/A	N/A
Tadham Moor (31/8/08)	12669.70	904.98	-1.209	0.007
East Waste (30/8/08)	17333.30	1238.10	-2.405	4.349

Since the data showed high levels of variance, skewness and kurtosis Kruskal-Wallis Tests were performed to analyse how sites varied one from another. The results are summarised in Table 6.13.

Table 6.13: Kruskal-Wallis Tests to analyse variance within floating vegetation data obtained from six study sites in August 2008
H = Kruskal-Wallis Test Statistic d of f = Degrees of Freedom
P = Probability of Null Hypothesis s/ns = Significant/Not Significant
*** = P ≤ 0.001 ** = P ≤ 0.01 * = P ≤ 0.05

Glyceria cover (% area). Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 90)	27.32	5	5.01×10^{-5}	s**
Shaded sites only (n = 45)	3.02	2	0.221	ns
Unshaded sites only (n = 45)	7.14	2	0.028	s*
Duckweed cover (% area). Estimated on one occasion at each trapping station (n = 15 at each site) in August 2008.				
	H	d of f	P	s/ns
All sites (n = 90)	20.50	5	0.00101	s**
Shaded sites only (n = 45)	10.57	2	0.00508	s**
Unshaded sites only (n = 45)	7.35	2	0.0254	s*

The *Glyceria* sp. at Catcott North, Tadham Moor and East Waste was predominantly *G. maxima*. At the other sites the smaller *G. fluitans* was found. I did not record the species identity of the floating duckweed-like vegetation systematically at each site because my main concern was the blanketing and shading effect of floating plants. Most of the Duckweed comprised the commoner *Lemna* sp. (e.g. *L. minor*) although *L. trisulca* (Ivy-leaved Duckweed) and *Wolffia arrhiza* were recorded at some of the unshaded trapping locations. Frogbit (*Hydrocharis morsus-ranae*) was also recorded at some sites.

6.2.4 *Post hoc* hypothesis testing

In section 6.2.3 the results were presented of multiple comparisons made using the Kruskal-Wallis Tests. As explained in section 2.7.2, in situations where such multiple comparisons are performed on data drawn from the same dataset there is a danger of non-significant results being identified as significant. To avoid this problem I applied the most conservative correction method (the Sequential Bonferroni Correction) to the statistical results reported in Tables

6.7, 6.10 and 6.13 above. The results of the Sequential Bonferroni Corrections are given in Table 6.14 where I compare the probability of the null hypothesis (P) with a corrected confidence level (α') which is calculated from the formula:

$$\alpha'_n = \alpha_{ec} / k - (n - 1)$$

Where: α'_n = the nth value of α' to be calculated in the sequence
α_{ec} = the confidence level set for the tests being compared
k = Number of tests

In this instance α_{ec} was the 95% confidence level (i.e. P = 0.05). The Null Hypothesis (no significant difference between sites) was accepted when P ≥ α'.

Table 6.14: Bonferroni Correction applied to P values calculated from Kruskal-Wallis Tests of data on environmental parameters.

	P	Rank	Inverse Rank	Corrected confidence level (α')
Width All sites	5.64×10^{-7}	3	22	0.00227
Width Shaded sites only	0.000116	8	17	0.00294
Width Unshaded sites only	6.68×10^{-7}	4	21	0.00238
Depth All sites	8.40×10^{-7}	5	20	0.00250
Depth Shaded sites only	0.00224	15	10	0.00500
Depth Unshaded sites only	0.000209	11	14	0.00357
Gradients All sites	4.70×10^{-7}	2	23	0.00217
Gradients Shaded sites only	4.15×10^{-7}	1	24	0.00208
Gradients Unshaded sites only	0.000160	9	16	0.00313
Oxygen All sites	0.0731	22	3	0.01667 NS
Oxygen Shaded sites only	0.229	24	1	0.05000 NS
Oxygen Unshaded sites only	0.0327	21	4	0.01250 NS
Conductivity All sites	1.34×10^{-5}	6	19	0.00263
Conductivity Shaded sites only	0.00145	13	12	0.00417
Conductivity Unshaded sites only	0.00259	16	9	0.00556
pH All sites	0.00017	10	15	0.00333
pH Shaded sites only	0.0059	18	7	0.00714
pH Unshaded sites only	0.0020	14	11	0.00454
Glyceria All sites	5.01×10^{-5}	7	18	0.00278
Glyceria Shaded sites only	0.221	23	2	0.02500 NS
Glyceria Unshaded sites only	0.028	20	5	0.01000 NS
Duckweed All sites	0.00101	12	13	0.00385
Duckweed Shaded sites only	0.00508	17	8	0.00625
Duckweed Unshaded sites only	0.0254	19	6	0.00833 NS

The results shown in Table 6.14 indicate that there were no significant differences between sites with regards to average amounts of dissolved Oxygen recorded in ditches. The average *Glyceria* cover was not significantly different between the shaded sites themselves or between the unshaded sites alone. However, there was a significant difference in *Glyceria* cover at shaded sites compared with unshaded ones.

6.2.5 Canonical Correspondence Analysis (CCA)

CCA was undertaken to see if any strong relationships could be found between the environmental parameters described above and the numbers of *Dytiscus* species recorded from each ditch. The dependent variables onto which the environmental data were regressed were the mean average counts per ditch in the following categories: male *D. marginalis*; female *D. marginalis*; male *D. dimidiatus*; female *D. dimidiatus*; *D. dimidiatus* larvae and *Dytiscus* larvae. The sexes were separated in order to see to what extent their relationship with the variables differed. The last category of those listed ('Dytiscus larvae') included 11 *Dytiscus* larvae that had not been positively identified due to CO1 sequence as *D. dimidiatus*. However, removing the larvae not positively identified did not alter the plot shown in Figures 6.3a and 6.3b in any fundamental way. As reported in Chapter 4, failure to identify larvae from sequence data was invariably connected with issues connected with amount and/or quality of DNA and, since analysis of the CO1 sequences did not suggest that Dytiscus spp. other than *D. dimidiatus* and *D marginalis* were present in the samples, and as there was no reason to think that there was a greater proportion of *D. dimidiatus* larvae among the unidentified larvae, it seemed reasonable to assume that the greater majority of the unidentified larvae were specimens of *D. marginalis*.

Since CCA is unreliable if there are more independent than dependent variables separate CCAs were conducted for physical, chemical and biotic (vegetation) factors. The first step in each CCA was to consider whether any strong correlations existed between any of the chosen independent variables.

6.2.5.1 CCA with physical parameters as independent variables

The physical parameters measured and estimated at each trapping station were width of waterbody, depth of water, degree of poaching and gradient of both banks. As there was very little poaching at any site this variable was dropped from the CCA. As noted in section 6.2.3, there was evidence of differences in variances and departures from a normal distribution in the three datasets width of waterbody, depth of water and summed gradients. For this reason correlations were performed using the Spearman Rank Correlation. The three independent variables were correlated one with another to produce the values for the corrected Spearman Rank Correlation Coefficent (r_s) and the results are set out in Table 6.15.

6.15: Values of corrected Spearman Rank Correlation Coefficient (r_s) for correlations of means of physical data collected for 18 linear waterbodies from six study sites.

	Summed Gradient	Depth of Water
Summed Gradient	-	
Depth of Water	-0.195 (P > 0.05)	-
Width of Waterbody	-0.148 (P > 0.05)	0.179 (P > 0.05)

None of the correlations in Table 6.15 were statistically significant; therefore all parameters could be used in the CCA plot. The values for depth of water were modified by dividing each by 10 in order that they were of the same rough order of magnitude as the summed gradients. The plots obtained from this CCA are reproduced in Figures 6.3a and 6.3b below.

Axis 1 of the plots in both Figures 6.3a and 6.3b was heavily influenced by summed gradient and water body width, while axis 2 was more influenced by water depth. The first axis was relatively successful at explaining overall variance in the data, accounting for 26.9%. The first two axes together explained 30.5% of variance but a Monte Carlo test (1000 replicates) suggested that only the eigenvalue for the first axis was unlikely to have been generated by chance (P = 0.0014). This suggested that the variability in abundance of *Dytiscus* beetles might

have been influenced by the gradient of the banks and width of waterbody.

Figure 6.3a shows two of the Westhay Heath ditches (WHa and WHb) as extreme outliers so far as site features were concerned and Figure 6.3b with *D. dimidiatus* larvae to the far right of the plot indicating a strong influence of bankside gradient and/or width of waterbody. This reflects the fact that in 2008 *D. dimidiatus* larvae were found in greatest numbers at Westhay Heath where the bankside gradients were shallow compared with all other sites and the water bodies wider.

Figure 6.3a CCA Triplot of physical environmental factors and average counts of *Dytiscus* spp. in particular categories
The arrows represent the environmental variables (Summed gradient, Width of waterbody and depth of Water), the blue squares represent the samples (i.e. the physical measurements made in the 18 ditches) and the red triangles represent the species (i.e. the six categories of *Dytiscus* – *D. marginalis* male and female, *D. dimidiatus* male and female, *Dytiscus* larvae and *D. dimidiatus* larvae). The plot has been scaled with the species at site centroids which emphasises the differences between samples (sites).

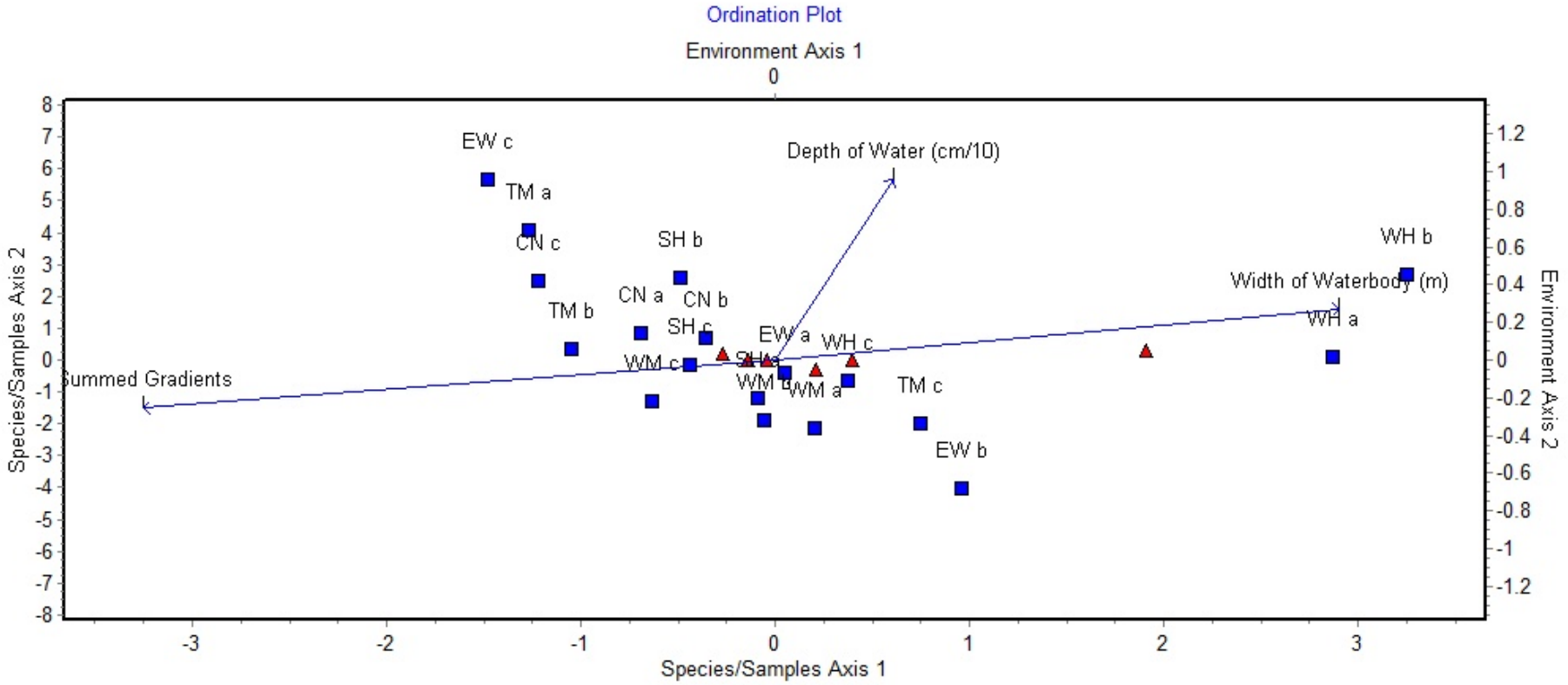
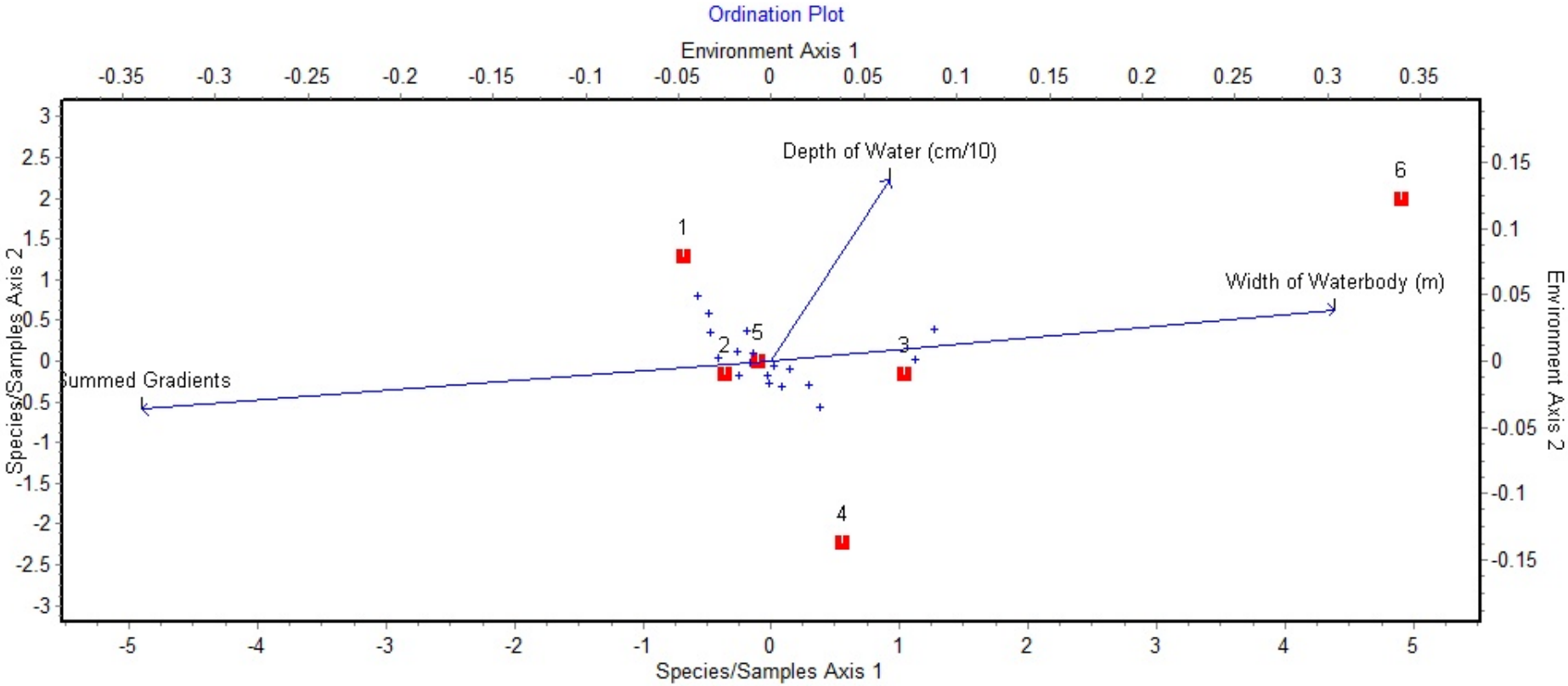


Figure 6.3b CCA Triplot of physical environmental factors and average counts of *Dytiscus* spp. in particular categories
This is the same ordination as shown in Figure 6.3a but in this case the plot has been scaled with the sites at species centroids which emphasises the differences between species. Sites are shown as crosses, species as squares. (1 = *D. marginalis* female, 2 = *D. marginalis* male, 3 = *D. dimidiatus* female, 4 = *D. dimidiatus* male, 5 = *Dytiscus* larvae (not *D. dimidiatus*) and 6 = *D. dimidiatus* larvae.)



6.2.5.2 CCA with water chemistry parameters as independent variables

The water chemistry parameters recorded at each trapping station were dissolved oxygen, conductivity and pH. The results of Spearman Rank Correlations of these parameters are reported in Table 6.16 below.

6.16: Values of corrected Spearman Rank Correlation Coefficient (r_s) for correlations of means of water chemistry data collected for 18 linear waterbodies from six study sites.

	Dissolved Oxygen	Conductivity
Dissolved Oxygen	-	
Conductivity	0.355 (P > 0.05)	-
pH	0.250 (P > 0.05)	0.648 (P< 0.01)

There was a significant positive correlation between pH and conductivity such that I decided it was unsafe to use the variables together in the CCA. I performed a $\log_{10}$ transformation on the values for conductivity so that they were of the same magnitude as those for dissolved oxygen concentration.

CCA using $\log_{10}$ transformed conductivity values and dissolved Oxygen as independent variables produced a plot that explained so little of the variance (Axis 1 – 7.2%, Axis 2 – 3.1%) as to be of no practical value. A Monte Carlo Test (1000 replicates) indicated significant probabilities (P = 0.56 for Axis 1 and P = 0.31 for Axis 2) that the eigenvalues for both axes could have arisen by random chance. For these reasons the plot is not reproduced here.

6.2.5.3 CCA with vegetation parameters as independent variables

The independent variables I considered using were the average estimates of tree and shrub cover, *Glyceria* cover and duckweed cover. Spearman Rank Correlations produced the results shown in Table 6.17.

6.17: Values of corrected Spearman Rank Correlation Coefficient (r_s) for correlations of means of vegetation data collected for 18 linear waterbodies from six study sites.

	Tree & shrub cover	*Glyceria* cover
Tree & shrub cover	-	
Glyceria cover	-0.364 (P > 0.05)	-
Duckweed cover	-0.090 (P > 0.05)	0.130 (P > 0.05)

Given these correlation results I decided to conduct the CCA with all three parameters. The percentage covers were divided by 10 in each instance in order to bring their magnitudes down towards the values for average *Dytiscus* catches.

The plot which resulted had a first canonical axis that explained 10.2% of the variance. However, a Monte Carlo test (1000 replicates) indicated that there was a high degree of probability that the eigenvalue for Axis 1 could have been due to random chance (P = 0.54). No firm conclusions regarding effect of vegetation cover upon *Dytiscus* abundance could be drawn from this plot.

6.3 Conclusions

6.3.1 Influence of shade on *Dytiscus* spp.

The 2008 season's fieldwork yielded some evidence that *D. dimidiatus* displays a preference for shaded waterbodies. Adults of *D. dimidiatus* were found at all six study sites but, when paired visits to shaded/unshaded sites were undertaken on particular days, statistically significant numbers of adult *D. dimidiatus* were found at the shaded sites. Ten larvae caught during 2008 were positively identified as *D. dimidiatus*. Except for one, all of these larvae were captured at shaded sites. There was also an indication that larvae of *D.*

marginalis as well as of *D. dimidiatus* might be more commonly found in shaded waterbodies.

6.3.2 Duckweed cover

In the previous chapter it was noted that at Shapwick Heath in 2007 larval numbers were negatively correlated with duckweed cover. I advanced the theory that duckweed may exert an adverse effect upon early instar larvae. No strong association, either positive or negative, was discerned in 2008 between duckweed cover and adult or larval abundance. However, in contrast to the 2007 survey season, duckweed cover was estimated at sites only once in the season and at a time (August) of later larval instars and when it was at almost 100% coverage in most ditches.

A connection between duckweed cover and shading might have been expected, but the correlation between % tree and shrub cover and % duckweed was not significant in 2008 and the value of the Spearman Rank Correlation Coefficient was among the lowest obtained from correlating environmental variables (r_s = -0.09, n = 18, P = > 0.05).

6.3.3 Other environmental factors

The ditches selected for investigation in 2008 varied from site to site in terms not only of degree of shading but also with regard to other environmental variables recorded. Canonical Correspondence Analysis (CCA), however, indicated that the only parameters that were strongly associated with numbers of *Dytiscus* caught were related to the physical characteristics of the waterbody (i.e. width, bankside gradient and depth of water). It was noticeable that the waterbodies where a disproportionate number of *D. dimidiatus* larvae were found (i.e. two of the linear features at Westhay Heath) stood out from those at other sites by virtue of their greater width, comparatively deep water and gently shelving sides. It was noted earlier in this chapter that the ‘ditches’ studied at Westhay Heath were more akin to the shallow margins of a lake particularly as these waterbodies were very closely associated with former peat workings.

This observation suggests that more former peat workings in the Brue Valley ought to be investigated as potential *D. dimidiatus* breeding grounds.

Chapter 7: Predator – Prey Relationships

Introduction

In this chapter several hypotheses were tested:

- That there was a clear linear relationship between occurrence and abundance of *Dytiscus* spp. in ditches and the macro-invertebrate diversity and/or the abundance of particular macro-invertebrate taxa in the ditches. (Such relationships would need to be demonstrated if it were to be claimed that Dytiscus spp. are good biodiversity indicators);
- *Dytiscus* spp. will predate certain taxa but not others when presented with them in artificial conditions. (If differences in prey preferences could be shown this would help distinguish the niches occupied by *D. dimidiatus* and *D. marginalis*);
- There could be inter-specific and intra-specific predation among *Dytiscus* spp. living in the same ditches.

To test the possible interactions within and between *Dytiscus* species and other aquatic macro-invertebrates I pursued three different sorts of investigation. These were:

- Examining if a relationship could be discerned between macro-invertebrate species-richness and *Dytiscus* abundance in waterbodies;
- Running a series of experiments in aquaria in which individual *Dytiscus* were presented with potential prey items; and
- By analysing the results from trapping studies to assess whether there was evidence for inter-specific and intra-specific predation in *Dytiscus* spp.

The results are compared with observations by other authors gleaned from the scientific and specialist literature.

7.1 Methods

7.1.1 Estimating macro-invertebrate species richness at the 2008 study sites

Each of the three ditches investigated in the six study sites was sampled during May 2008 using a standard pond net as described in Chapter 2. Animals caught were identified in the field as far as possible to species level. An estimate was made later of the species-richness of each sample by counting 'Recognisable Taxonomic Units' (RTUs) noted during timed sorting of the netted material. Each RTU was assumed to be an individual species, although the precise species identity was not established in all cases. In some instances identifications were made with confidence to species level (e.g. for the larger water beetles such as *Hydrophilus piceus*) in other cases I could only identify the RTU to a very superficial extent (e.g. 'Oligochaete worm'). Since an accurate inventory of each ditch was not needed, I did not regard it as a problem that not all individuals caught were assigned to a definite species. Oliver & Beattie (1993) championed the use of RTUs in rapid assessments of biodiversity although other workers [e.g. Krell (2004)] have questioned the reliability of species-richness estimates derived using them.

7.1.2 Experiments using aquaria

The protocol used to set up aquaria was described in Chapter 2. The exact configurations of the aquaria that could be set up depended on the availability of predators and potential prey caught in traps and by netting respectively. The configurations are illustrated in Figures G1a to G1e in Appendix G1.

On each occasion that aquaria were set up, for every aquarium stocked with a beetle predator there was a corresponding one immediately next to it with potential prey only. Because of their close proximity to each other it was considered highly probable that the two tanks experienced roughly the same conditions (such as exposure to the sun) and could be analysed as paired experimental treatments (+ Beetle, - Beetle).

7.1.3 Use of data collected during trapping and biometric assessment to investigate inter-specific and/or intra-specific predation in *Dytiscus* species

230 larval specimens were recorded in traps over the course of fieldwork. Of these 78 were released after removal of a leg. The usual reason that larvae were retained rather than released was that they had died in the traps. This indicated high trap mortality for larvae (up to two thirds of animals caught). It was thought that one of the reasons for this high mortality might be due to predation by other adults or larvae.

In order to investigate whether this was the case, an assessment of larval condition was made during the microscopic examinations of the larvae that were described in Chapter 4. Each larva examined was assigned to one of four categories on the basis of its overall condition: 1. Whole; 2. Injured/damaged; 3. Exoskeleton only; 4. Fragments only. Categories 2, 3 and 4 were considered as evidence that predation had occurred and trap records were checked to see whether there were other animals in the traps that could be responsible.

7.2 Results

7.2.1 Macro-invertebrate species richness

The results of RTU counts for each ditch sample are presented in Tables G2a to G2f in Appendix G2. One *D. marginalis* adult and three *Dytiscus* larvae were caught during netting. Apart from this a total of 36 different RTUs were recorded during the sampling, including three vertebrates. The macro-invertebrate RTU count at each site is summarised in Table 7.1 below.

To compare the RTU counts from different sites, I performed One-way ANOVAs that showed there were significant differences between the sites in terms of RTU counts per site ($F_{5,12} = 5.9$, $P = 0.006$). When only the shaded sites were compared they too displayed significant differences between each other ($F_{2,6} = 6.5$, $P = 0.03$) as did unshaded sites ($F_{2,6} = 7.8$, $P = 0.02$).

Table 7.1: Summary of results from timed netting at study sites in May 2008.

Site	Date	Total RTU Count	Mean RTUs per ditch	Range
Shapwick Heath	5/5/08	13	5.7	3 - 9
Westhay Moor	7/5/08	10	5.0	3 - 8
Westhay Heath	9/5/08	20	11.7	10 - 13
Catcott North	11/5/08	8	4.7	2 - 7
Tadham Moor	10/5/08	16	8.7	7 - 11
East Waste	11/5/08	22	12.3	10 - 15

The site that was estimated to be richest in terms of the macro-invertebrate species it supported (East Waste) was the only one for which no evidence of breeding by *Dytiscus* species was found. No *Dytiscus* larvae were trapped there although adult *D. marginalis* and *D. dimidiatus* of both sexes were recorded. However, breeding by both *D. marginalis* and *D. dimidiatus* was demonstrated at the second richest site (Westhay Heath). Some caution needs to be exercised in interpreting the results, given that species-richness was estimated purely on the basis of netting experiments conducted in May which would have missed any macro-invertebrate species that become more abundant later in the summer and autumn.

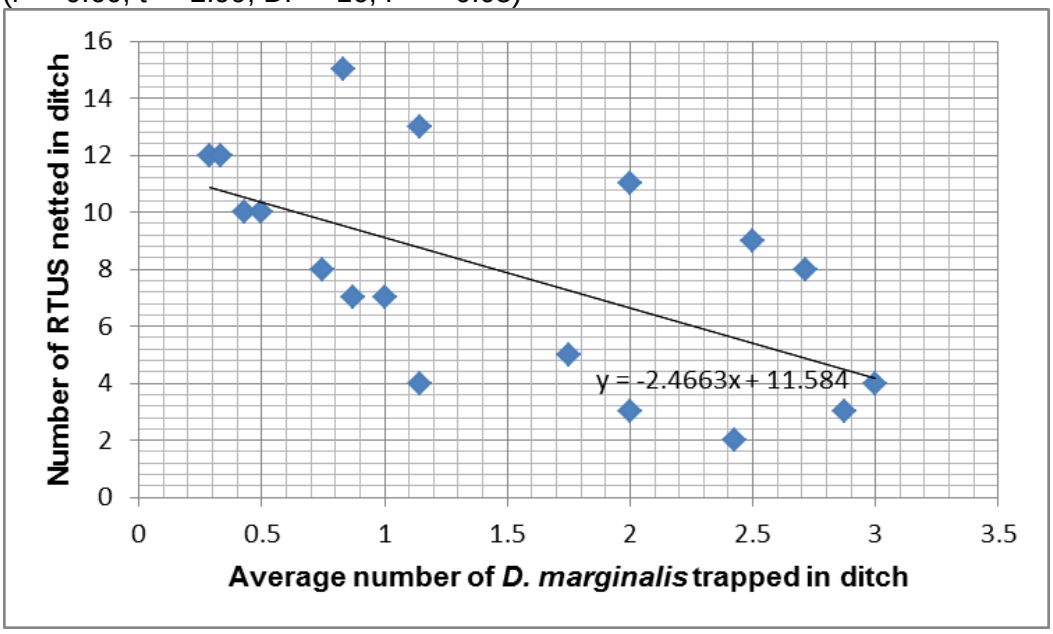
The average numbers of *D.marginalis*, *D. dimidiatus* and *Dytiscus* larvae caught in each ditch in 2008 were calculated. These data, (except for *D. dimidiatus* counts) and those on RTU numbers were normally distributed as determined using a Shapiro-Wilk Test (Chapter 2). The results of correlations between beetles and potential prey abundance are given in Table 7.2 below.

The only demonstrable significant relationship was a negative correlation between average numbers of *D. marginalis* adults trapped in ditches and the macro-invertebrate species richness of the ditches (as estimated from netting data). This relationship is graphed in Figure 7.1 below.

Table 7.2: Correlations between macro-invertebrate species richness and average numbers of Dytiscus spp. in 18 linear waterbodies on the Somerset Levels and Moors. Species-richness was estimated from counts of Recognisable Taxonomic Units (RTUs) obtained during netting in May 2008. * = significant correlation between the two variables (t = -2.99, DF = 16, P = <0.05)

	RTUs	Average *D. marginalis*	Average *D. dimidiatus*	Average *Dytiscus* Larvae
Average *D. marginalis*	**r = -0.60***	-		
Average *D. dimidiatus*	r_s = -0.05	r_s = 0.22	-	
Average *Dytiscus* Larvae	r = -0.21	r = 0.32	r_s = 0.34	-

Figure 7.1: Graph showing correlation between mean average numbers of *D. marginalis* trapped in a ditch and number of RTUs netted in the same ditch (r = -0.60, t = -2.99, DF = 16, P = <0.05)



I conducted a series of Spearman Rank Correlations between average numbers of individuals of *D.marginalis*, *D. dimidiatus* and *Dytiscus* larvae in the ditches and particular RTUs as follows: Other beetles including larvae (pooled data for all non-*Dytiscus* beetles netted); *Notonecta* sp.; *Ilyocoris cimicoides*; *Asellus* sp.; gammarid shrimps; planorbid snails; lymnaeid snails and *Sphaerium* sp. bivalves. In cases where RTU numbers were estimated, I chose the lowest value for the correlation (e.g. 100 where abundance was recorded as >100 <1000). No significant correlations were found between the RTU abundances and those of *D. dimidiatus*. Significant correlations were discovered involving *D. marginalis* and *Dytiscus* larvae and some RTUs and these are summarised in Table 7.3 along with the results of a Bonferroni Correction carried out. The

Correction was applied because with multiple comparisons there is the possibility of 'false' significant correlations due to chance.

Table 7.3: Sequential Bonferroni Correction applied to apparently significant Spearman Rank Correlations. (16 comparisons, P = Probability of Null Hypothesis. The P Value threshold was calculated sequentially according to the formula α/16, α/15, α/14….etc., where α is the 95% confidence interval = 0.05)

Correlation (n =20 in each case)	r_s Value	P	P Value threshold
Null hypothesis rejected (Significant correlation)			
D. marginalis vs Planorbid snail	-0.78	< 0.001	0.0031
Dytiscus larvae vs Gammarid shrimp	0.72	< 0.001	0.0033
D. marginalis vs *Sphaerium* sp. bivalve	-0.65	< 0.01	0.0036
Dytiscus larvae vs *Lymnaeid* snails	-0.62	< 0.01	0.0038
Null hypothesis accepted (No significant correlation)			
Dytiscus larvae vs *Asellus*	0.57	0.005	0.0042
D. marginalis vs *Asellus*	0.56	c.0.005	0.0046

The strong negative correlations in some instances between *Dytiscus* sp. and mollusc RTUs might indicate that the beetles were exerting an effect on mollusc numbers, although an alternative explanation could be that environmental parameters that favoured the beetles had the opposite effect on the molluscs. The significant positive correlation between abundances of *Dytiscus* larvae trapped and of gammarid shrimp netted might indicate a food preference exercised by the larvae or it might be due to similar responses to environmental parameters to those displayed by the shrimps.

7.2.2 Aquaria experiments

The number of paired aquaria (+beetle, -beetle) that could be set up in the time available for experimental work between October 2010 and June 2011 was limited by the number of *Dytiscus* beetles that could be trapped to stock +beetle aquaria and also by the number of potential prey items that could be obtained to place in even numbers in both + beetle and –beetle aquaria. In total I set up 50 aquaria equivalent to 25 combinations of + beetle and –beetle treatments. The numbers of individual beetles that were used to stock the +beetle aquaria were as follows: 14 *D.marginalis*; 6 *D. dimidiatus* and 5 *Dytiscus* larvae I.e. a total of 25 individuals. All the *Dytiscus* larvae used were subsequently demonstrated by COI sequencing to be *D. marginalis*.

Initially, experiments were allowed to run for one week before being stopped, but this was extended to a fortnight later in the study. The timings of the experiments are summarised in Table 7.2 below. The average maximum and minimum temperatures recorded over each period of time are given in the Table also.

Table 7.4: Summary details regarding the feeding experiments set up at the Peat Moors Centre, Shapwick 2008 – 9.

Time period 2010 - 11	Numbers of +beetle aquaria (For each of these there was an adjacent –beetle aquarium)			Average Temperature (ºC) in aquaria	
	Adult *D. marginalis*	Adult *D. dimidiatus*	*Dytiscus* Larvae	Minimum	Maximum
10 -17 October	4	-	-	10.8	21.7
17 – 23 October	4	-	-	3.0	20.3
17 April – 1 May	3	-	2	9.3	31.3
1 – 15 May	3	6	-	12.0	32.0
5 – 19 June	-	-	3	12.0	34.0
Totals	14	6	5		

On the first two occasions that experiments were conducted the bugs *Notonecta glauca* and *Ilyocoris cimicoides* were included among potential prey items because mature specimens were particularly abundant in watercourses at the time of year. This was not repeated subsequently for two main reasons. Firstly, abundance of mature specimens of these two species dropped appreciably so they became less easy to catch. Secondly, since these two species are both potential predators themselves, I decided to omit them from experimental set-ups so as not to complicate interpretation of results.

Potential prey items introduced to aquaria on a consistent basis throughout the period were the molluscs *Lymnaea stagnalis*, planorbid snails and *Sphaerium* sp. bivalves. A valved snail (*Bithynia* sp.) was introduced when it became available in sufficient numbers towards the end of the period.

The numbers of potential prey introduced to aquaria at the start of each experiment is contrasted in Table 7.5 with the numbers that remained in +beetle (*D. marginalis* adult) aquaria and –beetle aquaria when the experiments were halted.

Table 7.5: Numbers of potential prey surviving in 14 aquaria with adult *D. marginalis* as the + beetle treatment contrasted with survivorship in adjacent – beetle aquaria.

		+ Beetle aquaria		- Beetle aquaria	
Potential Prey	Number at start	Surviving at end	Mortality (%)	Surviving at end	Mortality (%)
Notonecta glauca	16	11	31.3	9	43.8
Ilyocoris cimicoides	16	11	31.3	14	12.5
Lymnaea stagnalis	72	51	29.2	67	6.9
Planorbid sp	24	20	16.7	22	8.3
Bithynia sp.	12	12	0.0	12	0.0
Sphaerium sp.	54	43	20.4	52	3.7
Totals	194	148	23.7	176	9.3

Only in the case of *L. stagnalis* and the *Sphaerium* sp. were there appreciably fewer specimens in the +beetle aquaria at the end of the experiment compared with at the beginning. In Table 7.6 the results are given for experiments in which the +beetle aquaria contained adult *D. dimidiatus*.

Table 7.6: Numbers of potential prey surviving in 6 aquaria with adult *D. dimidiatus* as the + beetle treatment contrasted with survivorship in adjacent – beetle aquaria.

		+ Beetle aquaria		- Beetle aquaria	
Potential Prey	Number at start	Surviving at end	Mortality (%)	Surviving at end	Mortality (%)
Lymnaea stagnalis	36	23	36.1	36	0.0
Planorbid sp.	12	10	16.7	11	8.3
Bithynia sp.	12	11	8.3	9	25.0
Sphaerium sp.	6	6	0.0	5	16.7
Totals	66	50	24.2	61	7.6

The result for *L. stagnalis* indicates that there was considerable predation of this mollusc by *D. dimidiatus* adults. There was little difference observed between +beetle and –beetle treatments with regards to other potential mollusc prey. In Table 7.7 the results are presented for pairs of aquaria where the +beetle treatment comprised *Dytiscus* larvae.

Table 7.7: Numbers of potential prey surviving in 5 aquaria with *Dytiscus* larvae as the + beetle treatment contrasted with survivorship in adjacent – beetle aquaria

		+ Beetle aquaria		- Beetle aquaria	
Potential Prey	Number at start	Surviving at end	Mortality (%)	Surviving at end	Mortality (%)
Lymnaea stagnalis	36	29	19.4	33	8.3
Planorbid sp.	10	7	30.0	10	0.0
Bithynia sp.	10	10	0.0	10	0.0
Sphaerium sp.	2	2	0.0	2	0.0
Totals	58	48	17.2	55	5.2

Caution needs to be exercised in interpreting the larval results since all of the +beetle mortality of *L. stagnalis* occurred in one aquarium.

Chi-squared tests were performed to establish if the there was a statistically significant difference between potential prey survival in the +beetle aquaria and –beetle aquaria. Only in the case of *D. marginalis* were enough experiments performed over the study period to allow valid tests to be conducted. In the case of *Lymnaea stagnalis*, $\chi^2 = 9.6$, DF = 4, P = <0.05. Therefore the null hypothesis was rejected. The +Beetle and –Beetle survivorship means were significantly different with survivorship in the –Beetle aquaria higher than in the +Beetle aquaria. However, there was no significant difference in the case of *Sphaerium* species ($\chi^2 = 0.8$, DF = 1, P = >0.05).

7.2.3 Trap data

143 larvae retained from traps were examined microscopically. Six of the smaller larvae were identified as non-*Dytiscus* species (1 *Acilius* sp., 5 *Colymbetes* sp.)**.** 65 of the larvae definitely identified as *Dytiscus* sp. were placed in condition categories 2, 3 and 4 described in section 7.1.3 and thought to be indicative of predation. This represented 47.7 % of larvae examined microscopically and 28.3% of the total number of larvae caught. The categories are shown Table 7.8.

Table 7.8: Results of condition assessment for 136 dead *Dytiscus* larvae.
Specimens were collected from traps placed at sites in the Somerset Levels and Moors 2006 – 2011.

Condition category	Number of larvae
Category 1 – Whole (No signs of injury or damage)	71
Category 2 – Injured/Damaged	16
Category 3 – Exoskeleton only	28
Category 4 – Fragments (specimen in pieces)	21
Total	136

Many of the larvae placed in Category 2 showed signs of damage to the ventral surface of the thorax but, other than this, were intact. Larvae in Category 3 typically were intact but lacked any internal organs or structure. There were no signs of a split in the skin, so specimens in Category 3 were assumed not to be cast-off exuviae but predated animals. Most of the specimens assigned to Category 4 were partial, sometimes comprising only the head capsule and its appendages.

For 42 of the specimens showing possible signs of predation, records were examined of other macro-invertebrates caught in the same traps. A summary of taxa caught along with these larvae is provided in Table G3 in Appendix G3. There were other *Dytiscus* specimens (adults, larvae or both) in all but four of the 35 traps from which I recorded larvae that appeared to be predated. It cannot be assumed automatically that other taxa present in the traps were responsible for the possible larval predation. In one case fragmentary larval remains were found in a trap without any other macro-invertebrates. Nevertheless, the presence of other *Dytiscus* larvae and/or adult *Dytiscus* beetles in so many traps in which predation was indicated inter-specific and intra-specific predation, particularly in situations where no other taxa were recorded. In many of the cases where other taxa were present, such as Horse Leech (*Haemopsis sanguisuga*) and medium-sized water beetles (e.g. *Ilybius ater*), these seemed unlikely predators of large *Dytiscus* larvae.

If the results from trapping experiments reflect predation of larvae by *Dytiscus* spp., it may be possible to infer both inter-specific and intra-specific predation by *D. marginalis* and possible inter-specific predation by *D. dimidiatus.*

In the one case where larval remains in a trap were identified as *D. dimidiatus*, the only other taxon recorded in that trap was an adult *D. marginalis* male. Similarly, there was circumstantial evidence for predation of *D. marginalis* larvae by *D. dimidiatus* adults from traps where only individuals of *D. dimidiatus* were recorded along with single dead *D. marginalis* larvae. *D. dimidiatus* larvae may have been responsible for predation of *D. marginalis* larvae in some traps and, in particular, one in which the only other taxon recorded was a specimen of *Hydaticus transversalis*, a medium-sized water beetle species.

With regards to intra-specific predation, no evidence was found of this by *D. dimidiatus*, although the numbers of larvae discovered was very low, so the possibility cannot be ruled out. By contrast, several examples were found of traps where damaged *D. marginalis* larvae were found alongside live specimens of either adults alone or solely larvae of the same species.

Given the possibilities that larval condition assessments mistakenly identified predation where it has not occurred or that other predators escaped from traps before being recorded, it is not feasible to 'prove' inter-specific or intra-specific predation by *Dytiscus* species. However, if it is assumed that predation occurred, it is possible to test if this was more likely to occur in traps with relatively high numbers of adults or larvae of *Dytiscus* spp. I examined data from 30 traps set in 2007 and 2008 where live *Dytiscus* specimens were found alongside larvae which, from their condition, I assumed were predated. I calculated means of *Dytiscus* numbers caught for all traps set on a particular date, for all traps where *Dytiscus* sp. were caught on that date, for traps in which predation was assumed to have occurred and for traps with *Dytiscus* but with no evidence of predation. The results are summarised in Table 7.9 below.

Table 7.9: Catches of *Dytiscus* spp. on dates on which predation was assumed.

Date	A. Average per trap	B. Average per trap with *Dytiscus*	C. Av. per trap with signs of predation	D. Av. per trap without signs of predation
22/04/2007	1.6	2.4	4.0	2.1
05/05/2007	3.5	4.3	7.0	3.8
20/05/2007	2.1	2.8	6.3	2.2
03/06/2007	2.7	3.3	5.3	2.7
17/06/2007	3.3	4.1	4.4	3.1
02/07/2007	1.4	2.6	3.3	2.3
16/07/2007	0.7	1.6	2.0	1.6
08/05/2008	0.7	1.4	1.0	1.5
10/05/2008	0.4	1.2	2.0	1.0
08/06/2008	1.0	1.9	5.0	1.4
15/06/2008	1.7	2.9	2.0	3.0
29/06/2008	0.5	1.3	2.0	1.2
13/07/2008	1.9	2.4	3.7	2.0
23/07/2008	0.6	1.3	2.0	1.0

To investigate whether there was a significant difference between the two sets of averages for traps with and without signs of predation on larvae I performed a two-tailed Wilcoxon Matched Pairs Test, making use of the fact that data was available on particular dates that could be paired for the purposes of the Test.

I compared the average numbers of *Dytiscus* individuals in traps with signs of predation (column B in Table 7.9) to the average numbers of *Dytiscus* in traps where *Dytiscus* were caught (column C in Table 7.9). In this instance the null hypothesis of no difference between the two medians was rejected. The two medians were significantly different ($t = 2.63661$, $P = <0.01$) with significantly higher numbers of *Dytiscus* in the traps where predation was assumed to have occurred.

7.3 Discussion

7.3.1 Relationship between *Dytiscus* abundance and macro-invertebrate species-richness

Various authors have remarked on how large macro-invertebrate predators such as dytiscid beetles, dragonfly larvae or hempiteran bugs are often the top predators in temperate wetlands, particularly in systems that lack fish or other vertebrate predators [e.g. Cobbaert *et al.* (2010), Vinnersten *et al.* (2009),

Fairchild *et al.* (2000), Batzer & Wissinger (1996), Mallory *et al.* (1994)]. Wellborn and his co-workers showed that, in shallow freshwater habitats lacking fish, it is these large macro-invertebrate predators that are the critical determinants of the species composition of prey communities [Wellborn *et al.* (1996)]. Recent studies of *Dytiscus alsakanus* in fish-free ponds in Alberta, Canada have shown that total macro-invertebrate biomass was less in systems with *D. alaskanus* than without [Cobbaert *et al.* (2010)]. Although overall species richness was not altered significantly, the composition of communities did appear to be affected by the beetle. Other macro-invertebrate predators, snails and *Gammarus lacustris* decreased in numbers when the dytiscid was introduced to ponds. The correlations reported in Table 7.3 above suggested a negative linear relationship between adults of the commonest *Dytiscus* species in ditch systems (i.e. *D. marginalis*) and planorbid snails and Pea Mussels (*Sphaerium* spp.) and a similar relationship between pond snails (Lymnaeidae) and Dytiscus larvae.

While the smaller water drainage ditches of the Somerset Levels and Moors are completely fishless, the fish species that were observed at the study sites tended to be the smaller species – mainly Sticklebacks (either Three-spined [*Gasterosterus aculeatus]* or Ten-spined [*Pungitius pungitius*]) – that can tolerate shallow, frequently oxygen-depleted water . Because of this one might anticipate that large dytiscids could exert an appreciable influence over macro-invertebrate communities and this may be the explanation for the observation that macro-invertebrate species-richness was negatively correlated with numbers of the most abundant *Dytiscus* species, *D. marginalis*.

7.3.2 Food preferences and predation of gastropod molluscs

Vinnersten *et al.* (2009) characterised dytiscids as “generalist predators” but pointed out that some species show definite prey preferences. Johansson & Nilsson (1992) offered a choice of food items to larvae of *D. latissimus* and *D. circumcinctus* and found that they preferred cased caddis (Trichoptera) to other prey. They concluded that these species – not present in the Somerset Levels – and one that is (*D. semisulcatus*) are specialist predators of cased caddis

larvae. In similar experiments the larvae of some North American *Dytiscus* species that co-exist in the same habitats exhibit definite prey preferences: *D. verticalis* for tadpoles; *D. harrisii* for caseless caddis flies [Leclair *et al.* (1986)].

With regards to *D. semisulcatus*, Beebee (1991) commented on the larva's habit of "*scuttling across the bottom of the pond*" which is connected, it was assumed, with searching for caddis larvae. The targeting of cased caddis larvae may also explain an aspect of larval morphology that *D. semisulcatus* shares with larvae of *D. latissimus,* namely that of a relatively narrow head compared with neck width. Figures in Johansson and Nilsson (1992) indicate that when attacking *Limnephilus* sp., a caddis larva with a fairly robust case, the head of the *D. latissimus* larva is often pulled inside the narrow entrance of the case as the caddis fly withdraws into its refuge. The absence of the narrow-headed trait among any of the larvae collected during this study was one argument used to dismiss the idea that any belonged to *D. semisulcatus*. The relative rarity of *D. semisulcatus* adults and absence of any clearly identifiable larvae among the material collected in this investigation may be related to the low frequencies at which cased caddis larvae tended to be found at the study sites.

Although a particular species of Dytiscus may demonstrate a pronounced preference for certain classes of prey and may even be somewhat specialised to prey upon that item, this does not mean that the beetle will not exploit other sources of food when they are in abundance. Cobbaert *et al.* (2010) found that *D. alaskanus* showed a distinct preference for preying upon large macro-invertebrate predators (e.g. *Notonecta* spp.) to the extent that a positive effect could be discerned upon zooplankton due to predator removal. However, it was also shown that large numbers of molluscs were taken by *D. alaskanus*. Predation of molluscs occurred to such an extent that the *Dytiscus* species influenced the freshwater community structure by eliminating grazing snails and increasing periphyton.

It has been suggested that gastropod molluscs may form an important part of the diet of *D. dimidiatus*. In his review of the scarce and threatened Coleoptera of Great Britain, Foster (2010) mentions that: "*Rondelaud (1979) demonstrated that* D. dimidiatus *will consume the dwarf pond snail* Galba truncatula *(Müller) both in the field and in the laboratory.*" Although this is true, Rondelaud concluded that in nature the snails do not form anything more than an incidental source of food for the beetles. The aquaria experiments conducted as part of my study indicate that both *D. marginalis* and *D. dimidiatus* adults will predate pond snails (in this case *Lymneaea stagnalis*) if presented with them in artificial conditions, but it remained unclear whether either *Dytiscus* species are specialist predators of these molluscs. The observation that *D. dimidiatus* were more abundant at sites (e.g. Shapwick) that were heavily shaded argues against such a specialisation in this species, as the shaded areas tended to be those without large numbers of pond snails. There was a significant negative correlation between numbers of lymnaeid snails netted and average percentage shade cast by trees and shrubs on ditches (r_s = -0.67, DF = 18, P < 0.01).

7.3.3 Evidence for intra- and inter-specific predation among *Dytiscus*

The extent of injury in some specimens of *Dytiscus* larvae collected during this study ranged from localised damage to part of the body to almost total destruction of the animal. Many instances were encountered of larvae that lacked any internal structure at all but which, in all other respects appeared intact. A plausible interpretation of these observations is that those larvae with partial damage and those which had been hollowed out were victims of attacks by other *Dytiscus* larvae whereas the fragmented larvae had been killed by adult beetles or other kinds of predator.

Dytiscus larvae possess highly specialised mouthparts with large jaws or mandibles that are folded under the clypeus when not in use. During an attack by the larva, the mandibles stab deep into the prey and paralysing toxins and digestive enzymes are pumped into the prey interior from openings near the tip of each mandible. The larva can only take in the dissolved tissues from the prey by sucking these back through the mandibles using a 'pharyngeal pump'.

This pump works only when the mandibles are folded back into their resting position [Weber (1933), Wichard *et al.* (2002)]. These details explain the observation made during fieldwork that live larvae were often found in baited traps clamped by their jaws to the bait. Dead larvae placed in category 3 in Table 7.6 ('Exoskeleton only') were therefore likely to have been predated by other *Dytiscus* larvae. A few other aquatic predators (notably hemipteran bugs) pierce their prey and inject digestive enzymes but (as Table G3 shows) the traps that contained exoskeletal remains of *Dytiscus* larvae rarely contained bugs and almost invariably contained other *Dytiscus* larvae.

Many of the category 2 larvae ('injured/damaged') were also likely to have been predated by other *Dytiscus* larvae. Foramanowicz (1984) studied the phenomenon of partial consumption of prey by *D. verticalis* larvae and concluded that the amount of each prey item ingested by a predating larva decreased as a function of prey density. From this it might be predicted that category 2 larvae would be found most frequently in traps with the highest numbers of trapped larvae. As reported in 7.3.3, there was evidence to suggest that predation of *Dytiscus* larvae by other *Dytiscus* individuals was indeed most often seen in traps with a higher than average catch of *Dytiscus* specimens.

So far as fragmentary larvae were concerned, adult beetles were often found in the traps with such remains (see Table G3 in Appendix G3). However in some cases no adults occurred, although other possible predators were recorded such as *Dytiscus* larvae or, in one instance an adult Smooth Newt (*Triturus vulgaris*). In only one trap were fragmentary remains found but no signs of any other organism. However, the escape rate of dytiscids from traps was not investigated and during the course of the study Water Shrews (*Neomys fodiens*) were found dead in three traps and it is possible that these very active predators might have been able to subdue and kill larvae in the traps, eat them *in situ* and escape.

Chapter 8: Study Findings

8.1 Techniques

It was recognised at the beginning of the investigation that two important technical challenges had to be overcome to achieve the study's primary objectives (see Chapter 1):

a. A technique had to be found to capture relatively large numbers of the subject animals for study; and
b. A means had to be found to distinguish the larvae of *D. marginalis* and *D. dimidiatus* from each other and from any other *Dytiscus* species that might be encountered in the Somerset Levels and Moors.

8.1.1 Trapping was shown to be a useful technique to capture adults and larvae of the target species

During fieldwork for this study 208 *D.dimidiatus* and 434 *D. marginalis* adults were trapped between 2007 and 2008. This compared with 21 records for D. dimidiatus and 37 for D. marginalis in the Somerset Environmental Records Centre (SERC) database (Tony Price, *pers. comm*.). Most of the records for *D. dimidiatus* in SERC's possession are due to Duff (1993) who gave about 60 records also for *D. marginalis*. As reported in Chapter 3 (Table 3.2), these figures for trapping compared well with the results from contract surveys that have employed netting as a technique to survey ditches, not only in terms of total catch but also with regards to average numbers caught per sample.

889 netted samples taken during 15 contract surveys obtained *17 D. dimidiatus* and 32 reported *D. marginalis* - capture rates of 0.02 beetles per sample and 0.04 beetles per sample for the respective species. In 2007 I set 500 baited traps and 645 in 2008, making a total of 1145 baited traps set. If each trap is taken to represent a sample taken then the relative capture rates per sample were: *D. dimidiatus* 0.18 beetles per sample and *D. marginalis* 0.38 beetles per sample. In each case there was an almost tenfold difference in capture rates between netting and trapping in favour of the latter.

Between 2007 and 2008 I trapped a total of 233 larvae (or 0.20 larvae per trap sample). Only 17 larval specimens (7.3% of the total catch) were positively identified as *D. dimidiatus* whereas this species formed 32.4% of the adult captures. From this it would have appeared that trapping was a less effective means to catch larval *D. dimidiatus* than it was to capture adult specimens, but the lower proportion of larvae caught was probably more a reflection of the concentration of larvae in a few sites and in a relatively few locations within those sites.

8.1.2 DNA Sequencing was shown to be a reliable tool for distinguishing *Dytiscus* larvae

My study has shown that it is possible to extract DNA from single legs taken from *Dytiscus* larvae and to determine species identity in 90% of cases using sequences from the Cytochrome Oxidase 1 (CO1) gene. This molecular ecological approach yielded positive identifications with a degree of certainty and reliability that contrasted with methods relying on keys and biometrical analysis. It had the advantage also that determinations of the species identity of live larvae could be made using material collected in the field in a non-lethal manner, whereas approaches relying on morphology tend to require that specimens are killed for subsequent analysis. Beebee (*pers. comm.*) has estimated that the cost of identification by sequencing was about £7 per specimen at current prices, making this also a relatively cheap option.

There are records of *D. dimidiatus* over many decades at some well-studied wetland sites in the UK [e.g. Wicken Fen, Askham Bog] so successful breeding within this country may be inferred, but, so far as is known, my study is the first that definitely proved breeding in the UK by positively identifying larvae as belonging to *D. dimidiatus*. Breeding was shown to have occurred at three localities – Shapwick Heath, Westhay Heath and Tadham Moor – although adults of this species are known to range much further afield than within the Somerset Levels and Moors. The contrast between the numbers of known adult locations and those of larvae may be an artefact of sampling that might be corrected with further collection of larvae, or it could indicate that conditions for

successful breeding occur in only a handful of places in the Levels and Moors. If the latter possibility is the case, this would have profound implications for the maintenance of the species as a feature of the Ramsar wetland.

8.2 Niche breadth and overlap

Two of the primary objectives of the study were:

- To investigate whether ecological niche separation can be observed in populations of *D. marginalis* and *D. dimidiatus* living in the Somerset Levels and Moors;
- If niche separation can be demonstrated, to identify how separation is achieved. Is the separation primarily on a temporal basis; is it habitat-based, or is it due to food preferences? To what extent can it be said that either species display the traits of a 'generalist' or 'specialist'? (Answering these questions will involve measurements of niche breadth and niche overlap of the two species.).

8.2.1 Niche Metrics

The theoretical basis of the niche measurements made was explained in Chapter 2 (section 2.7). There it was noted that all similarity indices are sensitive to a greater or lesser degree to the effects of sample size. Although it is one of the least sensitive to sample size, Morisita's Measure is not completely immune to bias (in the sense of poorly estimating the true value of the similarity index) if sample sizes are very small. With regards to adult beetles the smallest total number of beetles used in the analyses was 63 *D. dimidiatus* trapped at six sites during 2008. This compared with 204 individuals of *D. marginalis* recorded in the same year at the same study sites. Fewer larvae than adults of the corresponding species were caught in both fieldwork seasons and the numbers of *D. dimidiatus* larvae positively identified as such was especially low (7 in 2007, 10 in 2008). Given such low numbers of individuals, an accurate estimate of niche breadth is not easily obtained for *D. dimidiatus* larvae and the niche overlap between this species and *D. marginalis* cannot be measured with confidence for the larvae.

8.2.2 Temporal niche separation and seasonality

In Chapter 5 data was presented about the numbers of individual adults of *D. marginalis* and *D. dimidiatus* active on particular days spread over a year (January 2007 to January 2008). Taking the days when traps were set as resource states that the species might utilise, temporal niche breadth and niche overlap calculations were performed. The results are given in Table 8.1 below.

Table 8.1: Temporal niche breadths and niche overlap in relation to numbers of adult *Dytiscus* species trapped at Shapwick Heath National Nature Reserve on 25 days spread over one year. B = Levins's Measure of Niche Breadth; B_A = Standardised Levins's Measure; and C = Morisita's Index of Similarity/Measure of niche overlap.

	B	B_A
D. marginalis adults (n = 230)	12.1	0.46
D. dimidiatus adults (n = 145)	14.5	0.56
D. marginalis larvae (n = 121)	5.3	0.18
D. dimidiatus larvae (n = 7)	3.3	0.10
C for *D. marginalis* and *D. dimidiatus* adults = 1.00		
C for *D. marginalis* and *D. dimidiatus* larvae = 1.00		

As illustrated in the Graphs in Figures 5.2 to 5.4, the activity patterns of the adults of the two species appeared similar over the study period. There were distinct peaks in activity in spring and late summer but both species were active to some degree over the whole year, which explains the mid-range values for the standardised niche breadth measurement.

The activity of late instar larvae was much more confined to a period during the summer months than that of the adults. This is reflected in the relatively low values for the measures of niche breadth. It should be noted that the category '*D. marginalis* larvae' used in Table 8.1 comprises all larvae collected in the study period less those positively identified as *D. dimidiatus* through sequencing of the COI gene. If it is assumed the ratio of *D. marginalis*: *D. dimidiatus* is the same for unidentified larvae as it is for identified ones, it is estimated that no more than three of the 121 total were unrecognised *D. dimidiatus*.

Morisita's Measure indicated there was no discernible temporal differentiation between the niches occupied by *D. marginalis* and *D. dimidiatus* in terms of adult or larval activity.

8.2.3 Micro-habitat, niche separation and trap location preferences

In this analysis each of the permanent trap locations at Shapwick Heath used between January 2007 and January 2008 was considered as a separate resource state. Some authors have objected to the use of "*arbitrarily defined states like …artificial sampling units*" to measure niches [Krebs (1998)]. In basing one of my niche measurements on use of trapping locations, I have assumed that, where species displayed a clear preference for particular trap locations at a study site, this was due to the characteristics of the micro-habitat at the trap locations. An obvious objection to this would be if the trapped individuals were lured far away from their preferred habitat by the bait placed in the traps. An additional assumption that I have made is that animals caught in traps have not moved very far from the locations they prefer and that, therefore, the habitat and even the micro-habitat at the trap site bears close resemblance to those preferred by the trapped individual.

There appears to be little information in the literature about mobility of the target species, but I note that, in the species account for *D. dimidiatus* in his review of scarce Coleoptera, Foster quoted a mark-recapture study by Brancucci (1980) which Foster interpreted to show "*movements* [occurred] *between three ponds in a 150 metre long row but not into ponds a further 100 metres away*". This would tend to support my underlying assumption that the animals caught in traps are not very mobile and have not come from so great a distance away that inferences may not be drawn concerning habitat or even micro-habitat preferences.

The number of adults and larvae caught at each trapping location varied over the year (see section 5.2.6) and it was demonstrated earlier that there were statistically significant differences between trap locations at least in catches of adult male and larval *D. marginalis*. (Chi-squared statistical tests could not be performed with regards to other categories of catch.) Total adult catches at trap locations varied between 6 individual *Dytiscus* beetles caught at location 9 to 31 records from location 12. At two trap locations (4 & 20) only one larva was caught during 2007 contrasting with trap location 11 where 24 larvae were trapped.

Trap niche measurements based upon how the species apportioned themselves among the traps are summarised in Table 8.2 below.

Table 8.2: Trap Niche breadths and niche overlap in relation to numbers of *Dytiscus* species trapped at Shapwick Heath National Nature Reserve at 20 trapping locations. B = Levins's Measure of Niche Breadth; B_A = Standardised Levins's Measure; and C = Morisita's Index of Similarity/Measure of niche overlap.

	B	B_A
D. marginalis adults (n = 230)	17.1	0.85
D. dimidiatus adults (n = 145)	15.2	0.75
D. marginalis larvae (n = 121)	11.0	0.53
D. dimidiatus larvae (n = 7)	5.4	0.23
C for *D. marginalis* and *D. dimidiatus* adults = 0.91		
C for *D. marginalis* and *D. dimidiatus* larvae = 1.67		

From Table 8.2 it can be seen that the niche breadth measures indicate a quite even usage of the trap locations by the adult beetles, with *D. dimidiatus* having a slightly narrower niche than *D. marginalis*. The *D. marginalis* larvae displayed a much more skewed distribution among the trap locations (four traps accounting for over 50% of the total catch). The calculations for the larvae indicated that *D. dimidiatus* larvae showed an even greater preference for particular trap locations, although it must be remembered that overall numbers caught were very low.

In sections 5.2.7 a series of Canonical Correspondence Analyses (CCAs) were reported that investigated the association between beetle activity and a range of different environmental parameters measured at Shapwick Heath. Only in one case could a negative correlation be discerned - between duckweed cover and larval frequency.

The value of Morisita's Measure calculated for the adult beetles is evidence of a very strong overlap between *D. marginalis* and *D. dimidiatus* in terms of the trap locations preferred. The value of the Measure calculated for the larvae greatly exceeded 1.0 and in such circumstances it was difficult to interpret the result, a problem identified by Chao *et al.* (2006). The high value is due to the high number of *D. marginalis* larvae caught relative to the low number of *D.*

dimidiatus larvae. Chao *et al* (Op. cit.) point out that the Morisita measure is highly sensitive to the most abundant species.

8.2.4 Habitat preferences, niche separation and shade

In Chapter 6 evidence was presented that *D. dimidiatus* adults and *Dytiscus* larvae prefer shaded ditches to more open waterbodies. It was demonstrated that there were significant differences between the six study sites investigated in 2008 not only as regards tree cover and shade but also with respect to a range of other environmental parameters (see section 6.3.2.2). Each of the study sites could be considered as different habitat resources available to *Dytiscus* species living within the Brue Valley. Realistically, only the adult beetles would be able to access all of these resources by flying between sites and the mark-recapture studies reported earlier [i.e. Brancucci (1980)] tend to suggest that *D. dimidiatus* at least is not very mobile. However, assuming that there are no barriers to free interchange of individuals between the sites and the distances between them are not too great, then it is possible to consider each site as a resource state that the populations of adult beetles could exploit.

If niche metric calculations are performed treating the sites as separate resource states then one obtains the results summarised in Table 8.3 below.

Table 8.3: Habitat Niche breadths and niche overlap in relation to numbers of *Dytiscus* species trapped at six study sites in 2008. B = Levins's Measure of Niche Breadth; B_A = Standardised Levins's Measure; and C = Morisita's Index of Similarity/Measure of niche overlap.

	B	B_A
D. marginalis adults (n = 204)	4.4	0.68
D. dimidiatus adults (n = 63)	3.5	0.50
D. marginalis larvae (n = 65)	4.0	0.60
D. dimidiatus larvae (n = 10)	1.5	0.10
C for *D. marginalis* and *D. dimidiatus* adults = 0.94		
C for *D. marginalis* and *D. dimidiatus* larvae = 0.35		

While it is tempting to view these results as providing further evidence that *D. marginalis* is more of a generalist species than is *D. dimidiatus*, the underlying assumptions concerning free accessibility of the habitats remains untested.

Until data is collected to support the view that the sites are all equally accessible to adults, then these results ought to be treated with some caution. These remarks apply with equal force to the larval results since it is seems logical that larvae will be found only at sites that adults have been able to access.

The high degree of niche overlap in the adults seems to contradict the results from the 2008 season's fieldwork reported in Chapter 6. There evidence was presented that adults of *D. dimidiatus* displayed a preference for shaded waterbodies. It was noted that: "*Adults of D. dimidiatus were found at all six study sites but, when paired visits to shaded/unshaded sites were undertaken on particular days, statistically significant numbers of adult D. dimidiatus were found at the shaded sites.*" One possible explanation for the high overlap is that the niche calculations were performed using the whole year's data and not just that collected on the dates of the paired visits. It may be the case that the preference for shaded sites is a relatively weak one that does not extend over the whole year.

The niche overlap for the larvae in relation to sites/habitats was far less than for adults. The niche breadth measures indicated that *D. marginalis* was more of a generalist than *D. dimidiatus* in relation to the sites at which it bred. Eight out the ten larvae caught during 2008 that were positively identified as *D. dimidiatus* were trapped at Westhay Heath, so a very high proportion of the total number of larvae of this species were found in one place compared with *D. marginalis*.

All but one of the *D. dimidiatus* larvae were captured at shaded sites, which I have interpreted as supporting the view that breeding sites tend to be ones with shaded waterbodies. However, in Chapter 6 I acknowledged that: "*There was also an indication that larvae of D. marginalis as well as of D. dimidiatus might be more commonly found in shaded waterbodies".*

8.2.5 Prey preferences and niche separation

As reported in Chapter 7, feeding experiments were conducted by introducing live potential prey to aquaria and comparing prey item survivorship rates between those aquaria with beetles and those without. As explained previously, problems were encountered in obtaining sufficient specimens of potential prey, so there is too little data available to use in a meaningful analysis of niche in terms of food. Despite this, evidence was obtained to suggest that adults of both *D. marginalis* and *D. dimidiatus* as well as larvae of *D. marginalis* will take the pond snail *Lymnaea stagnalis* in artificial conditions.

In section 7.3.1 significant negative correlations were reported between numbers of *D. marginalis* trapped and numbers of certain mollusc taxa collected in netted samples, including lymnaeid snails. There was also a significant negative correlation noted between beetle abundance and overall macro-invertebrate diversity. These results indicated a possible preference shown by some *Dytiscus* species for lymnaeid snails and they are similar to those obtained by Rondelaud (1979), who found significant predation of *Galba (Lymnaea) truncatula* (Müller) by *D. dimidiatus* both in the field and in the laboratory in the Vendée Department of south-western France (an area with may similarities to the Levels and Moors in terms of landscape and land use). In this study no particularly strong differentiation was observed between *D. marginalis* or *D. dimidiatus* in respect of their predation upon pond snails, so I consider it unlikely that this is a defining aspect of niche separation between the two species. The suppression of snail populations by *Dytiscus* beetles is, however, an important aspect of their role within eco-systems that I have remarked upon in Chapter 1 in relation to disease control.

8.3 Competition

One of the objectives set at the beginning of the study was to determine whether there is good evidence to suggest that there was inter-specific or intra-specific competition occurring between *D. marginalis* and *D. dimidiatus.* One aspect of competition that was to be assessed was inter-specific and/or intra-specific predation.

8.3.1 Applying Wiens' criteria

Wiens' criteria for the assessment of evidence for inter-specific competition were reproduced in Table 1.1 in Chapter 1 [Wiens (1989)]. Taking each criterion in turn I have attempted to apply them to what has been discovered in this study concerning *D. dimidiatus* and *D. marginalis* in the Somerset Levels and Moors.

8.3.1.1 Have 'checkerboard patterns' of distribution 'consistent with predictions' been observed?

Considerable overlap was observed in terms of the sites and locations within sites (i.e. trapping stations) where adults of the two species were found. With regards to the larvae, the results are somewhat inconclusive, bearing in mind that relatively few larvae of *D. dimidiatus* have been positively identified, although the lack of specimens of this species could be taken as evidence suggesting the larvae are largely absent in locations where *D. marginalis* is present.

8.3.1.2 Do the species overlap in resource use?

Considerable overlap in terms of space occupied and seasonality were indicated by some of the niche measurements. There was evidence also that adults at least and, possibly larvae, of the two species both predate upon pond snails.

8.3.1.3 Has Intra-specific competition been demonstrated in either species?

This has not been definitively shown. Some evidence suggestive of larval predation by conspecific adults and larvae was produced in Chapter 7 (see sections 7.2.3 and 7.3.3).

8.3.1.4. Does resource use by one species reduce its availability to the other species?

This is difficult to answer without knowledge concerning the abundance of resources. For example, it is not known what numbers of pond snails might be available in the wild to *Dytiscus* species or the extent to which different *Dytiscus* species feed upon them.

8.3.1.5. Is there evidence of one species being negatively affected?

Without population data on *D. marginalis* and *D. dimidiatus* in the Somerset Levels this is not possible to answer.

8.3.1.6. Are there alternative process hypotheses that are consistent with patterns of distribution?

Since the patterns of distribution are poorly understood as yet (particularly with regards to breeding sites), it is difficult to frame alternative hypotheses.

From the above, I would conclude that no strong evidence has been produced to indicate that inter-specific competition is occurring between *D. dimidiatus* and *D. marginalis* in the Somerset Levels and Moors, although some potential lines of enquiry suggest themselves whereby this could be investigated (see the discussion under section 8.5).

8.3.2 Evidence for inter-specific and intra-specific predation

Inter-specific or intra-specific predation was not witnessed as such, but, as discussed in section 7.3.3, the examination of injuries to larval specimens and analysis of trapping results provided persuasive evidence that the larvae of both *D. dimidiatus* and *D. marginalis* were predated in traps by adults and larvae of both species. It is not known to what extent similar predatory behaviour occurs in the wild and there was an indication that instances of such predation assumed to have happened occurred in traps with higher than average densities of larvae, a situation that is unlikely to occur in nature except in very exceptional circumstances, such as in temporary waterbodies that dry out towards the end of the summer period when they may still be occupied by considerable numbers of larvae.

8.4 Conservation status of *D. dimidiatus*

An objective set for this study was to consider how this study might throw some light on the conservation status of *D. dimidiatus* at an international, national (UK) and local (Somerset Levels and Moors) scale. This is examined below alongside an evaluation of the characteristics of the *Dytiscus* species that occur in the Somerset Levels and Moors to gauge if any would make good candidates as 'flagship' and/or 'indicator' species.

8.4.1 In what sense is D. dimidiatus 'rare'?

In Chapter 1 (section 1.1.5), I drew attention to how it was feasible for an organism to be 'rare' in the wild yet not to be under any sort of threat that would merit conservation measures to prevent it becoming extinct. The 'seven forms of rarity' in Table 1.2 that were identified by Rabinowitz *et al.* (1986) provide a useful starting point for considering whether conservation measures are appropriate. I would place *D. dimidiatus* tentatively in 'category 3' of rarity as defined by Rabinowitz *et al.*, which is to say it is an organism with a wide geographical range within north western Europe (as demonstrated by the distributional maps in Appendix A3) but a restricted habitat (fen-type wetlands).

For a rare species in category 3 to persist, Rabinowitz *et al.* assumed that somewhere there must be relatively large populations. My study indicated that the Somerset Levels and Moors might be such a location; since *D. dimidiatus* approached the same levels of adult abundance in catches at some study sites as the commonest European *Dytiscus* species (*D. marginalis*). However, there is no reliable population estimate for the species in the Levels and Moors and there would be considerable merit in attempting to gauge the numbers that might occur typically in the ditch systems of some key sites (see section 8.6 below).

Earlier (in section 5.3.2) it was suggested that the ratio of *D. dimidiatus*: *D. marginalis* in traps might be a more a reflection of differences in activity levels between the species rather than, for example, of it being an indication of relative abundance of the species at particular locations. Differential activity levels were invoked previously to explain observed differences in sex ratios in traps, so a similar explanation might account for inter-specific ratios. Mean average *D. dimidiatus* caught per visit: mean average *D. marginalis* per visit was 1:1.59 at Shapwick Heath in 2007 and 1:2.84 in 2008. If the interspecific ratio was influenced mainly by differences in activity levels one might expect these to be similar between years. There was a difference in the ratios at this one site when two years' worth of data are compared, but while this is suggestive that differential activity levels might not be influencing ratios greatly, it is not conclusive. Data from trapping over a longer period would be needed to reach sound conclusions.

Despite being a large and readily identifiable water beetle it is conceivable that populations of *D. dimidiatus* could be missed due to lack of sufficient survey or, possibly, the use of inappropriate survey methods. Thus even at an extremely well-recorded site such as Wicken Fen in Cambridgeshire there can be long gaps in recorded occurrence. Omer-Cooper and Tottenham listed *D. dimidiatus* among the beetle species present at Wicken Fen in the early 1930s [Omer-Cooper & Tottenham (1932)] but the National Trust reported four captures (by A & G Foster) in 2007 and noted that: "*The last Wicken records of this rare*

(RDB2) species were 1998 and 1946" [National Trust (2007)]. It is possible for the species to be overlooked in whole regions of a country, as demonstrated by the announcement in 1997 of the "rediscovery" in Flanders of *D. dimidiatus* after a period of over 30 years (Vijver *et al.* (1993)].

Despite the above, it seems likely that the map for *D. dimidiatus* in the UK that is reproduced in Appendix A4, with a small, and probably diminishing, number of 10 km squares with recent records, offers a fair reflection of the true distribution of the species. Therefore, so far as the UK is concerned, the species should be considered under a degree of threat and its RDB3 status is probably merited.

8.5 Implications of study for conservation practice

8.5.1 Would any *Dytiscus* species make good 'flagship' or 'indicator species for conservation in the Somerset Levels and Moors?

Dytiscus water beetles are large insects with the potential to be of interest to the public. In my opinion, *D. marginalis* is too widespread and common for it to be a useful flagship for focussing attention on the Somerset Levels and Moors *per se*, but it could have use as a flagship for general freshwater conservation. *D. dimidiatus* has one of its UK strongholds in the Levels and Moors (see UK distribution map in Appendix A4) and it is mentioned in the Ramsar Citation for the internationally designated wetland (see Appendix A8). Another large, charismatic beetle that has a UK stronghold is the Greater Silver Water Beetle (*Hydrophilus piceus*) and a strong case was made by Beebee & O'Neil (2005) for this to be regarded as a flagship species on account of its clear association with a diminishing and threatened habitat, namely well-vegetated drainage ditches in floodplain grazing marsh. The linkage of *D. dimidiatus* with old fenland sites and grazing marsh drainage ditches could make it a useful species for consideration alongside *H. piceus* and, possibly, other water beetles as a flagship for the Levels and Moors. The possible association noted by Boyce (2004) of *D. dimidiatus* with locations used for breeding by the Lesser Silver

Water Beetle (*Hydrochara caraboides*) might indicate that a suite of water beetle species could be adopted for the purpose of promoting aspects of aquatic invertebrate conservation in the Levels and Moors.

There is some evidence from my study that *D. dimidiatus* abundance in localities may be negatively correlated with high duckweed cover and high electrical conductivity of waters. Since high duckweed cover and high conductivity may both be associated with agricultural pollution, it is possible that absence of *D. dimidiatus* from otherwise suitable habitats could be an indicator of water quality problems. It would be probably more accurate and cost effective, however, to measure water quality parameters directly or to use a suite of aquatic invertebrates as indicators (as is used, for example, by the Environment Agency in river water quality monitoring) than to rely on this species alone (particularly as the detailed relationship between *D. dimidiatus* abundance and shade, conductivity and duckweed cover is yet to be fully worked out).

As demonstrated in Chapter 7, I found some evidence that *D. marginalis* numbers might be negatively correlated with overall aquatic invertebrate diversity, while other authors [e.g. Cobbaert *et al.* (2010)] have shown that predatory *Dytiscus* can significantly affect invertebrate community structure. While this might encourage a view that numbers of *Dytiscus* species might be negative predictors of diversity, the effect on some parts of the community might be to increase diversity through the removal of other insect predators [see Cobbaert *et al.* (*Op. cit*.)]. Given this, it would seem better to measure the taxonomic richness of a community directly rather than to rely on one species for an indication of diversity.

8.5.2 Habitat requirements of *D. dimidiatus*

With respect to habitat selection by *D. dimidiatus* in Germany, Braasch (1989) concluded that larvae and adults had different requirements. So far as adult beetles were concerned, Braasch reported that the size of a water body and its

permanence was relatively unimportant. Over the course of three years (1986 - 89) Braasch found adult beetles in a range of different still waters and he formed the view that *D. dimidiatus* adults were relatively flexible with regards to the water bodies they would frequent so long as these habitats catered for some aspect of adult requirements (in terms of breeding, food, hibernation, dispersal, etc), whether this was over the long term or in the short-term [Braasch (*Op. cit*.]. Nevertheless, according to Braasch, the adults displayed some preferences for particular types of macro- and micro-habitat, tending to favour richly-vegetated still waters and being found more often in fens than in peat bogs. In contrast to the adults, size of water body seemed important to the larvae identified as *D. dimidiatus* in Braasch's study and he reported that: "*Nymphs require temporary, semipermanent* [sic] *and permanent waters of mostly small size*". It was crucial that water should remain over the whole period of larval development (which in his study area, like mine, was generally between May and June). Braasch also concluded that larval waterbodies must have "*little cover of Lemnaceae*" (i.e. duckweed), provide an abundance of prey organisms and support a diversity of vegetation; water bodies with dense algal cover and/or eutrophic conditions were not favourable to *D. dimidiatus* larvae [Braasch (*Op. cit*.)].

My findings agree with many of Braasch's. In particular, I found the adults of *D. dimidiatus* in all six study sites that I investigated and I could not detect a strong niche separation in the adults between *D. dimidiatus* and *D. marginalis,* the latter being considered a good example of a generalist species with a wide ecological niche. The larvae of *D. dimidiatus* were far more restricted than the adults and were found in much fewer numbers than were *D. marginalis* both in overall terms and in proportion to the adults, which would support the hypothesis that the larval needs of *D. dimidiatus* are different from those of the adult beetles. A relationship between *D. dimidiatus* larvae and reduced duckweed cover was also suggested by my fieldwork at Shapwick Heath in 2007, although this was not replicated in 2008.

With regards to eutrophication, breeding at Tadham Moor (the most eutrophic of the study sites) appears to be not wholly consistent with Braasch's findings. However, it must be appreciated that the Moor is not grossly polluted and it does match some of the other criteria that Braasch identified as important, having well-vegetated waterbodies with an abundance of potential prey (including many aquatic snails).

So far as *D. dimidiatus* is concerned, the key to maintaining a viable population appears to be retaining landscapes across the Levels and Moors with a diversity of aquatic habitats. For the moment, blocks of wet woodland in close association with waterbodies (whether these are ditches, shallow ponds or lakes), appear to be crucial to breeding success, as the vast majority of larvae that I confirmed as *D. dimidiatus* occurred in heavily-shaded wetland sites. Ditches in more open areas with rich vegetation and diverse invertebrate communities may provide other breeding opportunities (as testified by the finding of a *D. dimidiatus* larva at Tealham Moor) and certainly offer rich foraging areas for adult beetles. Generally, an improvement in water quality would probable favour *D. dimidiatus*, particularly if this resulted in a reduction in the prevalence of duckweed-choked ditches.

8.6 Recommendations for further study

The main priority for further research ought to be the finding of more breeding sites for *D. dimidiatus* both in the Brue Valley and in the wider Levels and Moors area. Not only would this be likely to help define the essential characteristics of breeding sites to shape land management practice but it would establish where the key sites are in the Ramsar wetland that are critical for the conservation of the species. Mark – recapture experiments should be prioritised also in order to attempt to establish population size of *D. dimidiatus* at some key sites as a basis for future monitoring.

My use of trapping as a primary means of data collection has focussed the attention of this study inevitably on adult beetles and on the later larval stages

of the *Dytiscus* species. More focus on egg-laying behaviour, the earliest stages of the larval development (i.e. L_1 instar) and on pupation would be likely to furnish much important information relevant to conservation of *D. dimidiatus*. For example, since large stands of emergent plants are not as prevalent in shaded sites as they are in open ones, it would be instructive to find out whether *D. dimidiatus* females need to lay eggs in the stems of live plants (as reported by Régimbart for *D. marginalis* and observed under laboratory conditions by Inoda in D. sharpi) [Régimbart (1875), Inoda (2011a, 2011b)].

The elucidation of the role of shade in all stages of the *D. dimidiatus* lifecycle would be assisted if a more precise and, arguably, less subjective means could be devised to measure shade than estimating percentage cover. (Perhaps the use of digital fish-eye camera technology could be investigated in this regard.) Further research would be desirable also into whether a relationship exists in the Levels and Moors ditches between water chemistry and shade. The possible link postulated by Boyce (2004) between *D. dimidiatus* occurrence and the shaded breeding sites used by *Hydrochara caraboides* would be worthy of more research.

Other avenues for fieldwork that suggest themselves include an investigation into nocturnal activity of *Dytiscus* species in Levels and Moors ditches to see if there are differences between species or between adults and larvae that could be the basis of niche separation. More laboratory-based research could be justified also looking into predator – prey relationships and/or conducting physiological experiments on larvae and adults to investigate their activity under varying water temperature conditions (which might help explain shade-mediated effects).

Lastly, it must be acknowledged that species of *Dytiscus* other than *D. dimidiatus* and *D. marginalis* occur in the wetlands that are a part of or are associated with the Somerset Levels and Moors. *D. semisulcatus* was encountered rarely during the study, despite its status as a supposedly widespread and relatively common species while there are inland records for *D.*

circumflexus that would merit investigation. Even in a relatively well-recorded locality such as the Somerset wetlands there remains much still to be learned about the distribution and ecology of Great Diving Beetles (*Dytiscus* spp.).

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THE ECOLOGY OF GREAT DIVING BEETLES (*DYTISCUS* SPP.) IN THE SOMERSET LEVELS AND MOORS

SUBMITTED BY
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UNIVERSITY OF SUSSEX

APPENDICES

NOVEMBER 2013

Appendix A1

Table A.1: Threat categories used to evaluate the conservation status of British beetles. Source: Hyman & Parsons (1992).

Category	Qualifying attributes
RDB[1] 1 (Endangered[2])	Taxa in danger of extinction [in Britain] and whose survival is unlikely if causal factors [that have contributed to their rarity] continue to operate.
RDB 2 (Vulnerable[2])	Taxa believed likely to move into the Endangered category in the near future if the causal factors [that have contributed to their rarity] continue operating.
RDB 3 (Rare[2])	Taxa with small populations that are not at present Endangered or Vulnerable, but are at risk [of becoming so].
RDB App (Extinct[3])	Taxa that were formerly native to Britain but which have not been recorded since 1900.
RDB I (Indeterminate[2])	Taxa considered to be Endangered, Vulnerable or Rare[2], but where there is not enough information to say which of the three categories (RDB1 to 3) is appropriate.
RDB K (Insufficiently known[2])	Taxa that are suspected but not definitely known to belong to any of the above categories, because of lack of information.
Na (Nationally Notable A)	Taxa that do not fall within RDB categories but which are none-the-less uncommon in Great Britain and thought to occur in 30 or fewer 10km squares of the National Grid or, for less well-recorded groups, within seven or fewer vice-counties.
Nb (Nationally Notable B)	Taxa that do not fall within RDB categories but which are none-the-less uncommon in Great Britain and thought to occur in between 31 and 100 10km squares of the National Grid or, for less well-recorded groups, between eight and twenty vice-counties.
Notable	Species that are estimated to occur within the range of 16 to 100 10 km squares.

Notes

1. RDB = Red Data Book

2. The terms 'Endangered', 'Vulnerable', 'Rare', 'Indeterminate' and 'Insufficiently known' were taken from the then current IUCN criteria for categorising threatened species (see Wells *et al*, 1983). The latest IUCN criteria are to be found in IUCN (2001).

3. According to IUCN criteria (Wells et al, op cit.), to be considered extinct a species should not have been definitely located in the wild during the past 50 years. A longer timescale was thought appropriate for the review presumably because of the relative under-recording of insect species compared with vertebrates and 'higher' plants.

Appendix A2

Genus *Dytiscus* from Catalogue of Palearctic Dytiscidae (Coleoptera) after Nilsson, A.N. (University of Umeå, Sweden)

List re-formatted from: Nilsson A.N. (2003) Dytiscidae, pp. 35-78. - In: I. Löbl & A. Smetana (editors): Catalogue of Palaearctic Coleoptera. Vol. 1. Stenstrup: Apollo Books, 819 pp. as updated by Nilsson A.N. (2011) **-** Update distributed as a PDF file via Internet; version 2011-01-01.
Available from: http://www2.emg.umu.se/projects/biginst/andersn/Cat_main.htm

KEY

The following abbreviations are used: (CN) conserved name, (HN) homonym, (NO) nomen oblitum, (NP) nomen protectum, (RN) replacement name, and (SN) suppressed name.]

Biogeography

E - Europe: AB Azerbaijan, AL Albania, AN Andorra, AR Armenia, AU Austria, AZ Azores, BE Belgium, BH Bosnia Hercegovina, BU Bulgaria, BY Byelorussia, CR Croatia, CZ Czech Republic, DE Denmark, EN Estonia, FA Faeroe Islands, FI Finland, FR France, GB Great Britain, GE Germany, GG Georgia, GR Greece, HU Hungary, IC Iceland, IR Ireland, IT Italy, KZ Kazakhstan, LA Latvia, LS Liechtenstein, LT Lithuania, LU Luxembourg, MA Malta, MC Macedonia, MD Moldavia, NL The Netherlands, NR Norway, PL Poland, PT Portugal, RO Romania, RU Russia (CT Central European Territory, NT North European Territory, ST South European Territory), SK Slovakia, SL Slovenia, SP Spain, SR Svalbard, SV Sweden, SZ Switzerland, TR Turkey, UK Ukraine, YU Yugoslavia.

N - North Africa: AG Algeria, CI Canary Islands, EG Egypt (except Sinai), LB Libya, MO Morocco, MR Madeira Archipelago, TU Tunisia.

A - Asia: AE Arab Emirates, AF Afghanistan, AP Arunachal Pradesh, BA Bahrain, BT Bhutan, CH China [CE Central Territory (ANH Anhui, HUB Hubei, HUN Hunan, JIA Jiangsu, JIX Jiangxi, SHG Shanghai, ZHE Zhejiang), NE Northeastern Territory (HEI Heilongjiang, JIL Jilin, LIA Liaoning), NO Northern Territory (BEI Beijing, GAN Gansu, HEB Hebei, HEN Henan, NIN Ningxia, NMO Nei Mongol, SHA Shaanxi, SHN Shandong, SHX Shanxi, TIA Tianjin), NW Northwestern Territory (GAN Gansu, NMO Nei Mongol, XIN Xinjiang), SE Southeastern Territory (FUJ Fujian, GUA Guangdong, GUX Guangxi, HAI Hainan, HKG Hong Kong, MAC Macao, TAI Taiwan), SW Southwestern Territory (GUI Guizhou, SCH Sichuan, YUN Yunnan), WP Western Plateau (QIN Qinghai, XIZ Xizang)], CY Cyprus, HP Himachal Pradesh, IN Iran, IQ Iraq, IS Israel, JA Japan, JO Jordan, KA Kashmir, KI Kyrgyzstan, KU Kuwait, KZ Kazakhstan, LE Lebanon, MG Mongolia, NP Nepal, NC North Korea, OM Oman, PA Pakistan, QA Quatar, RU Russia (ES East Siberia, FE Far East, WS West Siberia), SA Saudi Arabia, SC South Korea, SD Sikkim, Darjeeling, SI Sinai (Egyptian part), SY Syria, TD Tadzhikistan, TM Turkmenistan, TR Turkey, UP Uttar Pradesh, UZ Uzbekistan, YE Yemen.

AFR Afrotropical region, AUR Australian region, NAR Nearctic region, NTR Neotropical region, ORR Oriental region.

EXTRACT FROM CATALOGUE [adapted after Nilsson (2011)]

Family: **DYTISCIDAE** Leach, 1815
Subfamily: **Dytiscinae** Leach, 1815
Tribe: Dytiscini Leach, 1815
Genus: ***Dytiscus* Linnaeus, 1758: 411** type species *Dytiscus marginalis* Linnaeus, 1758

= *Leionotus* Kirby, 1837: 76 type species *Dytiscus conformis* Kunze, 1818 (=*Dytiscus marginalis* Linnaeus, 1758)

= *Macrodytes* Thomson, 1859: 12 type species *Dytiscus marginalis* Linnaeus, 1758

Species:

circumcinctus Ahrens, 1811: 67(=55) **E:** AU BE BU BY CR CZ DE EN FI FR GB GE GG HU IR IT LA LT NL NR PL RU (CT NT ST) SK SL SV SZ UK **A:** KZ RU (ES FE WS) TR **NAR**
= *angustatus* Curtis, 1826: 99
= *angustatus* Stephens, 1828: 88
= *anxius* Mannerheim, 1843: 218
= *circumscriptus* Lacordaire, 1835: 300 [HN]
= *confusus* Motschulsky, 1860: 101
= *dubius* Gyllenhal, 1827: 372 [HN]
= *flavocinctus* Hummel, 1823: 17
= *fuscostriatus* Motschulsky, 1859: 167
= *ooligbukii* Kirby, 1837: 74

circumflexus Fabricius, 1801: 258 **E:** AB AR AU BE BH BU BY CR CZ DE EN FR GB GE GG GR HU IR IT LA LT LU MA MD NL PL PT RU (ST) SK SL SP SV UK YU **N:** AG EG LB MO TU **A:** CY IN IS KZ LE RU (WS) SY TR
= *dubius* Audinet-Serville, 1830: 90
= *flavomaculatus* Curtis, 1826: 99
= *flavoscutellatus* Latreille, 1804: 162
= *kunstleri* Peytoureau, 1894: xlii
= *perplexus* Lacordaire, 1835: 303

dauricus dauricus Gebler, 1832: 39 **A:** CH (HEI JIL XIN) JA MG RU (ES FE) **NAR**
= *amurensis* J.Balfour-Browne, 1944: 356 [RN]
= *confluens* Say, 1830: 27 [HN]
= *confluens* Say, 1834: 440 [HN]
= *confluentus* LeConte, 1850: 212 [RN]
= *diffinis* LeConte, 1850: 212
= *franklinii* Kirby, 1837: 77
= *frontalis* Motschulsky, 1860: 101 [HN]
= *obscurus* Gschwendtner, 1922: 93
= *strigifrons* Motschulsky, 1860: 101
= *ventralis* Motschulsky, 1855: 79
= *vexatus* Sharp, 1882: 643
dauricus zaitzevi Nakane, 1990: 28 **A:** JA

delictus Zaitzev, 1906: 28 (*Macrodytes*) **A:** CH (HEI) RU (FE)

dimidiatus Bergsträsser, 1778: 33 **E:** AB AL AR AU BE BH BU BY CR CZ DE EN FI FR GB GE GG GR HU IT LA LT LU MC NL PL RO RU (CT NT ST) SK SL SV SZ UK YU **A:** IN SY TR

distantus Feng, 1936: 14 **A:** CH ("Manchuria")

lapponicus disjunctus Camerano, 1880: 120 **E:** IT
lapponicus lapponicus Gyllenhal, 1808: 468 **E:** BE BY CZ DE EN FI FR GB GE GG IR LA LT NL NR PL RU (CT NT) SK SV **A:** KZ RU (WS)
= *borealis* Motschulsky, 1860: 101
= *septemtrionalis* Gyllenhal, 1827: 373

latissimus Linnaeus, 1758: 411 **E:** AU BE BY CR CZ DE EN FI FR GE HU IT LA LT LU NL NR PL RO RU (CT NT ST) SK SV SZ UK **A:** RU (WS)
= *amplissimus* O.F. Müller, 1776: 69
= *anastomozans* Well, 1781: 386

latro Sharp, 1882: 644 **A:** CH (HEI) MG RU (ES FE WS)
= *piceatus* Sharp, 1882: 644
= *stadleri* Gschwendtner, 1922: 93

marginalis czerskii Zaitzev, 1953: 328 **A:** CH (HEB HEI HEN) JA NC RU (ES FE)
marginalis marginalis Linnaeus, 1758: 411 **E:** AB AL AU BE BH BU BY CR CZ DE EN FI FR GB GE GG GR HU IR IT LA LS LT LU MC NL NR PL PT RO RU (CT NT ST) SK SL SP SV SZ UK YU **A:** KZ RU (ES WS) TR
= *circumductus* Audinet-Serville, 1830: 90
= *conformis* Kunze, 1818: 58
= *semicostatus* Reineck, 1921: 117
= *semistriatus* Linnaeus, 1758: 412
= *submarginatus* Stephens, 1828: 90
= *totomarginalis* DeGeer, 1774: 391

mutinensis Branden, 1885: 97 **E:** CR GR IT
= *mutinensis* Pederzani, 1971: 220 [HN]

persicus Wehncke, 1876: 52 **E:** AR GG RU (ST) UK **A:** AF IN TR UZ

pisanus Laporte, 1835: 98 **E:** AN CR FR GR IT PT SP **N:** AG MO TU
= *ibericus* Rosenhauer, 1856: 47
= *nonsulcatus* Zimmermann, 1919: 233

semisulcatus O.F. Müller, 1776: 70 **E:** AL AU BE BU CR CZ DE EN FR GB GE GG GR IR IT LA LT LU NL NR PL PT SL SP SV SZ UK YU (Crna Gora) **N:** AG MO TU **A:** KZ RU (ES) TR **AUR**
= *exspectatus* Peyerimhoff, 1905: 229
= *frischii* Bergsträsser, 1778: 43
= *laevis* Engert, 1911: 19
= *maurus* Schaufuss, 1883: clxxiii
= *porcatus* Thunberg, 1794: 74
= *punctatus* Olivier, 1795: 12 [HN]
= *punctulatus* Fabricius, 1777: 238 [HN]
= *stagnalis* Geoffroy, 1785: 66

sharpi Wehncke, 1875: 500 **A:** CH (HUN SHA) JA
= *validus* Régimbart, 1899: 311

sinensis Feng, 1935: 182 **A:** CH (HEI SCH SHA)

thianschanicus Gschwendtner, 1923: 107 (*Macrodytes*) **E:** RU (ST) **A:** AF KA TD TR

SUMMARY TABLE

Table A.2: Summary of worldwide recorded distribution of *Dytiscus marginalis* Linnaeus, 1758 and *Dytiscus dimidiatus* Bergsträsser, 1778. (Source: Nilsson 2011.)

Species / Sub-species	***Countries with records***
D. marginalis marginalis	**Europe:** Azerbaijan; Albania; Austria; Belgium; Bosnia Herzogovina; Bulgaria; Byelorussia; Croatia; Czech Republic; Denmark; Estonia; Finland; France; Great Britain; Germany; Georgia; Greece; Hungary; Ireland; Italy; Latvia; Lichtenstein; Lithuania; Luxembourg; Macedonia; Netherlands; Norway; Poland; Portugal; Roumania; Russia (Central European Territory, Northern European Territory & Southern European Territory); Slovakia; Slovenia; Spain; Sweden; Switzerland; Ukraine; Yugoslavia. **Asia:** Kazakhstan; Russia (East Siberia, West Siberia); Turkey.
D. marginalis czerskii	**Asia:** China (Hebei, Heilongjiang, Henan); Japan; North Korea; Russia (East Siberia Far East).
D. dimidiatus	**Europe:** Azerbaijan; Albania; Armenia; Austria; Belgium; Bosnia Herzogovina; Bulgaria; Byelorussia; Croatia; Czech Republic; Denmark; Estonia; Finland; France; Great Britain; Germany; Georgia; Greece; Hungary; Italy; Latvia; Lithuania; Luxembourg; Macedonia; Netherlands; Poland; Roumania; Russia (Central European Territory, Northern European Territory & Southern European Territory) Slovakia; Slovenia; Sweden; Switzerland; Ukraine; Yugoslavia. **Asia:** Iran; Syria; Turkey.

N.B Older authorities such as Balfour- Browne (1950) mention the occurrence of *D.dimidiatus* in the Iberican Peninsula. Balfour-Browne cites Guignot (1933) as a source for saying that the species occurs on Mediterranean islands also (Balfour-Browne op cit.). This is not reflected in Nilsson's latest catalogue upon which Table A.2 is based (Nilsson 2011). Similarly, Nilsson's catalogue does not include records for *D. marginalis* from North Africa where Balfour-Browne states it may be found (Balfour-Browne op cit.).

Appendix A3

A3: Distribution maps for *Dytiscus* spp. in Western Europe after du Chatenet (2005).

Dytiscus circumcinctus (Ahrens, 1811)

Dytiscus circumflexus (Fabricius, 1801)

Dytiscus dimidiatus (Bergsträsser, 1778)

A3: Distribution maps for *Dytiscus* spp. in Western Europe after du Chatenet (2005).

Dytiscus lapponicus (Gyllenhal, 1808)

Dytiscus marginalis (Linnaeus, 1758)

Dytiscus semisulcatus (Müller, O.F., 1776)

Appendix A4

A4: Distribution maps for *Dytiscus* spp. in the British Isles after Sutton (2008).

The Enigma

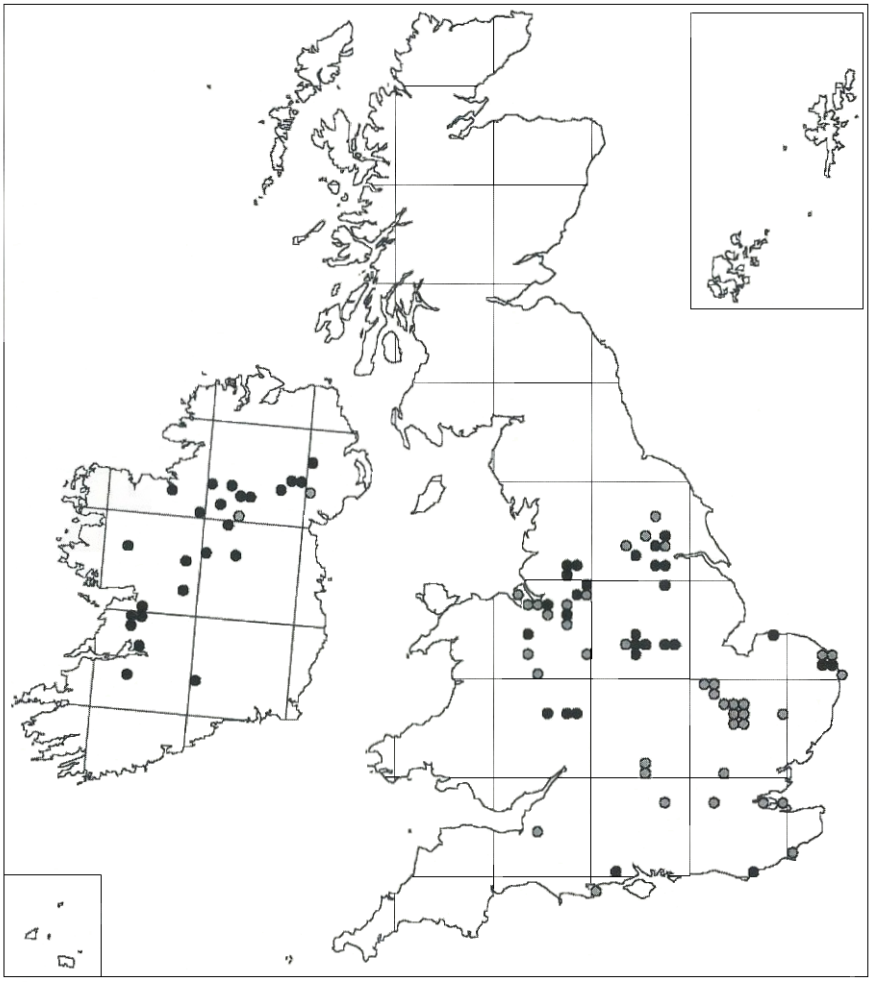


Figure 29. The distribution of The Enigma *Dytiscus circumcinctus* in the British Isles.

○ pre 1980 records ● post 1980 records

A4: Distribution maps for *Dytiscus* spp. in the British Isles after Sutton (2008).

The Wasp

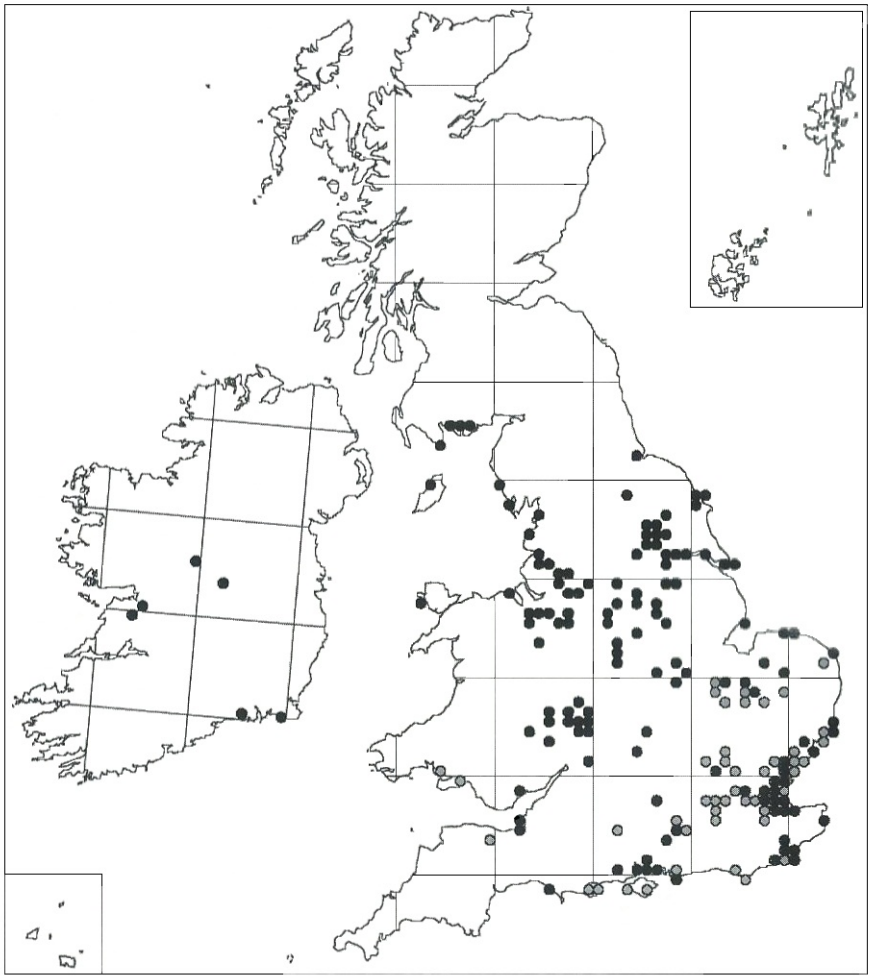


Figure 28. The distribution of The Wasp *Dytiscus circumflexus* in the British Isles.

○ pre 1980 records ● post 1980 records

A4: Distribution maps for *Dytiscus* spp. in the British Isles after Sutton (2008).

King Diving Beetle

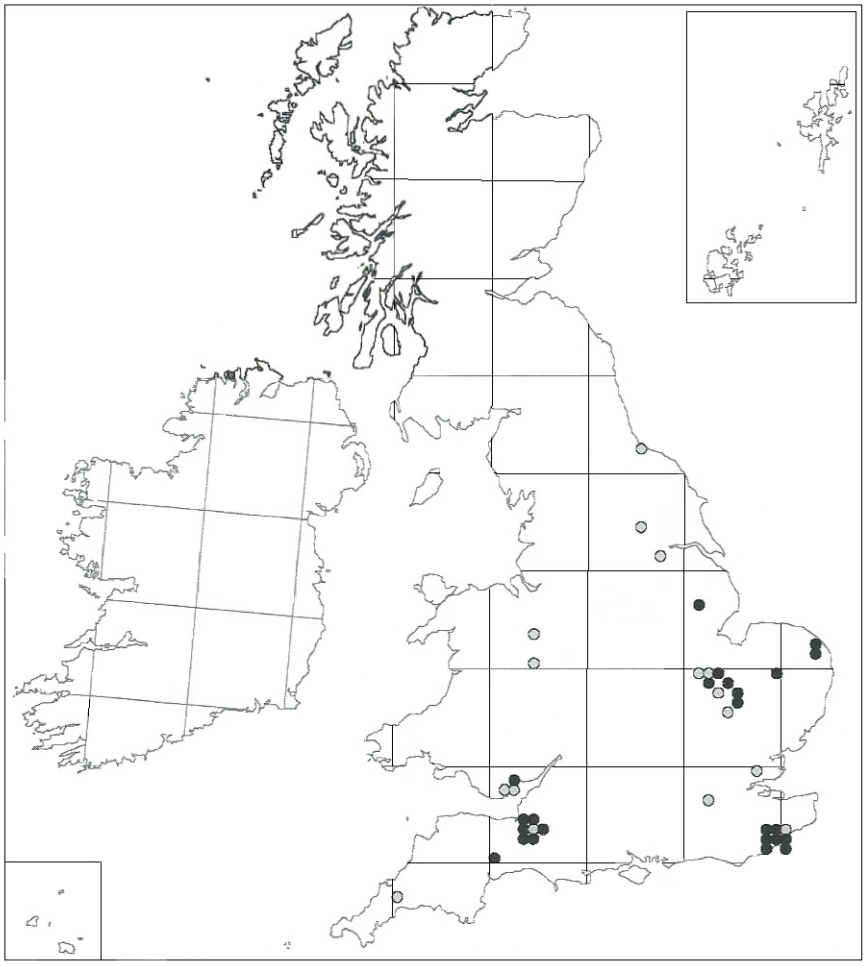


Figure 30. The distribution of the King Diving Beetle *Dytiscus dimidiatus* in the British Isles.

○ pre 1980 records ● post 1980 records

A4: Distribution maps for *Dytiscus* spp. in the British Isles after Sutton (2008).

Highland Diving Beetle

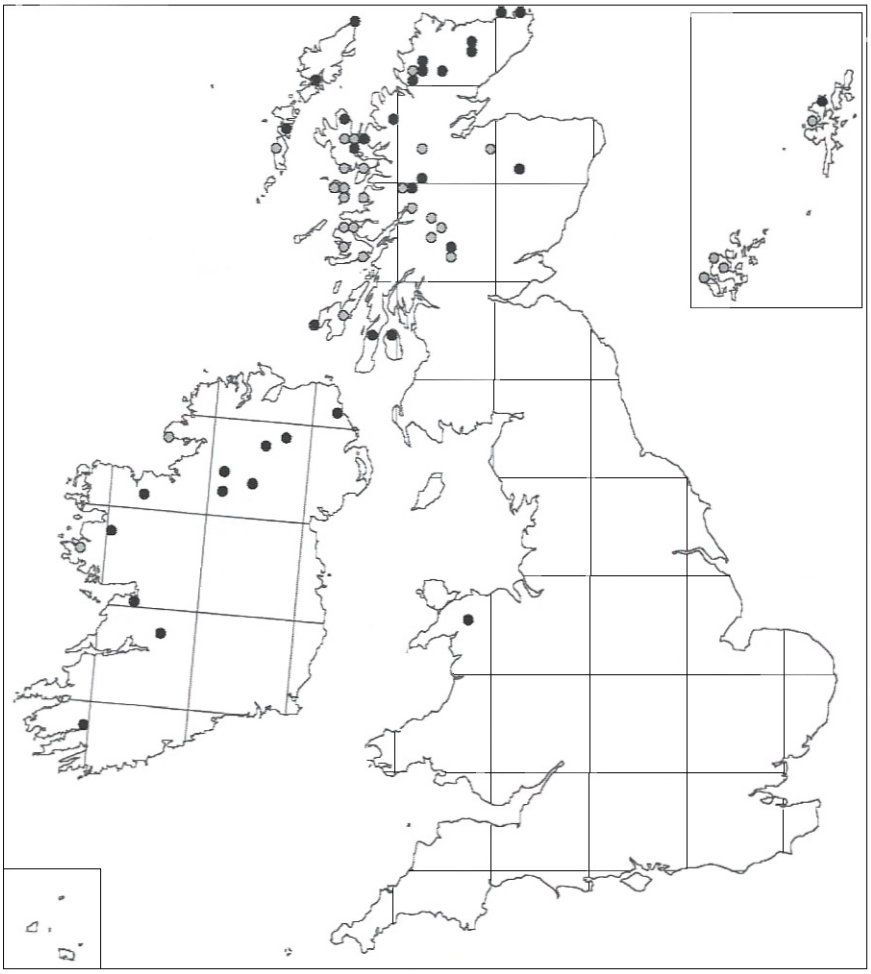


Figure 32. The distribution of the Highland Diving Beetle *Dytiscus lapponicus* in the British Isles.

○ pre 1980 records ● post 1980 records

A4: Distribution maps for *Dytiscus* spp. in the British Isles after Sutton (2008).

Great Diving Beetle

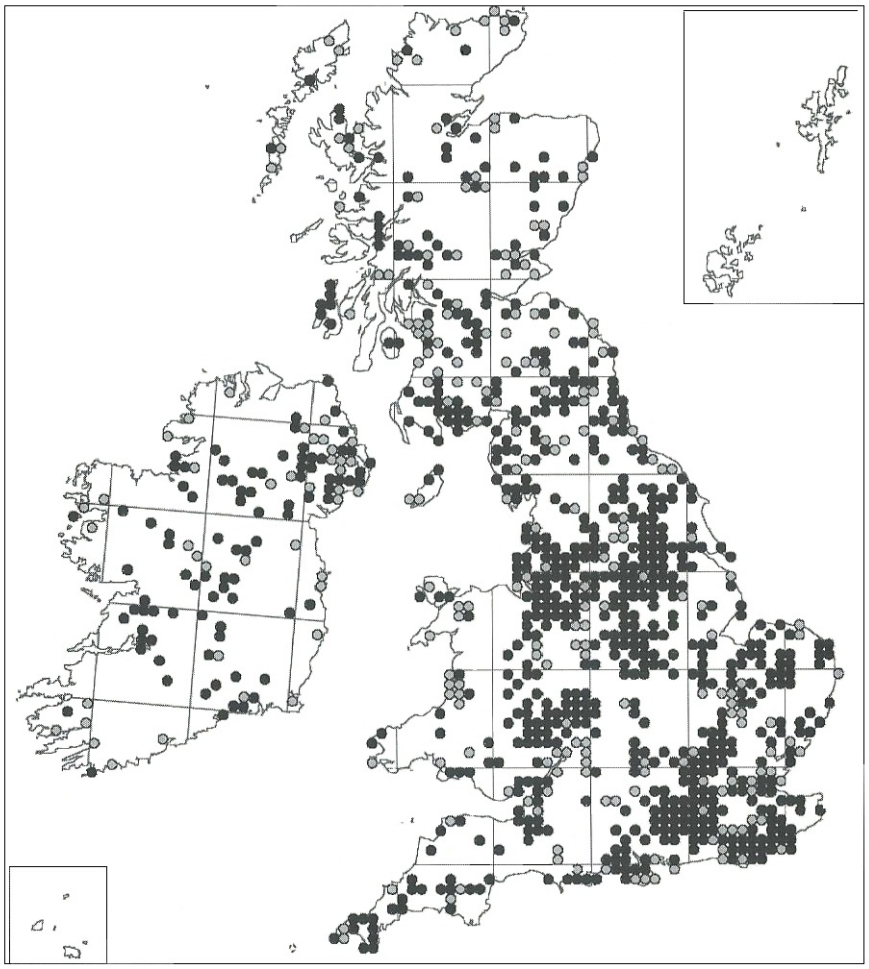


Figure 26. The distribution of the Great Diving Beetle *Dytiscus marginalis* in the British Isles.

○ pre 1980 records ● post 1980 records

A4: Distribution maps for *Dytiscus* spp. in the British Isles after Sutton (2008).

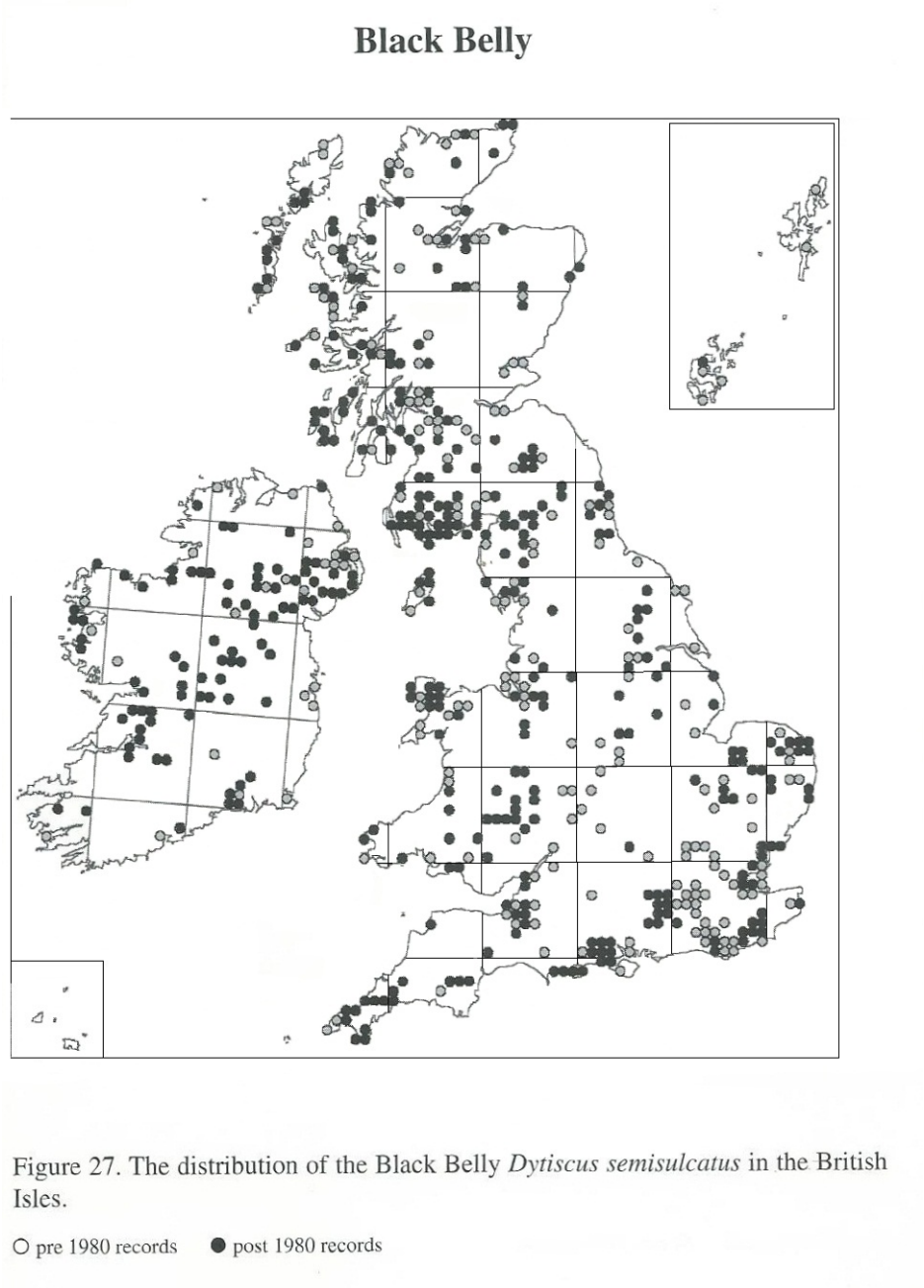


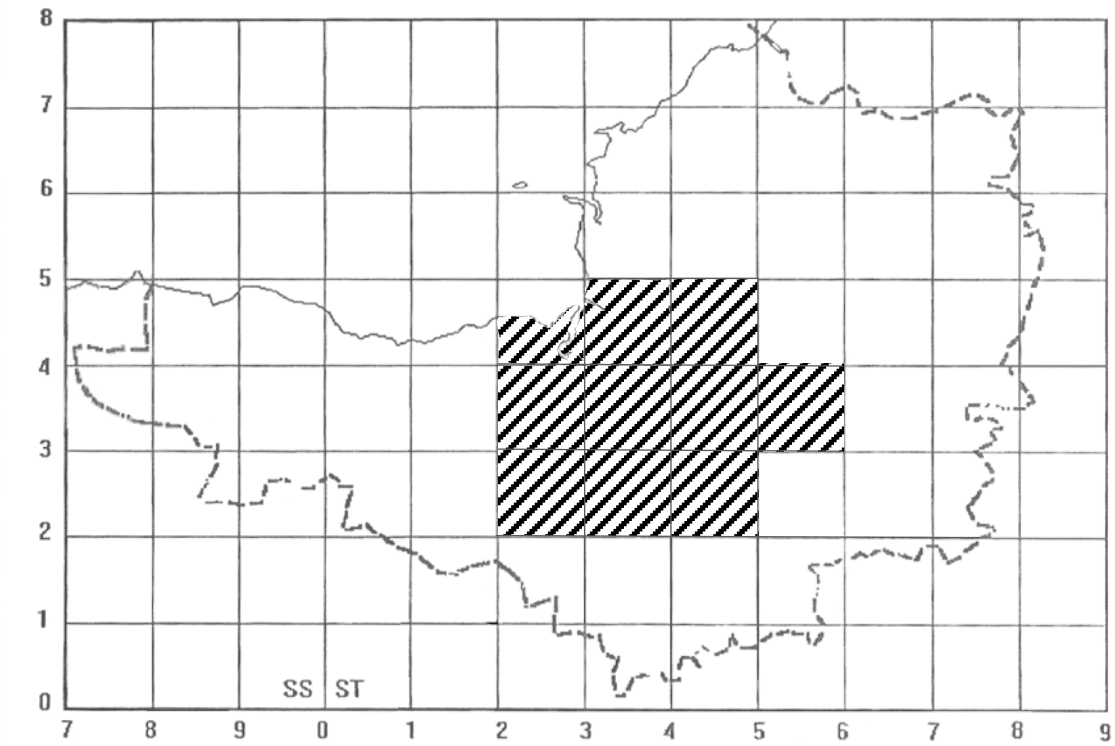
Figure 27. The distribution of the Black Belly *Dytiscus semisulcatus* in the British Isles.

○ pre 1980 records ● post 1980 records

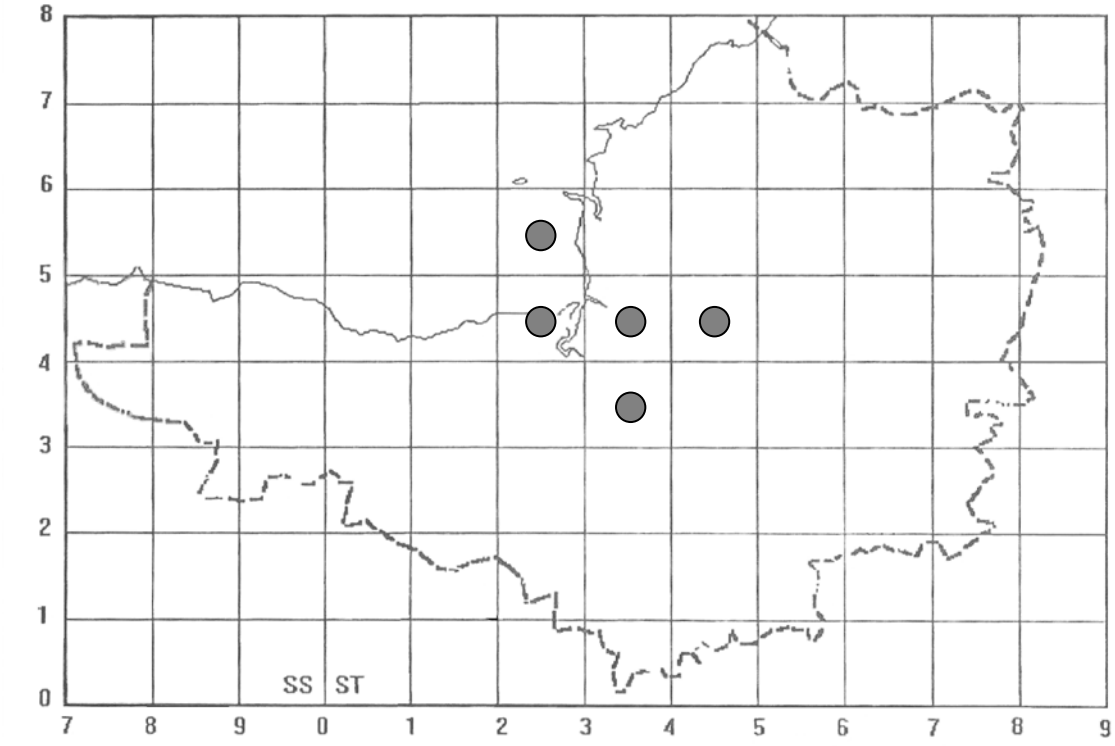
Appendix A5

Maps of records for *Dytiscus* spp. in Somerset [Sources: SERC, Duff (1993) and contact surveys listed in Appendix C1].

A5a: Map of Somerset indicating squares occupied by Somerset Levels and Moors

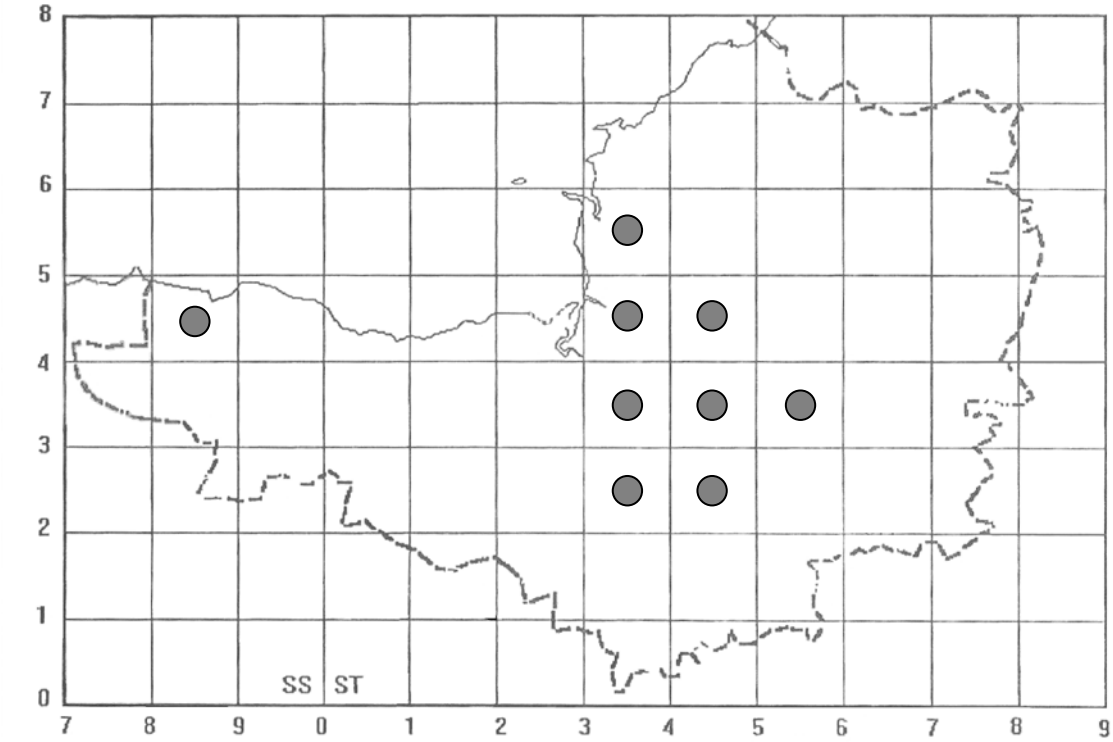


A5b: Somerset records of *Dytiscus circumflexus* by 10km OS square

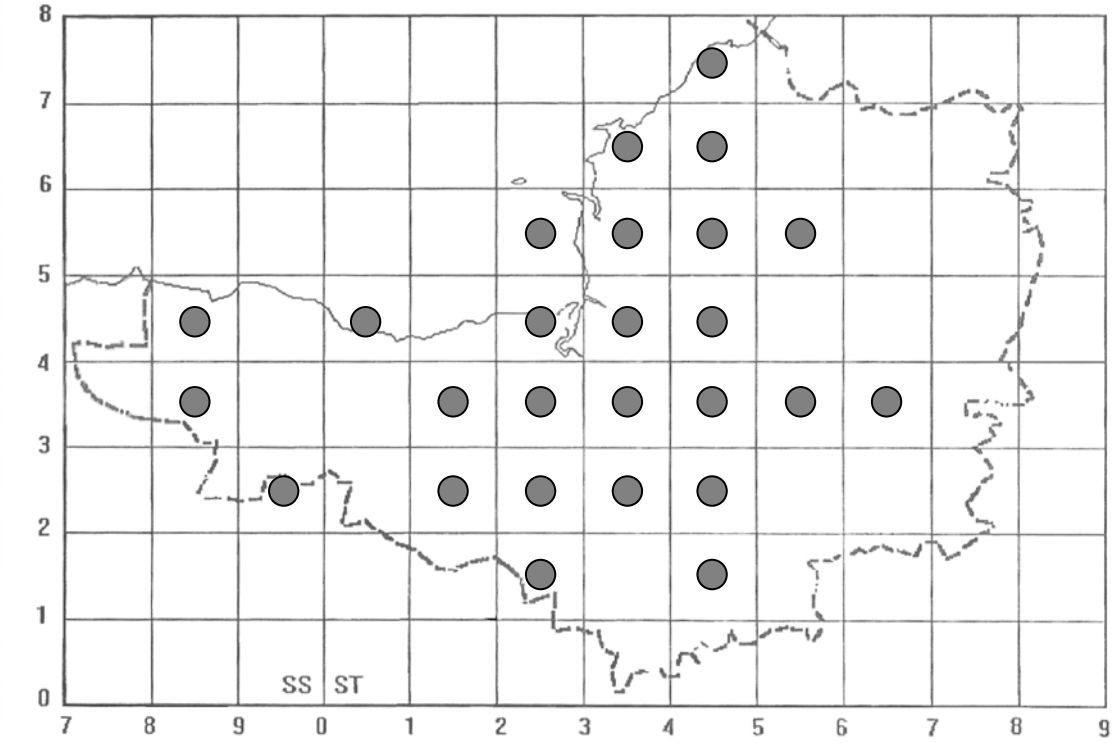


Appendix A5 (continued)

A5c: Somerset records of *Dytiscus dimidiatus* by 10km OS square

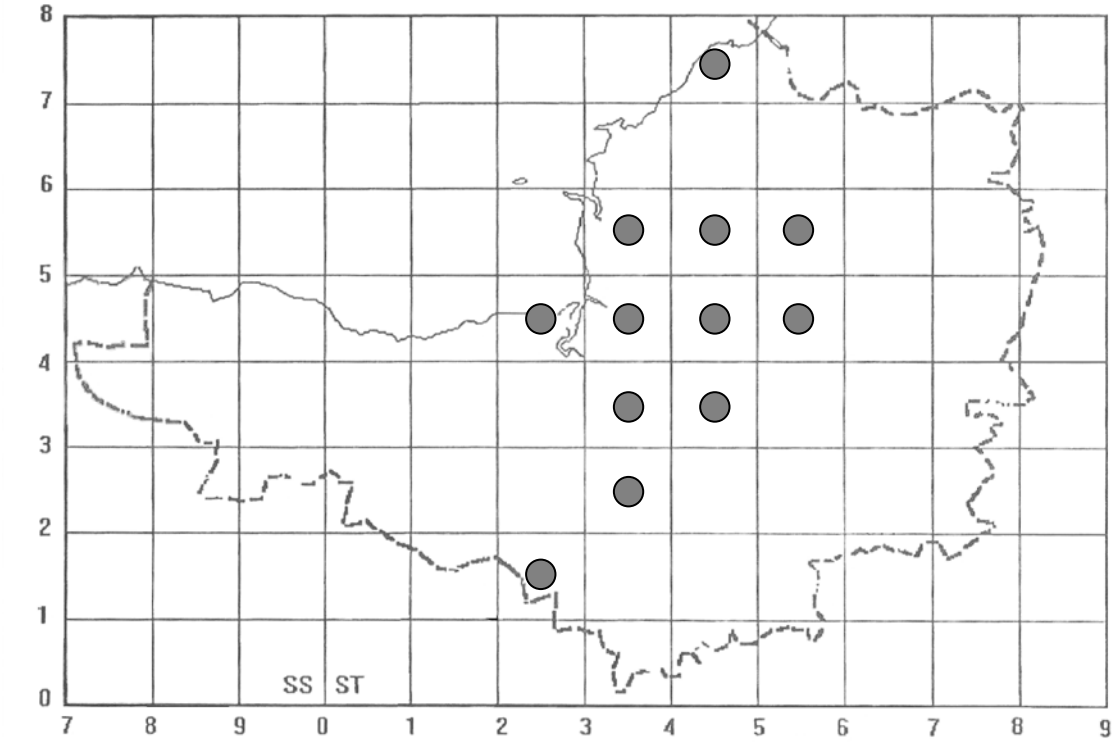


A5a: Somerset records of *Dytiscus marginalis* by 10km OS square



Appendix A5 (continued)

A5c: Somerset records of *Dytiscus semisulcatus* by 10km OS square



Appendix A6

A6: Key to adults of British *Dytiscus* spp. after Beebee (1991).

Key to British species of *Dytiscus*

In this key, the most obvious or important features are in bold. Ancillary information, useful in confirming identification, is also provided for each species.

1	**Beetle less than 20mm long**	Not a *Dytiscus* species
	Uniformly black underneath No yellow edges at front or back of pronotum Smallish by *Dytiscus* standards (22-30mm)	***D. semisulcatus***
	Abdominal sternae yellow or greenish but **with black edges** (giving a 'waspish' appearance underneath)	2
	Abdominal sternae **uniformly yellow**, sometimes with brown or reddish tinge, but without black banding effect	3
2	**Probably found in stony highland loch** Elytra brownish rather than black or dark green Distinct constriction at thorax/abdomen junction Smallish by *Dytiscus* standards (22-28mm)	***D. lapponicus***
	Black banding on abdominal sternae very pronounced Sharp postcoxal processes Normal *Dytiscus* size (26-32mm) Commonly found in coastal waters, including brackish ditches	***D. circumflexus***
3	**No yellow edges at front or back of pronotum**	4
	Postcoxal processes rather blunt No yellow rim around eye Normal *Dytiscus* size (26-32mm)	***D. marginalis***
	Postcoxal processes sharply pointed Normal *Dytiscus* size (26-32mm) Usually narrow yellow band around eye Females often without grooves in elytra	***D. circumcinctus***
4	Large by *Dytiscus* standards (30-38mm) Underside often with reddish tinge Grooves on elytra of female terminate about ⅔ way down back	***D. dimidiatus***

Colour patterns found in the various *Dytiscus* species are shown opposite, in which only regions important for identification are highlighted. From above, *dimidiatus* resembles *semisulcatus* (though the former is much larger), while all the rest have the '*marginalis*' pattern. From beneath, *marginalis*, *circumcinctus* and *dimidiatus* are similar to each other; *lapponicus* resembles *circumflexus* though the black markings are less pronounced; and *semisulcatus* is uniquely all-black. If in doubt, close examination of the postcoxal processes should clarify the matter (see oppposite).

Virtually all of these characters are easily seen, and *Dytiscus* species are simple to identify with the single exception of *D. circumcinctus*. At first sight this beetle looks like the much more abundant *marginalis*, and may often have been mistaken for it in the past. So it is always important to look carefully at what you initially suppose to be yet another common diving beetle, just to make sure.

Figure 1 Great Silver Beetle *Hydrophilus piceus*

Figure 2 Anatomical features used to identify the larger water beetles. Dorsal (a) and ventral (b) surfaces of a typical adult dytiscid are shown. In each case, the right-hand side portrays a male (with smooth elytra and claspers on forelimb) and the left-hand side a female (with sulcated elytra and no claspers).

Figure 3 The unpaired ventral spine of *Hydrophilus* (1) and postcoxal processes of the six species of *Dytiscus*: *marginalis* (2), *circumcinctus* (3), *semisulcatus* (4), *dimidiatus* (5), *circumflexus* (6) and *lapponicus* (7).

Figure 4 Colours of adult dytiscids. Dorsal surfaces of the '*marginalis*' type (a) and the '*semisulcatus*' type (b); note that the left side of (a) has a yellow margin around the eye typical of *circumcinctus*, while the right-hand side lacks this margin (as in all the other species).

Figure 5 Ventral surfaces of the '*marginalis*' type (a), '*circumflexus*' type (b) and '*semisulcatus*' type (c).

A6: Key to adults of British *Dytiscus* spp. after Beebee (1991).

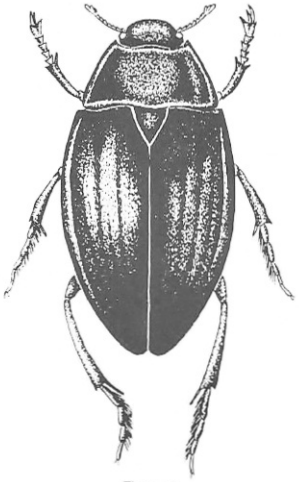
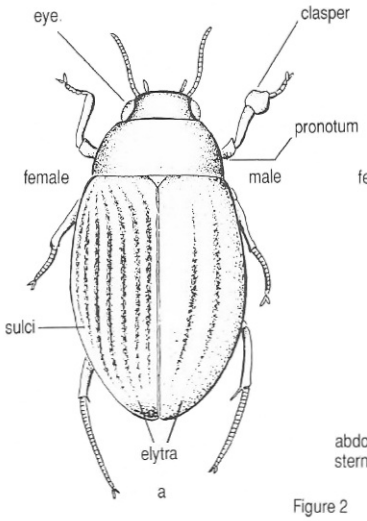
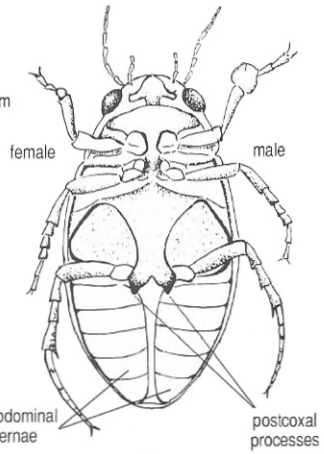


Figure 1



a



b

Figure 2

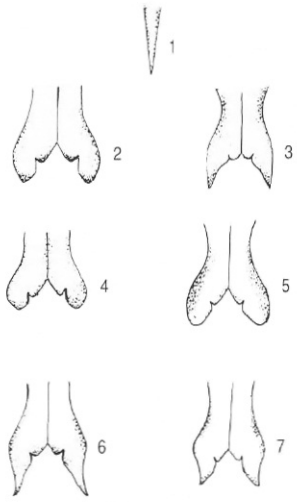
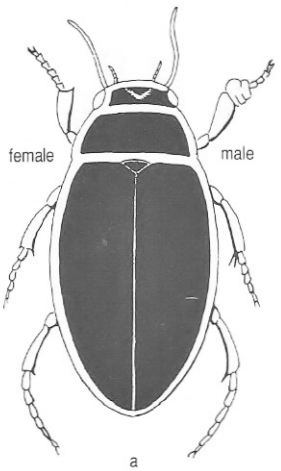
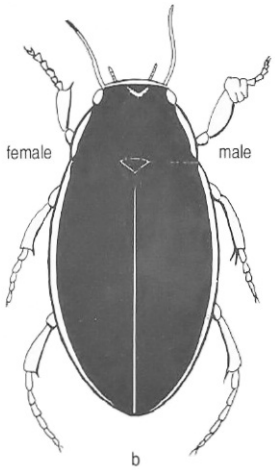


Figure 3

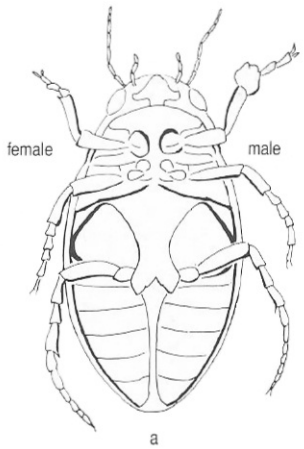


a

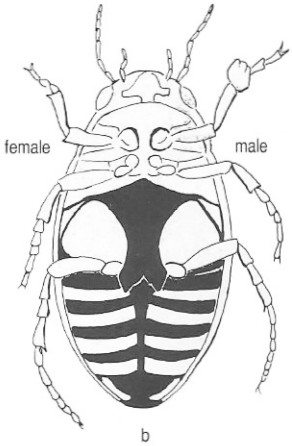


b

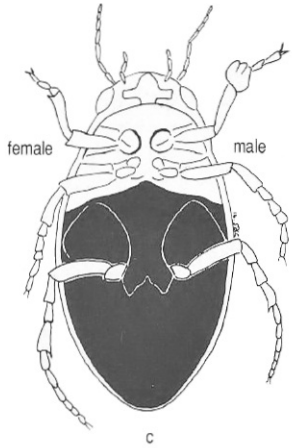
Figure 4



a



b

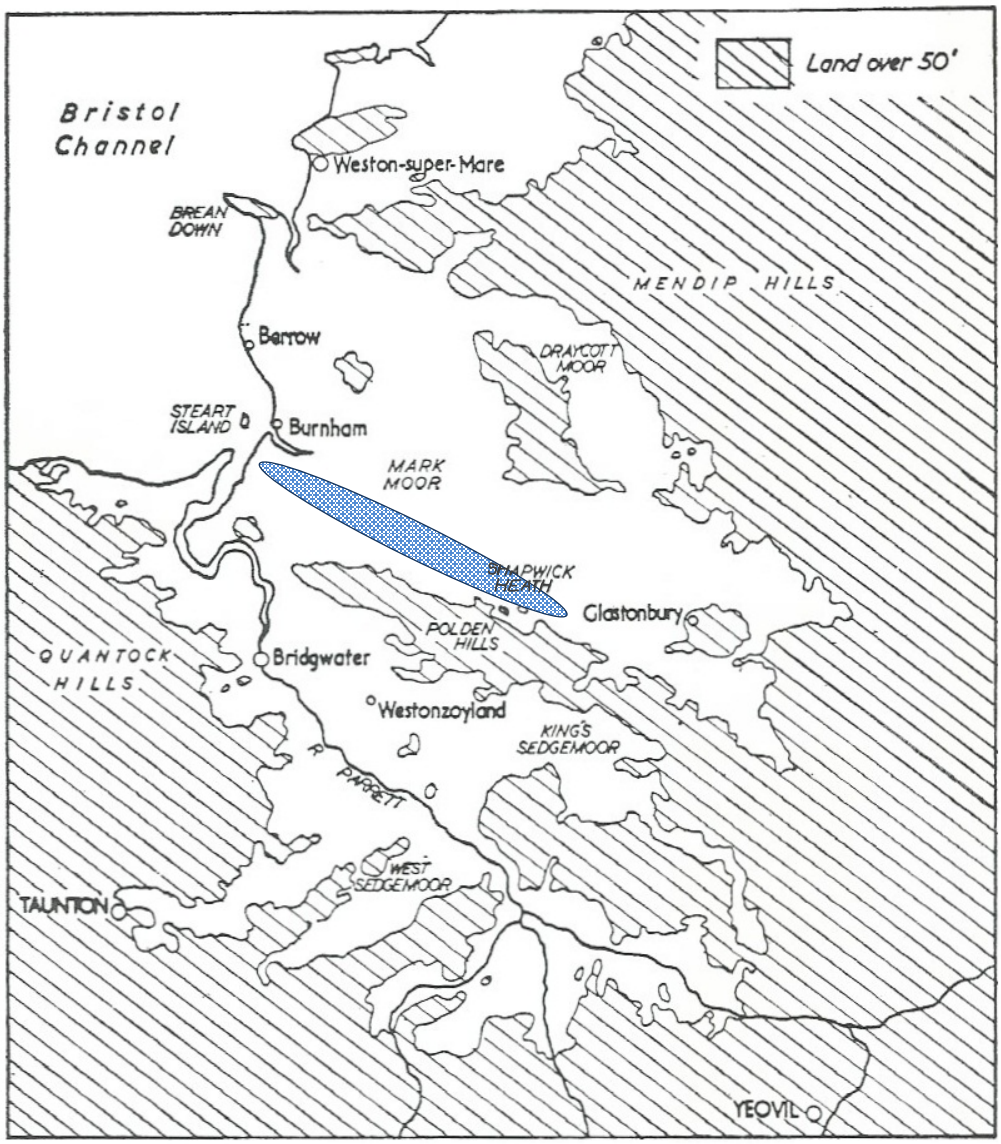


c

Figure 5

Appendix A7

A7: Map of the Somerset Levels & Moors after Storer (1985). The location of the Brue Valley is indicated by the area of blue shading.



Appendix A8

A8: Extracts from Ramsar citation for the Somerset Levels and Moors

[JNCC (2006)]. [Highlighted text – My emphasis]

12. General overview of the site:
Provide a short paragraph giving a summary description of the principal ecological characteristics and importance of the wetland. The Ramsar site consists of a series of Sites of Special Scientific Interest (SSSI) within the largest area of lowland wet grassland and associated wetland habitat remaining in Britain. It covers about 35,000 ha in the flood plains of the Rivers Axe, Brue, Parrett, Tone and their tributaries. The majority of the site is only a few metres above mean sea level and drains through a large network of ditches, rhynes, drains and rivers. Flooding may affect large areas in winter depending on rainfall and tidal conditions. Parts of the site in the Brue Valley include areas of former raised peat bog which have now been substantially modified by agricultural improvement and peat extraction which has created areas of open water, fen and reedbed. The site attracts internationally important numbers of wildfowl in winter and is one of the most important sites in southern Britain for breeding waders. The network of rhynes and ditches support an outstanding assemblage of aquatic invertebrates, particularly beetles.

14. Justification for the application of each Criterion listed in 13 above:
Provide justification for each Criterion in turn, clearly identifying to which Criterion the justification applies (see Annex II for guidance on acceptable forms of justification).

Ramsar criterion 2
Supports 17 species of British Red Data Book invertebrates.

22. Noteworthy fauna:

Species Information
Nationally important species occurring on the site.
Invertebrates.
Hydrochara caraboides, Bagous nodulosus, Odontomyia angulata, Oulema erichsoni, Valvata macrostoma, Odontomyia ornata, Stethophyma grossum, Pteromicra leucopeza, Lejops vittata, Cantharis fusca, Paederus caligatus, Hydaticus transversalis, Dytiscus dimidiatus, Hydrophilus piceus, Limnebus aluta, Laccornis oblongus

Site-relevant references

Bratton, JH (ed.) (1991) *British Red Data Books: 3. Invertebrates other than insects*. Joint Nature Conservation Committee,
Peterborough

O'Neil, P & Beebee, TJC (2005) The great silver water beetle in Britain: a cry for help *British Wildlife*, **16**(4), 265-269

Shirt, DB (ed.) (1987) *British Red Data Books: 2. Insects*. Nature Conservancy Council, Peterborough

Appendix B1: Maps & Aerial Photographs (2007 & 1946)

B1a: Locations of study sites

B1b: Shapwick Heath

B1c: Westhay Moor

B1d: Westhay Heath

B1e: Catcott North

B1f: Tadham Moor

B1g: East Waste

B1b(i): Aerial photograph of Shapwick Heath 2007 (Source: Somerset County Council)

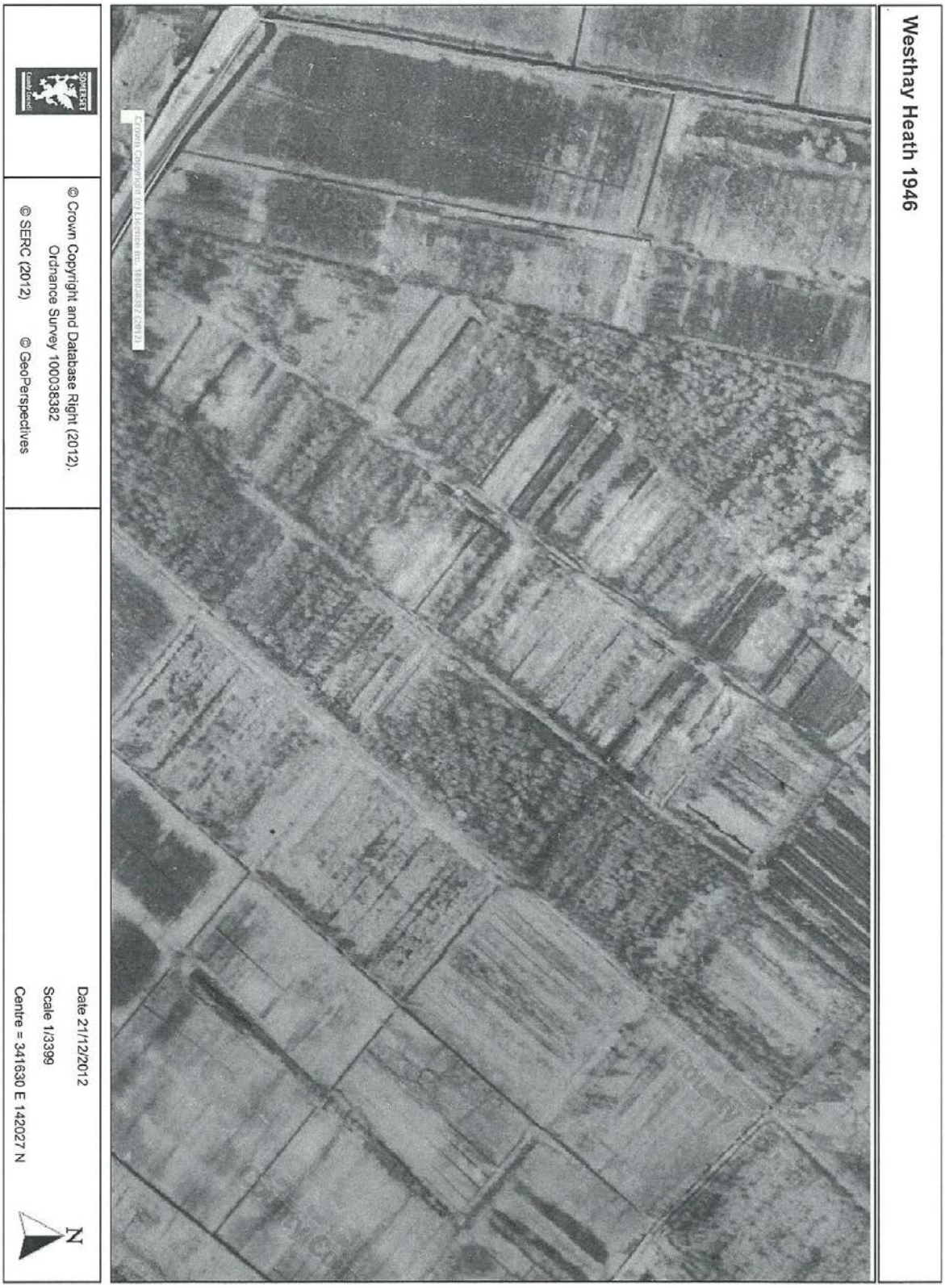
B1b(ii): Aerial photograph of Shapwick Heath 1946 (Source: Somerset County Council) [N.B. Not to same scale as B1b(i)]

B1c(i): Aerial photograph of Westhay Moor 2007 (Source: Somerset County Council)

B1c(ii): Aerial photograph of Westhay Moor 1946 (Source: Somerset County Council) [N.B. Not to same scale as B1c(i)]

B1d(i): Aerial photograph of Westhay Heath 2007 (Source: Somerset County Council)

B1d(ii): Aerial photograph of Westhay Heath 1946 (Source: Somerset County Council) [N.B. Not to same scale as B1d(i)]



B1e(i): Aerial photograph of Catcott North 2007 (Source: Somerset County Council)

B1e(ii): Aerial photograph of Catcott North 1946 (Source: Somerset County Council) [N.B. Not to same scale as B1e(i)]

B1f(i): Aerial photograph of Tadham Moor 2007 (Source: Somerset County Council)

B1f(ii): Aerial photograph of Tadham Moor 1946 (Source: Somerset County Council) [N.B. Not to same scale as B1f(i)]

B1g(i): Aerial photograph of East Waste 2007 (Source: Somerset County Council)

B1g(ii): Aerial photograph of Tadham Moor 1946 (Source: Somerset County Council) [N.B. Not to same scale as B1g(i)]

Appendix B2: SSSI Citations and ecological survey

B2a: Excerpt from SSSI Citation for Shapwick Heath SSSI

B2b: Excerpt from SSSI Citation for Westhay Moor SSSI

B2c: Excerpt from SSSI Citation for Westhay Heath SSSI

B2d: Excerpt from SSSI Citation for Catcott, Edington and Chilton Moors SSSI

B2e: Excerpt from SSSI Citation for Tealham and Tadham Moor SSSI

B2f: Excerpt from field notes made during Somerset Wildlife Trust survey of East Waste (Somerset Wildlife Trust 2010)

B2a: Excerpt from SSSI Citation for Shapwick Heath SSSI

National Grid Reference: ST 430403 **Area:** 393.99 (ha.) 973.55 (ac.)
Ordnance Survey Sheet 1:50,000: 182 1:10,000: ST 44 SW, ST 43 NW

Description and Reasons for Notification:

Shapwick Heath, part of the Somerset Levels Wetlands, is a former raised bog lying below 4 metres ODN in the basin of the River Brue. The site includes the last remnant of active raised bog on the Somerset Levels and Moors. The soils are principally of the Turbary series acid peats, modified in most parts by cutting. Altcar series reed peats occur on the western fringe of the site. Several low, sandy hillocks (burtles) occur in the Canada Farm area.

A variety of grassland communities has developed in the unimproved pastures and hay meadows. There are good examples of the nationally rare and threatened species-rich 'mire' type meadows characterised by Common Sedge *Carex nigra*, Carnation Sedge *Carex panicea*, Purple Moor-grass *Molinia caerulea*, Meadow Thistle *Cirsium dissectum* and Devil's-bit Scabious *Succisa pratensis*. Drier grasslands include the Common Knapweed *Centaurea nigra*/Crested Dog's-tail *Cynosurus cristatus* type, with frequent Meadowsweet *Filipendula ulmaris*, Quaking Grass *Briza media* and Oval Sedge *Carex ovalis*.

Wet heathy grassland is also present having many species in common with the mire community, but dominated by Purple Moor-grass and Common Bent *Agrostis capillaris* with large patches of Bog Myrtle *Myrica gale*, Creeping Willow *Salix repens* and Cross-leaved Heath *Erica tetralix*. *Sphagnum* moss forms carpets over the wettest parts of the heath.

Large populations of orchids are associated with the 'mire' type and heathy communities, notably Fragrant Orchid *Gymnadenia conopsea*, Lesser Butterfly Orchid *Platanthera bifolia* and Southern Marsh Orchid *Dactylorhiza praetermissa*. Other nationally restricted vascular plants include Marsh Cinquefoil *Potentilla palustris*, Marsh Fern *Thelypteris thelypteroides* and the national rarities Marsh Pea *Lathyrus palustris* and Milk Parsley *Peucedanum palustre*.

The remnant of active raised bog occurs on the eastern part of the site at Ashcott Heath. The bog is a mosaic of wet heath, *Sphagnum* moss carpet, Bog Myrtle, old peat cuts, ditches and scrub. Several plants with very restricted distributions in southern Britain are associated with the acid bog conditions including Small Bur-reed *Sparganium minimum*, Small Bladderwort *Utricularia minor* and Hare's-tail Cottongrass *Eriophorum vaginatum*. Other plants of interest are Bog Asphodel *Narthecium ossifragum*, Bog-bean *Menyanthes trifoliata*, Round-leaved Sundew *Drosera rotundifolia* and Ivy-leaved Bellflower *Wahlenbergia hederacea*. Drier parts of the ancient bog surface are now colonised by fen woodland and scrub dominated by Alder *Alnus glutinosa* and willows *Salix spp*, with Downy Birch *Betula pubescens*

and Pedunculate Oak *Quercus robur*. The marshy ground and open pools in this woodland support a rich flora including large stands of Royal Fern *Osmunda regalis* and Tussock Sedge *Carex paniculata*.

Although many field ditches on Shapwick Heath are infilled or overgrown, the open ditches and abandoned peat cuts have diverse aquatic and bank-side floras. Emergent species of note are Lesser Water-plantain *Baldellia ranunculoides*, Water-violet *Hottonia palustris* and Greater Water Parsnip *Sium latifolium*. Floating species include the nationally rare Rootless Duckweed *Wolffia arrhiza* and in the submerged layer are the nationally restricted species Whorled Water-milfoil *Myriophyllum verticillatum* and Fen Pondweed *Potamogeton coloratus*.

The site supports a diverse community of terrestrial and aquatic invertebrates. National rarities are the Large Marsh Grasshopper *Stethophyma grossum* found on *Sphagnum* moss bogs, the Greater Silver Diving Beetle *Hydrophilus piceus* and the Lesser Silver Diving Beetle *Hydrochara caraboides* which is now confined nationally to the Brue Basin Peat Moors. The nationally restricted Hairy Dragonfly *Brachytron pratense* and the Ruddy Darter *Sympetrum sanguineum* breed in the ditches and flooded peat cuts. The site has interesting butterfly populations including Marsh Fritillary *Euphydryas aurinia* and Marbled White *Melanargia galathia*.

The great diversity of habitats supports at least 64 species of breeding birds including Lapwing *Vanellus vanellus* and Snipe *Gallinago gallinago* in wet fields, Grasshopper Warbler *Locustella naevia* and Nightingale *Luscinia megarhynchos* in scrubby areas, and Water Rail *Rallus aquaticus* and Reed Warblers *Acrocephalus scirpaceus* in flooded old peat cuttings.

The flora and fauna communities associated with the wetland habitats present on Shapwick Heath are dependent generally on high water levels and to provide for hydrological management, the site covers a discrete hydrological block bounded on the south by high ground and on the east, west and north by the road to Ashcott, Black Ditch Rhyne and the South Drain respectively. Water on the site is derived from three main sources, rainfall, the South Drain and the Polden Hills to the south.

The site is used by Otters *Lutra lutra*.

B2b: Excerpt from SSSI Citation for Westhay Moor SSSI

National Grid Reference: ST 455445 **Area**: 513.66 (ha) 1269.25 (ac)
Ordnance Survey Sheet 1:50,000: 182 1:10,000: ST 44 NE, ST 44 NW, ST 44 SE, ST 44 SW

Description and Reasons for Notification:

Westhay Moor forms part of the nationally important grazing marsh and ditch systems of the Somerset Levels and Moors. The land lies below 5m ODN in the basin of the River Brue at the foot of the Liss Limestone slopes of the Wedmore Ridge. The soils are principally of the Turbary series acid peats modified in parts by cutting. Altcar series reed peat occurs on the fringes of the site.

Over much of the moor, the water table is high throughout the year with extensive winter flooding occurring regularly. Water tables in the peat excavations are artificially lowered during active working, but excavations often fill with water for much of the year.

The moor is a former raised bog, now for the most part modified to grassland by agricultural practice. A wide range of sward types has developed due to variation in soils and in management practice. Unimproved swards are well represented with many examples of the now nationally rare and threatened species-rich mire-type meadows characterised by meadow thistle *Cirsium dissectum,* carnation sedge *Carex panicea,* marsh pennywort *Hydrocotyle vulgaris* and devil's-bit scabious *Succisa pratensis.* Wetter swards contain ragged-Robin *Lychnis flos-cuculi,* common spike-rush *Eleocharis palustris,* marsh-marigold *Caltha palustris,* greater bird's-foot trefoil *Lotus uliginosus* and rushes *Juncus* spp*.* Semi-improved swards include a good diversity of grasses, often with components of the mire-type community present. Most swards contain a variety of sedges including hammer sedge *Carex hirta* and brown sedge C. *disticha;* together with typical wet grassland species such as meadowsweet *Filipendula ulmaria* and marsh ragwort *Senecio aquaticus.*

An area of birch *Betula* spp and *willow Salix* spp carr woodland, with purple moor-grass *Molinia caerulea,* bog myrtle *Myrica gale* and relict areas of the formerly extensive raised bog with *Sphagnum* spp, lies in the centre of the peat excavations in the southern part of the moor. This area contains bog plants with restricted distributions in southern England, including hare's-tail cottongrass *Eriophorum vaginatum* and royal fern *Osmunda regalis.*

136 aquatic and bankside vascular plant species have been recorded from the field ditches, IDB-maintained rhynes, arterial watercourses and abandoned peat workings. The diverse emergent and bankside flora, dominated by reed sweet-grass *Glyceria maxima* in overgrown channels and by bulrush *Typha latifolia* in abandoned peat excavations, includes nodding bur-marigold *Bidens cernua,* fine-leaved water-dropwort *Oenanthe aquatica,* water dock *Rumex hydrolapathum,* marsh dock *R. palustris ,* flowering rush

Butomus umbellatus, narrow-leaved water-plantain *Alisma lanceolatum* and marsh stitchwort *Stellaria palustris.* The ditches and rhynes contain a good range of submerged species; notably water-violet *Hottonia palustris,* greater bladderwort *Utricularia vulgaris* and hairlike pondweed *Potamogeton trichoides.* Floating species include frogbit *Hydrocharis morsus-range,* rootless duckweed *Wolffia arrhiza* and great duckweed *Lemna polyrhiza.*

Westhay Moor supports a nationally outstanding community of terrestrial and aquatic invertebrates. Thirteen Red Data Book species have been recorded in recent years including the endangered lesser silver diving beetle, *Hydrochara caraboides,* which preys on the rich molluscan fauna in the peaty ditches. It is now confined nationally to the peat moors in the Brue Basin. The large soldier fly, *Odontomyia ornata* and the rare marsh fly, *Pteromicra leucopeza* are also found on Westhay Moor. At least 28 nationally notable invertebrate species also occur on the moor.

Other habitats present include hedges and hedgerow trees of alder *Alnus glutinosa,* willow, hawthorn *Crataegus monogyna* and birch which together with the meadows provide valuable resting and feeding areas for the invertebrates. The meadows, ditches, abandoned peat workings and hedgerows provide suitable breeding habitats for a diverse and nationally important breeding bird community. At least 39 species breed on the moor including lapwing *Vanellus vanellus,* snipe *Gallinago gallinago,* redshank *Tringa totanus* and yellow wagtail *Motacilla flava* on the meadows and nightingale *Luscinia megarhynchos* and Little Owl *Athene noctus* in the scrub woodland. Flooded peat workings attract wintering and breeding waterfowl including little grebe *Tachybaptus ruficollis* and water rail *Rallus aquaticus.*

Other vertebrate species present include the otter *Lutra lutra,* grass snake *Natrix natrix* and common frog *Rana temporaria.*

B2c: Excerpt from SSSI Citation for Westhay Heath SSSI

National Grid Reference: ST 415422 **Area:** 25.9 (ha.) 64.0 (ac.)
Ordnance Survey Sheet 1:50,000: 182 1:10,000: ST 44 SW

Description and Reasons for Notification:

Westhay Heath is an area of tall fen vegetation containing scrub, marshy grassland, ditches and small ponds in the heart of the peat moors on the Somerset Levels. This mosaic of habitats has developed on areas previously used for peat extraction. The site is of importance for the presence of a nationally rare fen community, including a diverse assemblage of breeding and wintering birds and the presence of a nationally rare breeding bird species.

The moss and reed peats were originally approximately four metres thick, overlying neutral alluvial clays. In some areas all the peat has been removed, in others varying thicknesses remain. Some parts are now permanently flooded whilst others are kept free of surface water for most of the year by drainage ditches.

Within the flooded peat workings are small areas of open water, dense patches of scrub, mainly Grey Willow *Salix cinerea* and extensive stands of Common Reed *Phragmites australis*. Large areas are dominated by Bulrush *Typha latifolia*. Many other tall fen species grow amongst the Bulrush and on the edges of these areas. These include three plants with a very restricted national distribution: Golden Dock *Rumex maritimus*, Marsh Dock *Rumex palustris* and Milk-parsley *Peucedanum palustre*; together with Yellow Iris *Iris pseudacorus*, Water Plantain *Alisma plantgao-aquatica*, Fine-leaved Water-dropwort *Oenanthe aquatica*, Reed Canary-grass *Phalaris arundinacea*, Water Dock *Rumex hydrolapathum*, Cyperus Sedge *Carex pseudocyperus* and Trifid Bur-marigold *Bidens tripartita*, whilst the floating plant Frogbit *Hydrocharis morsus-ranae* is abundant.

This fen habitat supports breeding populations of at least 16 bird species. These include Little Grebe *Tachybaptus ruficollis*, CettiÕs warbler *Cettia cetti*, Whinchat *Saxicola rubetra*, Water Rail *Rallus aquaticus*, Mute Swan *Cygnus olor*, Reed warbler *Acrolephalus scirpaceus*, Sedge Warbler *Acrocephalus schoenobaenus* and Teal *Anas crecca*. This is the only Somerset site where the nationally rare Marsh Harrier *Circus aeruginosus* breeds. In addition Barn Owl *Tyto alba*, Kingfisher *Alcedo atthis*, Buzzard *Buteo buteo* and Grey Heron *Ardea cinerea*, regularly frequent the area. Bittern *Botaurus stellaris*, Bearded Tit *Panurus biarmicus* and Cetti's Warbler regularly over-winter, while Hobby *Falco subbuteo* is a frequent summer visitor.

Otters *Lutra lutra* are regularly recorded on Westhay Heath. Harvest mice *Micromysminutus* breed on the site, while Grass Snake *Natrix natrix* and Common Frog *Rana temporaria* are frequent.

B2d: Excerpt from SSSI Citation for Catcott, Edington and Chilton Moors SSSI

National Grid Reference: ST 390420 **Area:** 1083 (ha) 2676 (ac)
Ordnance Survey Sheet 1:50,000: 182 1:10,000: ST 44 SW, ST 43, NW, ST 34 SE

Description:

Catcott, Edington and Chilton Moors form part of the extensive grazing marsh and ditch systems of the Somerset Levels and Moors. The land lies below 8m ODN in the basin of the River Brue. The soils are principally of the Altcar series reed peats which are overlain in parts by remnants of the Turberry Moor series moss peats. On the northern and southern fringes of the site the peat soils are overlain by Midelney series alluvial clay.

The water table is high for most of the year with occasional winter flooding by overtopping of the River Brue. The complex of rhynes and ditches has a high penned water level in summer and drains freely to the arterial system in winter.

A wide range of sward types has developed due to the variation in soils and in management practice. Unimproved swards are well represented with many meadows dominated by species-rich mire-type communities characterised by Meadow Thistle *Cirsium dissectum*, Meadow Rue *Thalictrum flavum*, Quaking-grass *Briza media*, Heath-grass *Danthonia decumbens*, Carnation Sedge *Carex panicea*, Common Sedge *C. nigra* and Southern Marsh-orchid *Dactylorhiza praetermissa*. Wetter unimproved marshy grassland may, in addition, contain Rushes *Juncus* spp, Marsh marigold *Caltha palustris*, Marsh Pennywort *Hydrocotyle vulgaris*, Tubular Water-dropwort *Oenanthe fistulosa*, Ragged-Robin *Lychnis flos-cuculi* and Creeping Jenny *Lysimachia nummularia*. A few meadows also contain Devil's-bit Scabious *Succisa pratensis*. Many of the semi-improved *Festu-Lolium* grasslands include components of the mire-type community, often with Oxeye Daisy *Leucanthemum vulgare*, Autumn Hawkbit *Leontodon autumnalis* and Meadow Vetchling *Lathyrus pratensis*.

Catcott Heath, on the south-eastern part of the site, contains an area of Purple Moorgrass *Molinia caerulea*, Bog Myrtle *Myrica gale* and Cross-leaved Heath *Erica tetralix* heathland with Alder *Anlus glutinosa* carr woodland and mixed scrub, containing Common Cotton-grass *Eriophorum angustifolium* and Royal Fern *Osmunda regalis*. The heath is noted for its rare vascular plants including Marsh Pea *Lathyrus palustris* Milk-parsley *Peucedanum palustre* and Marsh Fern *Thelypteris thelypteroides*. Other species with restricted distributions nationally include Marsh Cinquefoil *Potentilla palustris*, Great Fen-sedge *Cladium mariscus*, Slender Sedge *Carex lasiocarpa* and Marsh Stitchwort *Stellaria palustris*. A similar but less species-rich area of alder carr and wet grassland is found on Burtle Whites on the Northeastern part of the site.

127 aquatic and bankside vascular plant species have been recorded in the field ditches, IDB-maintained rhynes and deep arterial watercourses. The diverse bankside flora, dominated by Reed Sweet-grass *Glyceria maxima*, includes Flowering Rush *Butomus umbellatus*, Bottle Sedge *Carex rostrata* and Water Dock *Rumex hydrolapathum*. Aquatic deep water species such as Yellow Water-lily *Nuphar lutea* and Arrowhead *Sagittaria sagittifolia* are largely confined to the eutrophic arterial channels. The ditches and rhynes contain a good range of submerged species: notably Fan-leaved Water-crowfoot *Ranunculus circinatus*, Spiked Water-milfoil *Myriophyllum spicatum*, Water-violet *Hottonia palustris* and Greater Bladderwort *Utricularis vulgaris*. Floating species include Frogbit *Hydrocharis morsus-ranea* and Rootless Duckweed *Wolffia arrhiza* with several notable emergent species including Mare's- tail *Hippuris vulgaris*, Greater Water-parsnip *Sium latifolium*, Lesser Waterplantain *Baldellia ranunculoides* and Fine-leaved-Water-dropwort *Oenanthe aquatica*.

A diverse invertebrate fauna is associated with these botanically rich water channels. The water beetle fauna is of exceptional interest, with the nationally rare species *Haliplus mucronatus* and *Hydrophilus piceus* present. The rare soldier fly *Stratiomys furcata* is found, and there are good numbers of dragonflies and damselflies, notably *Brachytron pratense* and *Sympetrum sanguineum*.

Other habitats present include hedges and hedgerow trees of Alder, Hawthorn *Crataegus monogyna* and willow *Salix spp*.

These diverse habitats provide suitable feeding and nesting sites for a wide range of birds. In winter, waterfowl such as Golden Plover *Pluvialis apricota*, Lapwing *Vanellus vanellus*, Snipe *Gallinago gallinago* and Dunlin *Calidris alpina* feed on the wet grasslands, whilst under flood conditions, wildfowl such as Teal *Anas crecca*, Wigeon *A. penelope* and Mallard *A. platyrhynchos* move on to the Moors. The pastures remain moist into spring and early summer when the tussocky fields support breeding Snipe, Lapwing, Curlew *Numenius arquata* and a few pairs of Redshank *Tringa totanus*, Yellow Wagtail *Motacilla flava* and Whinchat *Saxicola rubeta* breed on the moors and in spring, the pastures are an important feeding ground for Whimbrel *Numenius phaeopus* on migration.

Other vertebrate species present, include the Otter *Lutra lutra*, Grass Snake *Natrix natrix* and Common Frog *Rana temporaria*.

B2e: Excerpt from SSSI Citation for Tealham and Tadham Moor SSSI

National Grid Reference: ST 420450 **Area:** 917.6 (ha) 2267.3 (ac)
Ordnance Survey Sheet 1:50,000: 182 1:10,000: ST 34 NE ST 44 NW ST 34 SE ST 44 SW

Description:

Tealham and Tadham Moors form part of the extensive grazing marsh and ditch systems of the Somerset Levels and Moors. The land lies below 8m ODN in the basin of the River Brue at the foot of the Lias limestone slopes of the Wedmore Ridge. The soils are principally of the Altcar series reed peats which are overlain in parts by remnants of the Turbary Moor series moss peats, giving more acid surface conditions. The fringes of the moor comprise Midelney series alluvial clay over peat soils.

The water table is high throughout the greater part of the year with winter flooding occurring annually, by over-topping of the River Brue. The extensive system of rhynes and ditches has a high penned water level in summer and is free draining during the winter period.

A wide range of grassland types have developed due to the variation in soils and in management practice. The fescue/rye-grass swards are dominated by Meadow Fescue (Festuca pratensis) and Perennial Rye-grass (Lolium perenne) together with marsh Ragwort (Senecio aquaticus), Ragged-Robin (Lychnis floss-cuculi) and Meadowsweet (Filipendula ulmaria). Other more diverse swards contain Common Knapweed (Centaurea nigra), Crested Dog's-tail (Cynosurus cristatus) and Meadow Rue (Thalictrum flavum). The wetter grasslands have Marsh-marigold (Caltha palustris), Lesser Spearwort (Ranunculus flammula) and rushes (Juncus spp). There are some species-rich examples of the mire-type communities with Meadow Thistle (Cirsium dissectum), Carnation Sedge (Carex panicea) and Sweet Vernal-grass (Anthoxanthum odoratum).

113 aquatic and bankside vascular plant species have been recorded from the field ditches,IDB rhynes and deep arterial watercourses. Deep water species such as Yellow Water-lily (Nuphar lutea) and Shining Pondweed (Potamogeton lucens)are restricted to the eutrophic arterial channels whilst the ditches and rhynes contain a good range of submerged species: Water Violet (Hottonia palustris), Greater Bladderwort (Utricularia vulgaris),water-starworts (Callitriche spp) and stoneworts (Chars spp); floating species: Frogbit (Hydrocharis morsusranae), Least Duckweed (Wolffia arrhiza) and emergent species: Lesser Water-plantain (Alisma lanceolatum), Water Dock (Rumex hydrolapathum) and bur-reeds (Sparganium spp).

A diverse invertebrate fauna is associated in particular with ditches that have a good submerged plant community. The water beetle fauna is exceptionally rich, with the nationally rare species Hydrophilus piceus and Hydrochara

caraboides (abundant here at its only location in Britain); together with the rare soldier flies Stratiomys furcata and Odontomyia ornata. Good numbers of dragonflies and damselflies occur including the Hairy Dragonfly (Brachytron pratense) and the Variable Coenagrion (Coenagrion pulchellum).

Other habitats present include hedges and hedgerow trees of Alder (Alnus glutinosa), Hawthorn (Crataegus monogyna) and willow (Salix spp) together with a few areas of scrub. An area of mixed woodland is the site of a heronry - the only one located on the moors.

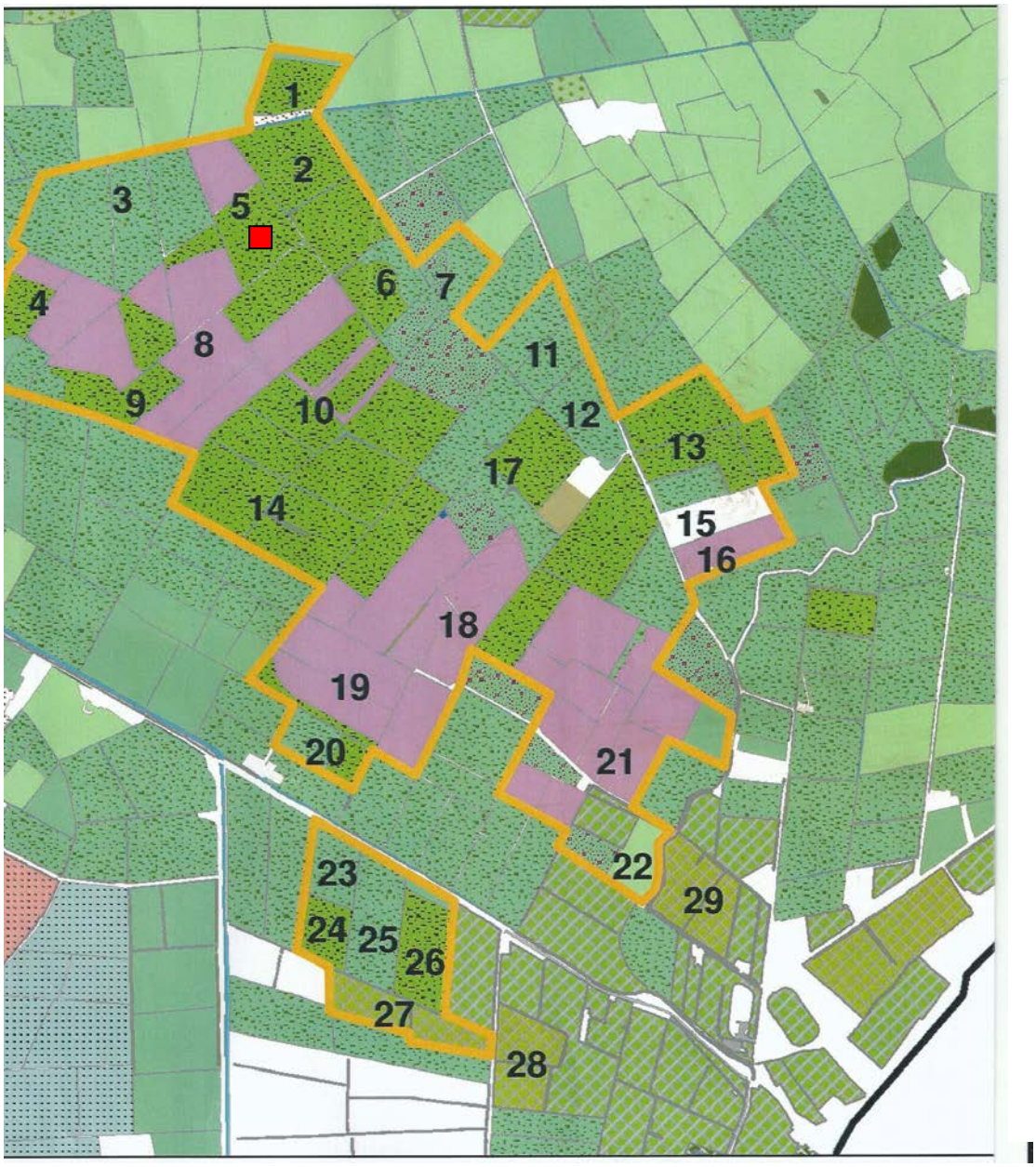
These habitats provide suitable feeding and nesting sites for a wide range of birds large numbers of waterfowl feed on the wet grasslands; including good Golden Plover (Pluvialis apricaria), Lapwing (Vanelius vanellus), Snipe (Gallinago gallinago) and Dunlin (Calidris alpina). Under flood conditions, populations of wildfowl move on to the moor: Bewick's Swan (Cygnus bewickii), Wigeon (Anas Penelope) and Teal (Anas crecca). Much of the moor remains moist into the spring and early summer when the low tussocky pastures provide suitable conditions for breeding Snipe, Lapwing, Curlew (Numenius arquata) and Redshank (Tringa totanus). Good numbers of Yellow Wagtail (Motacilla flava) and Whinchat (Saxicola rubetra) breed on the fringes of the moor. Tealham and Tadham Moors are also an important feeding ground for Whimbrel (Numenius phaeopus) on their spring migration in April/May.

A wide range of other vertebrate species have been recorded here, most notably the Otter (Lutra lutra).

B2f(i): Excerpts from field notes made during Somerset Wildlife Trust 2010 survey of East Waste. (Numbers refer to map reproduced below.)

1	Species-rich wet grassland. Common knapweed F, meadow vetchling F, jointed rush F, common marsh bedstraw O, greater bird's-foot trefoil O..
2	Species-rich wet grassland.
3	Probably good winter wader interest
4	Probably good winter wader interest
5	Probably good winter wader interest
7	Good ditches
8	A cluster of fields, all top quality
9	Winter wader interest (snipe)
10	Probably good winter wader interest
11	2 species-poor fields next to Godney Road. Species include common spike-rush, sharp-flowered rush, marsh thistle, common sorrel, greater bird's-foot trefoil (R). Much soft rush in 2010.
12	Species-poor fields.
13	Sharp-flowered rush, red clover, meadow buttercup, meadowsweet, common sorrel, cuckooflower, brown sedge, herb cover <30%, soft rush 30%.
14	2 species-rich wet grassland fields. Greater bird's-foot trefoil R, cuckooflower, red clover, meadowsweet O, brown sedge, sharp-flowered rush R, meadow vetchling, glaucous sedge, common spike-rush.
15	Abandoned, unmanaged field
16	Sharp-flowered rush, meadowsweet, common marsh bedstraw, red clover, amphibious bistort, cuckooflower, brown sedge, autumn hawkbit.
20	East part of field [contains]: meadow vetchling, common sorrel, ?oval sedge, common sedge all R. Red clover and meadow buttercup F, common spike-rush O.
22	Species-poor field
23	Wet grassland. Cuckooflower O, brown sedge O, common sorrel O, red clover F, meadowsweet R, meadow buttercup F, meadow vetchling O
24	AgCx - brown sedge A, meadow vetchling F, cuckooflower O, meadow buttercup, common sedge O, meadowsweet O, red clover O
25	Southern end: common sedge R, meadowsweet R, meadow vetchling F, brown sedge O, common spike-rush R.
26	North half of field: meadow buttercup F, meadow vetchling O, amphibious bistort R, cuckooflower O, brown sedge R/O, common sedge R/O, red clover O. Southern half more species-rich: as for northern half but brown sedge F, common sedge O/F, sharp-flowered rush R. Herbs >30% cover

B2f(ii): Phase 1 Habitat Survey Map East Waste (2010)
(Source: Somerset Wildlife Trust) Purple fields denote species-rich rush pasture, green fields with stippling are wet grassland or species-poor rush pasture. Ditches EWa, EWb and EWc were situated in the field marked by the red square.



Appendix C1: Contract aquatic invertebrate surveys conducted in the Somerset Levels and Moors 1984 – 2007

Table C1: Summary of contract surveys conducted 1984 – 2007.

Authors (Year)	Report	Moors surveyed (see Key)
Drake, C.M., Foster, A.P. & Palmer, M.A. (1984)	A survey of the invertebrates of the Somerset Levels and Moors. Unpublished report to Nature Conservancy Council by Chief Scientists Team, Peterborough	BB, CEC, GR, KM, KSM, ML, NM, PH, SL, TT, WT, WG, WSM, WM
Drake, C.M.(1989)	England Field Unit Project No.107 Somerset and Avon Levels Aquatic Invertebrate Survey, 1989 Unpublished report to Nature Conservancy Council by England Field Unit, Peterborough	CM, LM, KM, NaM,
Anderson, M.A., Hill-Cottingham, P. & Duff, A.G. (1991)	Avon Invertebrates Survey. Unpublished report for English Nature SW by Somerset Ecology Consultants Ltd	B, CG, C/T, GR, NaM, KM, Ksey, Pu, PuM
Hill-Cottingham, P. (1993)	Somerset Levels Hydrochara caraboides Survey 1993. Unpublished report for English Nature by Somerset Ecology Consultants Ltd	CEC, TT, WG
Anderson, M.A., Hill-Cottingham, P., Smith, T. & Duff, A.G. (1994)	Invertebrate survey of ditches on the Avon Levels and Moors. Unpublished report for English Nature SW by Somerset Ecology Consultants Ltd	BS, PuM
Gibbs, D. (1994)	A survey of the aquatic invertebrate fauna of ditches on the Somerset Levels and Moors. Unpublished report to English Nature	CEC, KSM, NM, SL, TT, WSM, WT
Hill-Cottingham, P. & Smith, T. (1996)	Pawlett Hams Ditch Survey 1996. Unpublished report to English Nature	PH
Hill-Cottingham, P. & Smith, T. (1997)	Avon Levels and Moors Ditch Invertebrates Survey. Unpublished report to English Nature	BS, C/T, KM, PuM
Hill-Cottingham, P. & Smith, T. (1998a)	Pawlett Hams Ponds Survey of Aquatic Invertebrates on Bridgwater Bay SSSI. Unpublished report to English Nature	PH
Hill-Cottingham, P. & Smith, T. (1998b)	Surveys of the aquatic macro-invertebrates in ditches on Wet Moor, West Moor, and Curry Moors SSSIs in 1997 and 1998. Unpublished report to English Nature	WM, WT, CM
Godfrey, A. (1999a)	Aquatic invertebrate survey of the Somerset Levels and Moors. Unpublished report to English Nature	CEC, KSM, NM, SL, TT, WSM, WT
Godfrey, A. (1999b)	Aquatic invertebrate survey of the North Somerset Levels 1999. Unpublished report to English Nature	BS, C/T, KM, NaM, PuM
Boyce, D.G. (2004)	A study of the distribution and ecology of the lesser silver water beetle Hydrochara caraboides on the Somerset Levels. English Nature Research Report No.591, English Nature, Peterborough	SH, WG
Drake, C.M.(2005)	The Effectiveness of Management of Grazing Marshes for Aquatic Invertebrate Communities. Unpublished report to Buglife - The Invertebrate Conservation Trust	SL, WW
Keystone Environmental (2011)	Somerset Peat Moors Invertebrate Report for Somerset County Council April 2011	GH, GM

Key to Table C1

Avon/North Somerset Levels

B = Banwell
BB = Berrow and Bleadon Levels
BS = Biddle Street
C/T = Clevedon/Tickenham Moors
CG = Congresbury Moor
GV = Gordano Valley
KM = Kenn Moor
KSey = Kingston Seymour
NaM = Nailsea Moor
Pu = Puxton Moor

Somerset Levels and Moors

CEC = Catcott, Edington and Chilton Moors
CM = Curry Moor
CR = Crannel Moor
GH = Glastonbury Heath/Westhay Level
GM = Godney Moor
KSM = Kings Sedgemoor
L = Langmead
ML = Moorlinch Moor
NM = North Moor
PH = Pawlett Hams
SH = Shapwick Heath
SL = Southlake Moor
TT = Tealham and Tadham Moors
WT = West Moor
WG = Westhay Moor
WSM = West Sedgemoor
WM = Wet Moor
WW =West Wastes

Appendix C2: Methods employed in contract aquatic invertebrate surveys conducted in the Somerset Levels and Moors 1984 – 2007

Table C2(i): Surveys 1984 – 1996 (See Key at end of Appendix)

Survey	Sampling methodology	Sorting methodology
1.Drake, C.M., Foster, A.P. & Palmer, M.A. (1984)	A. "A pond net with a 1mm mesh" B. April – May & September - October C. 20 metres D. Approx six sweeps on 4 occasions E. No set pattern of sweeps F. No fixed time for sweeping	A. Polythene sheet, plastic tray B. 30 - 45 minutes C. Some taken for later sorting (up to 30 minutes worth) of for laboratory ID D. Abundances scored on 1 - 4 scale
2. Drake, C.M.(1989)	A. "A pond net" B. May C. Up to 50 metres D. Not recorded E. Not recorded F. Not recorded	A. White sheet B. Not recorded C. Some taken for laboratory ID D. Abundances scored on 1 - 3 scale
3. Anderson, M.A., Hill-Cottingham, P. & Duff, A.G. (1991)	A. "A pond net" B. October - November C. 50 metres D. Three sweeps E. No set pattern of sweeps F. No fixed time for sweeping	A. Large metal tray, bucket B. One hour (including netting) C. Some taken for laboratory ID D. Abundances scored on 1 - 3 scale
4. Hill-Cottingham, P. (1993)	A. "A net" B. August - September C. Not recorded D. Minimum of three sweeps E. Not recorded F. Not recorded	A. Large metal tray B. Not recorded C. No material taken away D. Mollusca and presence of *Hydrochara. caraboides*
5. Anderson, M.A., Hill-Cottingham, P., Smith, T. & Duff, A.G. (1994)	A. "A pond net" B. September C. 50 metres D. Minimum of three sweeps E. No set pattern of sweeps F. No fixed time for sweeping	A. Large metal tray B. Not recorded C. Some taken for laboratory ID D. Abundances scored on 1 - 3 scale
6. Gibbs, D. (1994)	A. "A standard sized dip-net" B. May - June C. 30 metres D. Not recorded E. No set pattern of sweeps F. No fixed time for sweeping	A. White plastic sheet B. One hour (including netting) in blocs of 15 minutes C. Some taken for laboratory ID D. For RDB, notable & local spp. the numbers of samples from which recorded noted otherwise presence at a site only
7. Hill-Cottingham, P. & Smith, T. (1996)	A. "A pond net" B. October C. 50 metres D. Three sweeps E. No set pattern of sweeps F. No fixed time for sweeping	A. Large metal tray, bucket B. One hour (including netting) C. Some taken for laboratory ID D. Abundances scored on 1 - 3 scale

Appendix C2: Methods employed in contract aquatic invertebrate surveys conducted in the Somerset Levels and Moors 1984 – 2007

Table C2(ii): Surveys 1997 – 2004 (See Key at end of Appendix)

Survey	Sampling methodology	Sorting methodology
8. Hill-Cottingham, P. & Smith, T. (1997)	A. "A long-handled net" B. June B. 20 metres C. Not recorded D. No set pattern of sweeps E. No fixed time for sweeping	A. Large metal tray, bucket B. One hour (including netting) C. Some taken for laboratory ID D. Abundances scored on 1 - 3 scale
9. Hill-Cottingham, P. & Smith, T. (1998a)	A. As Hill-Cottingham & Smith (1996) B. June C. N/A - Pond survey D. Three sweeps E. No set pattern of sweeps F. No fixed time for sweeping	A. Large metal tray, bucket B. One hour (including netting) C. Some taken for laboratory ID D. Abundances scored on 1 - 3 scale
10. Hill-Cottingham, P. & Smith, T. (1998b)	A. "A long handled net" B. October, June C. 50 metres D. Minimum of three sweeps E. No set pattern of sweeps F. No fixed time for sweeping	A. Large metal tray B. Not recorded C. Some taken for laboratory ID D. Abundances scored on 1 - 4 scale .
11. Godfrey, A. (1999a)	A. "A pond net" B. July C. 50 metres D. Not recorded E. Not recorded F. 3 minutes fixed time	A. Sieve, white sorting tray B. Until no new taxa observed (c 30 - 45 minutes according to Godfrey) C. Some samples sorted in laboratory D. Actual numbers
12. Godfrey, A. (1999b)	A. "A pond net" B. July C. 50 metres D. Not recorded E. Not recorded F. 3 minutes fixed time	A. Sieve, white sorting tray B. Until no new taxa observed (c 30 - 45 minutes according to Godfrey) C. Some samples sorted in laboratory D. Actual numbers
13. Boyce, D.G. (2004)	A. "A pond net" B. April - June C. 25 square metres D. Not recorded E. Not recorded F. 15 minutes fixed time (may have included sorting?)	A. White tray B. Not recorded C. No material taken D. The objective of the survey was to study *H. caraboides*. The presence of "other large beetles" was noted.

Appendix C2: Methods employed in contract aquatic invertebrate surveys conducted in the Somerset Levels and Moors 1984 – 2007

Table C2(ii): Surveys 2005 – 2011 (See Key at end of Appendix)

Survey	Sampling methodology	Sorting methodology
14. Drake, C.M.(2005)*	A. "Standard FBA-quality design 24cm across x 27 cm tall and 39cm deep with flexible netting and a nominal 1mm diameter mesh" B. September - October C. 25 metres D. 6 sweeps & No fixed number E. Single 'horseshoe-shaped' sweep across ditch at intervals of 5 paces & No pattern F. No fixed time & fixed time	A. White polythene sheet (fertiliser sack), white tray, bucket B. 10 minutes C. Some taken for laboratory ID D. Abundances scored on 1 - 4 scale
15. Keystone Environmental (2011)	A. "Standard pond net" B. April C. Not recorded D. Not recorded E. Not recorded F. 3 minutes fixed time	A. Sieve, white sorting tray B. Not recorded C. Some taken for laboratory ID D. Abundances scored on 1 - 4 scale

* Drake (2005) set out to compare methodologies he characterised as the 'Gibbs Method' (fixed no. of sweeps, fixed pattern) with the 'Drake method' (vigorous netting for fixed time). Unfortunately, Drake does not record the time set but others have limited this to 3 minutes (e.g. Godfrey 1999 a & b, Pond Action 1992, 1994)

KEY

Sampling Methodology	Sorting Methodology
A. Type of net B. Time of year C. Length of ditch netted D. Number of sweeps per sample E. Pattern of sweeping F. Time spent sweeping	A. Equipment used (e.g. sheet, tray, sieve, bucket) B. Time spent sorting C. Material taken for later ID D. How counted

Appendix C3: Data from 2006 Fieldwork

Table C3(i): *Dytiscus* beetles caught in traps set at Shapwick Heath on 6/5/06 and collected on 7/5/06.

Trap No.	Baited/ Unbaited	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
6A	B	0	0	0	1	0
6B	U	0	0	0	0	0
7A	B	0	0	0	0	0
7B	U	1	0	0	0	0
8A	B	0	0	0	0	0
8B	U	0	0	0	0	0
9A	B	0	3	0	0	0
9B	U	0	0	0	0	0
10A	B	0	1	0	1	1
10B	U	0	0	0	0	0
5A	U	0	0	0	0	0
5B	B	0	0	0	0	0
4A	B	5	2	0	0	1
4B	U	0	0	0	0	0
3A	B	0	0	0	0	0
3B	U	0	1	0	0	0
2A	B	0	0	1	0	0
2B	U	0	0	0	0	0
1A	B	1	1	0	0	0
1B	U	0	0	0	0	0
	TOTALS	7	8	1	2	2

Maximum/Minimum Temperatures

Location of thermometer	Minimum (degrees C)	Maximum (degrees C)
2A/2B	6	25
4A/4B	6	21
7A/7B	7	25
9A/9B	7	26

Other species of note recorded: The water beetle, *Hydaticus transversalis* in Traps 1B, 2B & 6B; Male Smooth Newt (*Triturus vulgaris*) in Trap 10B

Appendix C3: Data from 2006 Fieldwork

Table C3(ii): *Dytiscus* beetles caught in traps set at Shapwick Heath on 13/5/06 and collected on 14/5/06.

Trap No.	Baited/ Unbaited	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
1A	B	0	1	0	0	1
1B	U	0	2	0	0	0
2A	B	0	1	0	0	0
2B	U	0	0	0	0	0
3A	B	1	0	0	0	0
3B	U	1	0	0	0	0
4A	B	0	1	0	0	0
4B	U	0	0	0	0	0
5A	B	0	2	0	0	1
5B	U	0	1	0	0	0
6A	B	0	0	0	0	0
6B	U	0	0	0	0	0
7A	B	0	0	0	0	0
7B	U	0	0	0	0	0
8A	B	0	0	0	0	1
8B	U	0	0	0	0	0
9A	B	1	1	0	0	0
9B	U	0	0	0	0	0
10A	B	0	0	0	0	0
10B	U	0	0	0	0	0
	Totals	3	9	0	0	3

Maximum/Minimum Temperatures

Location of thermometer	Minimum (degrees C)	Maximum (degrees C)
10A/10B	5	25
8A/8B	8	19
6A/6B	7	23
2A/2B	8	19

Other species of note recorded: Lesser Silver Water Beetle (*Hydrochara caraboides*) in Traps 1a & 4B; The water beetle, *Hydaticus transversalis* in Traps 4A, 5A, 5B, 8B, 9A & 10A; Male Smooth Newt (*Triturus vulgaris*) in Trap 3B, female in 8A; Ten-spined Stickleback (*Pungitius pungitius*) in Trap 2B, 3A & 3B (x2).

Appendix C3: Data from 2006 Fieldwork

Table C3(iii): *Dytiscus* beetles caught in traps set at Shapwick Heath on 27/5/06 and collected on 28/5/06.

Trap No.	Baited/ Unbaited	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
10A	B	0	1	0	0	1
10B	U	0	0	0	0	0
9A	B	0	0	0	0	3
9B	U	0	0	0	0	1
8A	B	0	2	0	0	0
8B	U	0	0	0	0	0
7A	B	1	0	0	0	5
7B	U	0	0	0	0	0
6A	B	0	2	0	0	1
6B	U	0	0	0	0	0
5A	B	1	0	0	0	0
5B	U	0	0	0	0	0
4A	B	0	1	0	0	1
4B	U	0	0	0	0	0
3A	B	0	0	0	0	0
3B	U	0	0	0	0	0
2A	B	0	0	0	0	0
2B	U	0	0	0	0	0
1A	B	0	0	0	0	3
1B	U	0	0	0	0	0
	Totals	2	6	0	0	15

Maximum/Minimum Temperatures

Location of thermometer	Minimum (degrees C)	Maximum (degrees C)
10A/10B	11	26
8A/8B	11	24
5A/5B	9	25
2A/2B	10	21

Other species of note recorded: The water beetle, *Hydaticus transversalis* in Traps 10A, & 10B; Male Smooth Newt (*Triturus vulgaris*) in Trap 10B (x2), female in 10B (x2) & 5A; Ten-spined Stickleback (*Pungitius pungitius*) in Trap 7B, 4A (x2) & 3B; Frog tadpoles (*Rana* sp.) in 5A, 5B (x3), 6B & 7B.

Appendix C3: Data from 2006 Fieldwork

Table C3(iv): *Dytiscus* beetles caught in traps set at Shapwick Heath on 9/6/06 and collected on 10/6/06.

Trap No.	Baited/ Unbaited	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
6A	B	0	0	0	0	2
6B	U	0	0	0	0	1
5A	B	0	0	0	0	1
5B	U	0	0	0	0	0
4A	B	0	0	0	0	2
4B	U	0	0	0	0	0
3A	B	0	0	0	0	0
3B	U	0	0	0	0	0
2A	B	0	0	0	0	1
2B	U	0	0	0	0	0
1A	B	0	0	0	0	3
1B	U	0	0	0	0	1
7A	B	0	0	0	0	2
7B	U	0	0	0	0	0
8A	B	0	0	0	0	4
8B	U	0	0	0	0	3
9A	B	0	1	0	0	4
9B	U	0	0	0	0	0
10A	B	1	0	0	0	1
10B	U	0	0	0	0	0
	Totals	1	1	0	0	25

Maximum/Minimum Temperatures

Location of thermometer	Minimum (degrees C)	Maximum (degrees C)
2A/2B	11	41
5A/5B	12	42
7A/7B	14	31
9A/9B	7	42

Other species of note recorded: The water beetle, *Hydaticus transversalis* in Trap 8B; The water beetle, *Acilius sulcatus* in Trap 5A; Newt larva (*Triturus* sp.) in Trap 5B.

Appendix C3: Data from 2006 Fieldwork

Table C3(v): *Dytiscus* beetles caught in traps set at Shapwick Heath on 23/6/06 and collected on 24/6/06.

Trap No.	Baited/ Unbaited	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
1	B	1	0	0	0	0
2	B	0	1	0	0	1
3	B	0	0	0	0	1
4	B	0	0	0	0	0
5	B	0	0	0	0	0
6	B	0	0	0	0	0
7	B	0	0	0	0	0
8	B	0	0	0	0	0
9	B	0	0	0	0	0
10	B	0	0	0	0	0
11	B	0	0	0	0	0
12	B	0	0	0	0	0
13	B	0	0	0	0	0
14	B	0	0	0	0	0
15	B	1	0	0	0	2
16	B	0	0	0	0	0
17	B	2	0	0	0	3
18	B	0	0	0	0	0
19	B	0	0	0	0	1
20	B	0	0	0	0	0
	Totals	4	1	0	0	8

Maximum/Minimum Temperatures

Location of thermometer	Minimum (degrees C)	Maximum (degrees C)
1	13	22
6	13	19
10	12	25
20	12	21

Other species of note recorded: The water beetle, *Hydaticus transversalis* in Trap 7; Ten-spined Stickleback (*Pungitius pungitius*) in Trap 17.

Appendix C3: Data from 2006 Fieldwork

Table C3(vi): *Dytiscus* beetles caught in traps set at Shapwick Heath on 15/7/06 and collected on 16/7/06.

Trap No.	Baited/ Unbaited	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
1	B	0	0	0	0	0
2	B	0	0	0	0	0
3	B	0	1	0	0	0
4	B	0	0	0	0	0
5	B	0	0	0	0	0
6	B	0	0	0	0	0
7	B	0	0	0	0	0
8	B	0	0	0	0	0
9	B	0	0	0	0	0
10	B	0	0	0	0	0
11	B	0	0	0	0	0
12	B	0	0	0	0	0
13	B	0	0	0	0	0
14	B	0	0	0	0	2
15	B	0	0	0	0	2
16	B	0	0	0	0	0
17	B	0	2	0	0	0
18	B	1	0	0	0	0
19	B	0	2	0	0	0
20	B	0	0	0	0	1
21	B	0	0	0	0	1
22	B	0	0	0	0	1
23	B	0	0	0	0	0
24	B	0	0	1	0	1
25	B	0	0	0	0	1
	Totals	1	5	1	0	9

Maximum/Minimum Temperatures

Location of thermometer	Minimum (degrees C)	Maximum (degrees C)
1	11	28
6	13	20
16	11	28
18	14	28

Other species of note recorded: Greater Silver Water Beetle (*Hydrophilius piceus*) larvae in Traps 16 & 19; Lesser Silver Water Beetle (*Hydrochara caraboides*) larva in Trap 12; The water beetle, *Hydaticus transversalis* in Traps 2 & 17 (x3); The water beetle, *Acilius sulcatus* in Trap 17; Three-spined Stickleback (*Gasterosterus aculeatus*) in Trap 22; Common Frog (*Rana temporaria*) in Trap 20; Water Shrew (*Neomys fodiens*) in Traps 14 & 15.

Appendix C3: Data from 2006 Fieldwork

Table C3(vi): *Dytiscus* beetles caught in traps set at Shapwick Heath on 9/9/06 and collected on 10/9/06.

Trap No.	Baited/ Unbaited	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
1	B	0	0	0	0	0
2	B	0	0	0	0	0
3	B	0	0	0	0	0
4	B	0	0	0	0	0
5	B	0	0	0	0	0
6	B	0	0	0	0	0
7	B	0	0	0	0	0
8	B	0	0	0	0	0
9	B	0	0	0	0	0
10	B	0	0	0	0	0
11	B	0	0	0	0	0
12	B	0	0	0	0	0
13	B	0	0	0	0	0
14	B	1	1	0	1	0
15	B	0	0	0	0	0
16	B	0	0	0	0	0
17	B	1	0	0	0	0
18	B	1	0	0	0	0
19	B	0	0	0	0	0
20	B	0	0	0	0	0
21	B	0	0	0	0	0
22	B	2	1	0	1	0
23	B	0	0	0	0	0
	Totals	5	2	0	2	0

Maximum/Minimum Temperatures

Location of thermometer	Minimum (degrees C)	Maximum (degrees C)
1	12	21
6	11	21
19	7	44
21	9	26

Other species of note recorded: The water beetle, *Hydaticus transversalis* in Trap 15; The water beetle, *Acilius sulcatus* in Trap 17.

Appendix C3: Data from 2006 Fieldwork

Table C3(vii): *Dytiscus* beetles *caught by netting at Shapwick Heath on 13/5/06.*

Traps in section of ditch swept	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
1A, 1B	0	0	0	0	0
2A, 2B	0	0	0	0	0
3A, 3B	0	0	0	0	0
4A, 4B	0	1	0	0	0
5A,5B	0	0	0	0	0
6A, 6B	0	0	0	0	0
7A, 7B	0	0	0	0	0
8A, 8B	0	0	0	0	1
9A, 9B	0	0	0	0	0
10A, 10B	0	0	0	0	0
Totals	0	1	0	0	1

Other species of note recorded: The water beetle, *Hydaticus transversalis* in section 3A/3B; Male Smooth Newt (*Triturus vulgaris*) in section 2A/2B; Common Frog (*Rana temporaria*) in sections 3A/3B & 4A/4B; Ten-spined Stickleback (*Pungitius pungitius*) in sections 8A/8B (x2) & 9A/9B.

Table C3(viii): *Dytiscus* beetles caught by netting at Shapwick Heath on 28/5/06.

Traps in section of ditch swept	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
10A, 10B	0	0	0	0	0
9A, 9B	0	0	0	0	1
8A, 8B	0	0	0	0	2
7A, 7B	0	0	0	0	0
6A,6B	0	0	0	0	0
5A, 5B	0	0	0	0	0
4A, 4B	0	0	0	0	0
3A, 3B	0	0	0	0	0
2A, 2B	0	0	0	0	0
1A, 1B	0	0	0	0	1
Totals	0	0	0	0	4

Other species of note recorded: The water beetle, *Hydaticus transversalis* in sections 1A/1B & 5A/5B; The water beetle, *Acilius sulcatus* in section 5A/5B; Frog tadpoles (*Rana* sp.) in sections 9A/9B, 7A/7B (x2), 6A/6B (x2), 5A/5B (x3), 4A/4B, 3A/3B & 2A/2B (x2) ; Ten-spined Stickleback (*Pungitius pungitius*) in sections 1A/1B (x2), 2A/2B (x2), 7A/7B (x2) & 10A/10B.

Appendix C3: Data from 2006 Fieldwork

Table C3(ix): *Dytiscus* beetles *caught by netting at Shapwick Heath on 5/6/06.*

Traps in section of ditch swept	*D. marginalis* adult ♀	*D. marginalis* adult ♂	*D. dimidiatus* adult ♀	*D. dimidiatus* adult ♂	*Dytiscus* larva
6A, 6B	0	0	0	0	0
5A, 5B	0	0	0	0	0
4A, 4B	0	1	0	0	0
3A, 3B	0	1	0	0	0
2A, 2B	0	0	0	0	1
1A, 1B	0	0	0	0	1
7A, 7B	0	0	0	0	0
8A, 8B	0	0	0	0	0
9A,9B	0	0	0	0	1
10A, 10B	0	0	0	0	1
Totals	0	2	0	0	4

Other species of note recorded: The water beetle, *Acilius sulcatus* in section 9A/9B; Female Smooth Newt (*Triturus vulgaris*) in section 3A/3B; Common Frog tadpoles (*Rana* sp.) in sections 1A/1B, 2A/2B, & 3A/3B.

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(i): *D. dimidiatus* (Sweden)

Species: D. dimidiatus Specimen ID: N/A

Sequence Length: 803 BP

Location: Öland, Sweden (Lat 56.79475251 Lon 16.59770927)

Source: J Bergsten (Pers comm.) Published: No

GenBank Accession No: Not submitted

```
1    AGGATTTGGA ATAATTTCAC ATATTATTAG ACAAGAAAGA GGAAAAAAGG
51   AAACTTTTGG TTCTTTAGGA ATAATTTATG CTATACTAGC AATTGGTTTA
101  TTAGGGTTTG TTGTATGAGC ACATCATATA TTTACTGTAG GGATAGATGT
151  AGACACACGA GCATATTTTA CTTCTGCTAC TATAATTATT GCCGTACCCA
201  CAGGAATTAA AATTTTTTCT TGATTAGCAA CTCTTCATGG ATCTCAAATT
251  AGTTATAGCC CATCTTTATT ATGAGCATTA GGATTTGTAT TTTTATTTAC
301  TGTAGGGGGT TTAACAGGAG TAGTATTAGC TAATTCATCA ATTGATATTA
351  TTCTTCATGA TACATACTAT GTAGTTGCCC ATTTTCATTA TGTATTATCT
401  ATAGGAGCAG TATTTGCAAT TTTAGCTGGA TTTATTCAAT GATTTCCCTT
451  ATTTACAGGA TTAACCTTAA ATTCTAATTT ATTAAAAATT CAATTTGTAG
501  TAATATTTGT AGGGGTAAAT TTAACTTTCT TTCCTCAACA CTTCTTAGGT
551  TTAAGAGGAA TACCTCGTCG ATATTCAGAT TATCCTGATG CTTATACTTC
601  ATGAAATGTA GTATCTTCAA TTGGATCGAC TATCTCATTT ATTGGGGTAA
651  TATTATTAAT TTATATTATC TGAGAAGCTT TTATTTCTCA ACGATTAGTA
701  ATTTTTTCTA ATCAAATACC AACTTCTATT GAATGATTCC AATCCCATCC
751  CCCAGCTGAA CATAGATATT CCGAACTTCC AATATTATCT AATTTCTGAT
801  ATG
```

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(ii): *D. marginalis* (Sweden A)

Species: D. marginalis Specimen ID: N/A

Sequence Length: 803 BP

Location: Öland, Sweden (Lat 56.71630256 Lon 16.63305167)

Source: J Bergsten (Pers comm.) Published: No

GenBank Accession No: Not submitted

1 TTGCACCCAG AAGTTTATAT TTTAATTCTT CCAGGGTTTG GGATAATTTC
51 TCACATTATT AGACAAGAAA GAGGAAAAAA GGAAACTTTT GGTTCTCTAG
101 GTATAATTTA TGCTATATTA GCAATTGGTC TATTAGGATT TGTTGTATGA
151 GCACATCATA TATTTACTGT AGGAATAGAT GTAGACACAC GGGCATATTT
201 TACTTCTGCT ACTATAATTA TTGCTGTACC CACAGGAATT AAAATTTTTT
251 CTTGGTTAGC AACTCTTCAT GGATCTCAAA TTAGATATAG TCCTTCTTTA
301 CTATGAGCAT TAGGGTTTGT ATTTTTATTT ACTGTAGGGG GTTTAACAGG
351 AGTAGTATTA GCTAACTCTT CGATTGATAT TATTCTTCAT GATACATACT
401 ATGTAGTTGC CCATTTTCAT TATGTATTAT CTATAGGAGC AGTATTTGCA
451 ATTTTAGCTG GATTTATTCA ATGATTCCCC TTATTTACAG GATTGACTTT
501 AAATTCTAAT TTATTAAAAA TTCAATTTGT AGTAATATTT ATTGGGGTTA
551 ATTTAACTTT CTTTCCTCAA CACTTCTTAG GTTTAAGAGG AATACCTCGT
601 CGATATTCAG ATTATCCTGA TGCTTATACT TCATGAAATG TAGTATCTTC
651 AATTGGATCT ACTATTTCAT TTATTGGAGT AATATTATTA ATTTATATTA
701 TCTGAGAAGC TTTTATTTCT CAACGATTAG TAATTTTTTC AAATCAAATA
751 CCAACTTCTA TTGAATGATT CCAATCCCAT CCCCCAGCGA ACACAGATAT
801 TCG

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(iii): *D. marginalis* (Japan A)

Species: D. marginalis czerskii Specimen ID: N/A

Sequence Length: 769 BP

Location: Gassan, Yamagata Prefecture, Japan

Source: GenBank Published: Inoda et al (2012)

GenBank Accession No: AB674738

1 ATAATTTCTC ATATTATTAG ACAAGAAAGA GGGAAAAAGG AAACTTTTGG
51 TTCTTTAGGG ATAATTTATG CTATATTAGC AATTGGTCTA TTAGGATTTG
101 TTGTATGAGC ACATCATATA TTTACTGTAG GAATGGATGT AGATACACGA
151 GCATATTTTA CTTCTGCTAC TATAATTATT GCCGTACCCA CAGGAATTAA
201 AATTTTTTCT TGATTAGCAA CTCTTCATGG ATCTCAAATT AGATATAGAC
251 CTTCCTTACT ATGAGCATTA GGGTTTGTAT TTTTATTTAC TGTAGGAGGT
301 TTAACAGGGG TGGTATTAGC TAATTCTTCA ATTGATATTA TTCTTCATGA
351 TACATATTAT GTAGTTGCCC ATTTCCATTA TGTATTATCT ATAGGGGCAG
401 TATTTGCAAT TTTAGCTGGA TTTATTCAAT GATTCCCTTT ATTTACAGGA
451 TTAACTTTAA ATTCTAATTT ATTAAAAATT CAATTTATAG TAATATTTGT
501 TGGAGTTAAT TTAACTTTTT TTCCTCAACA CTTCTTAGGT TTAAGAGGAA
551 TACCTCGTCG GTATTCAGAT TACCCTGATG CTTATACTTC ATGAAATGTA
601 GTATCTTCAA TTGGATCTAC TATTTCATTT ATTGGGGTAA TATTATTAAT
651 TTATATTATC TGAGAAGCTT TTATTTCTCA ACGATTAGTA ATTTTTTCAA
701 ATCAAATACC AACTTCTATT GAATGATTCC AATCTCATCC CCCAGCTGAA
751 CACAGATATT CTGAACTTC

This sequence is identical with that of ‘Japan B’

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(iv): *D. marginalis* (Japan B)

Species: D. marginalis czerskii Secimen ID: N/A

Sequence Length: 769 BP

Location: Choukaisan, Yamagata Prefecture, Japan

Source: GenBank Published: Inoda et al (2012)

GenBank Accession No: AB674737

1 ATAATTTCTC ATATTATTAG ACAAGAAAGA GGGAAAAAGG AAACTTTTGG
51 TTCTTTAGGG ATAATTTATG CTATATTAGC AATTGGTCTA TTAGGATTTG
101 TTGTATGAGC ACATCATATA TTTACTGTAG GAATGGATGT AGATACACGA
151 GCATATTTTA CTTCTGCTAC TATAATTATT GCCGTACCCA CAGGAATTAA
201 AATTTTTTCT TGATTAGCAA CTCTTCATGG ATCTCAAATT AGATATAGAC
251 CTTCCTTACT ATGAGCATTA GGGTTTGTAT TTTTATTTAC TGTAGGAGGT
301 TTAACAGGGG TGGTATTAGC TAATTCTTCA ATTGATATTA TTCTTCATGA
351 TACATATTAT GTAGTTGCCC ATTTCCATTA TGTATTATCT ATAGGGGCAG
401 TATTTGCAAT TTTAGCTGGA TTTATTCAAT GATTCCCTTT ATTTACAGGA
451 TTAACTTTAA ATTCTAATTT ATTAAAAATT CAATTTATAG TAATATTTGT
501 TGGAGTTAAT TTAACTTTTT TTCCTCAACA CTTCTTAGGT TTAAGAGGAA
551 TACCTCGTCG GTATTCAGAT TACCCTGATG CTTATACTTC ATGAAATGTA
601 GTATCTTCAA TTGGATCTAC TATTTCATTT ATTGGGGTAA TATTATTAAT
651 TTATATTATC TGAGAAGCTT TTATTTCTCA ACGATTAGTA ATTTTTTCAA
701 ATCAAATACC AACTTCTATT GAATGATTCC AATCTCATCC CCCAGCTGAA
751 CACAGATATT CTGAACTTC

This sequence is identical with that of ‘Japan A’

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(v): *D. marginalis* (Japan C)

Species: D. marginalis czerskii Specimen ID: 'Isolate MB1534'

Sequence Length: 628 BP

Location: Jyuniko, Aomori Prefecture, Japan

Source: GenBank Published: Inoda & Balke (2011)

GenBank Accession No: FR751062

```
1    GGTTCTTTAG GGATAATTTA TGCTATATTA GCAATTGGTC TATTAGGATT
51   TGTTGTATGA GCACATCATA TATTTACTGT AGGAATGGAT GTGGATACAC
101  GAGCATATTT TACTTCTGCT ACTATAATTA TTGCTGTACC CACAGGAATT
151  AAAATTTTTT CTTGATTAGC AACTCTTCAT GGATCTCAAA TTAGATATAG
201  ACCTTCTTTA CTATGAGCAT TAGGATTTGT ATTTTTATTT ACTGTAGGGG
251  GTTTAACAGG GGTGGTATTA GCTAATTCTT CAATTGATAT TATTCTTCAT
301  GATACATATT ATGTAGTTGC CCATTTCCAT TATGTATTAT CTATAGGGGC
351  AGTATTTGCA ATTTTAGCTG GATTTATTCA ATGATTCCCT TTATTTACAG
401  GATTAACTTT AAATTCTAAT TTATTAAAAA TTCAATTTAT AGTAATATTT
451  GTTGGAGTTA ATTTAACTTT TTTTCCTCAA CACTTCTTAG GTTTAAGAGG
501  AATACCTCGT CGATATTCAG ATTACCCTGA TGCTTATACT TCATGAAATG
551  TAGTATCTTC AATTGGATCT ACTATTTCAT TTATTGGGGT AATATTATTA
601  ATTTATACTA TCTGAGAAGC TTTTATTT
```

The same sequence was reported by the authors (Inoda & Balke 2011) from four other specimens of *D. marginalis czerskii*: 'Isolate: MB790' (Accession Number FR751044); 'Isolate: MB793' (Accession Number FR751047) 'Isolate MB794' (Accession Number FR751048) and 'Isolate MB799' (Accession Number FR751052). All the specimens were from Jyuniko, Aomori Prefecture, Japan.

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(vi): *D. marginalis* (Japan D)

Species: D. marginalis czerskii Specimen ID: 'Isolate: MB789'

Sequence Length: 628 BP

Location: Jyuniko, Aomori Prefecture, Japan

Source: GenBank Published: Inoda & Balke (2011)

GenBank Accession No: FR751043

```
1    GGTTCTTTAG GGATAATTTA TGCTATATTA GCAATTGGTC TATTAGGATT
51   TGTTGTATGA GCACATCATA TATTTACTGT AGGAATGGAT GTGGATACAC
101  GAGCATATTT TACTTCTGCT ACTATAATTA TTGCTGTACC CACAGGAATT
151  AAAATTTTTT CTTGATTAGC AACTCTTCAT GGATCTCAAA TTAGATATAG
201  ACCTTCTTTA CTATGAGCAT TAGGGTTTGT ATTTTTATTT ACTGTAGGAG
251  GTTTAACAGG GGTGGTATTA GCTAATTCTT CAATTGATAT TATTCTTCAT
301  GATACATATT ATGTAGTTGC CCATTTCCAT TATGTATTAT CTATAGGGGC
351  AGTATTTGCA ATTTTAGCTG GATTTATTCA ATGATTCCCT TTATTTACAG
401  ATTAACTTT AAATTCTAAT TTATTAAAAA TTCAATTTAT AGTAATATTT
451  GTTGGAGTTA ATTTAACTTT TTTTCCTCAA CACTTCTTAG GTTTAAGAGG
501  AATACCTCGT CGATATTCAG ATTACCCTGA TGCTTATACT TCATGAAATG
551  TAGTATCTTC AATTGGATCT ACTATTTCAT TTATTGGGGT AATATTATTA
601  ATTTATATTA TCTGAGAAGC TTTTATTT
```

T – Locus different from Japan C

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(vii): *D. marginalis* (Japan E)

Species: D. marginalis czerskii Specimen ID: 'Isolate: MB1535'

Sequence Length: 628 BP

Location: Hakodate, Hokkaido Prefecture, Japan

Source: GenBank Published: Inoda & Balke (2011)

GenBank Accession No: FR751063

```
1    GGTTCTTTAG GGATAATTTA TGCTATATTA GCAATTGGTC TATTAGGATT
51   TGTTGTATGA GCACATCATA TATTTACTGT GGGAATGGAT GTAGATACAC
101  GAGCATATTT TACTTCTGCT ACTATAATTA TTGCCGTACC CACAGGAATT
151  AAAATTTTTT CTTGATTAGC AACTCTTCAT GGATCTCAAA TTAGATATAG
201  ACCTTCTTTA CTATGAGCAT TAGGGTTTGT ATTTTTATTT ACTGTAGGGG
251  GTTTAACAGG GGTGGTATTA GCTAATTCTT CAATTGATAT TATTCTTCAT
301  GATACATATT ATGTAGTTGC CCATTTCCAT TATGTATTAT CTATAGGGGC
351  AGTATTTGCA ATTTTAGCTG GATTTATTCA ATGATTCCCT TTATTTACAG
401  GATTAACTTT AAATTCTAAT TTATTAAAAA TTCAATTTAT AGTAATATTT
451  GTTGGAGTTA ATTTAACTTT TTTTCCTCAA CACTTCTTAG GTTTAAGAGG
501  AATACCTCGT CGATATTCAG ATTACCCTGA TGCTTATACT TCATGAAATG
551  TAGTATCTTC AATTGGATCT ACTATTTCAT TTATTGGGGT AATATTATTA
601  ATTTATATTA TCTGAGAAGC TTTTATTT
```

T – Locus different from Japan C

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(viii): *D. marginalis* (Japan F)

Species: D. marginalis czerskii Specimen ID: 'Isolate MB1533'

Sequence Length: 628 BP

Location: Gassan, Yamagata Prefecture, Japan

Source: GenBank Published: Inoda & Balke (2011)

GenBank Accession No: FR751061

```
1    GGTTCTTTAG GGATAATTTA TGCTATATTA GCAATTGGTC TATTAGGATT
51   TGTTGTATGA GCACATCATA TATTTACTGT AGGAATGGAT GTAGATACAC
101  GAGCATATTT TACTTCTGCT ACTATAATTA TTGCCGTACC CACAGGAATT
151  AAAATTTTTT CTTGATTAGC AACTCTTCAT GGATCTCAAA TTAGATATAG
201  ACCTTCCTTA CTATGAGCAT TAGGGTTTGT ATTTTTATTT ACTGTAGGAG
251  GTTTAACAGG GGTGGTATTA GCTAATTCTT CAATTGATAT TATTCTTCAT
301  GATACATATT ATGTAGTTGC CCATTTCCAT TATGTATTAT CTATAGGGGC
351  AGTATTTGCA ATTTTAGCTG GATTTATTCA ATGATTCCCT TTATTTACAG
401  GATTAACTTT AAATTCTAAT TTATTAAAAA TTCAATTTAT AGTAATATTT
451  GTTGGAGTTA ATTTAACTTT TTTTCCTCAA CACTTCTTAG GTTTAAGAGG
501  AATACCTCGT CGGTATTCAG ATTACCCTGA TGCTTATACT TCATGAAATG
551  TAGTATCTTC AATTGGATCT ACTATTTCAT TTATTGGGGT AATATTATTA
601  ATTTATATTA TCTGAGAAGC TTTTATTT
```

T – Locus different from Japan C

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(ix): *D. marginalis* (UK)

Species: D. marginalis Specimen ID: 'Isolate: MB329'

Sequence Length: 628 BP

Location: England, United Kingdom

Source: GenBank Published: Inoda & Balke (2011)

GenBank Accession No: FR751067

```
1    GGTTCTCTAG GTATAATTTA TGCTATATTA GCAATTGGTC TATTAGGATT
51   TGTTGTATGA GCACATCATA TATTTACTGT AGGAATAGAT GTAGACACAC
101  GGGCATATTT TACTTCTGCT ACTATAATTA TTGCTGTACC CACAGGAATT
151  AAAATTTTTT CTTGGTTAGC AACTCTTCAT GGATCTCAAA TTAGATATAG
201  CCCTTCTTTA CTATGAGCAT TAGGATTTGT ATTTTTATTT ACTGTAGGGG
251  GTTTAACAGG AGTAGTATTA GCTAACTCTT CGATTGATAT TATTCTTCAT
301  GATACATACT ATGTAGTTGC CCATTTTCAT TATGTATTAT CTATAGGAGC
351  AGTATTTGCA ATTTTAGCTG GATTTATTCA ATGATTCCCC TTATTTACAG
401  GATTGACTTT AAATTCTAAT TTATTAAAAA TTCAATTTGT AGTAATATTT
451  ATTGGGGTTA ATTTAACTTT CTTTCCTCAA CACTTCTTAG GTTTAAGAGG
501  AATACCTCGT CGATATTCAG ATTATCCTGA TGCTTATACT TCATGAAATG
551  TAGTATCTTC AATTGGATCT ACTATTTCAT TTATTGGAGT AATATTATTA
601  ATTTATATTA TCTGAGAAGC TTTTATTT
```

T – Locus different from Japan C

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(x): *D. marginalis* (Sweden B)

Species: D. marginalis Specimen ID: 'UNM KBMDymg168'

Sequence Length: 1294 BP

Location: Täfteåhalvön, Västerbotten Province, Sweden

Source: GenBank Published: Miller et al (2007)

GenBank Accession No: DQ813691

1 TTTAATATTA GGAGCTCCAG ATATAGCATT CCCTCGAATA AATAATATAA
51 GATTTTGACT TCTCCCGCCT TCTTTAACTT TATTATTAAT AAGAAGAATA
101 GTAGAAAGAG GGGCAGGAAC AGGTTGAACA GTTTATCCCC TCTTTCAGC
151 AAGAATTGCC CATGGGGGGG CTTCAGTAGA TTTAGCTATT TTAGATTAC
201 ATTTAGCTGG GGTTTCTTCT ATTTTAGGGG CTGTGAATTT TATTACAACA
251 ATTATTAATA TACGATCAGT AGGAATAACT TTAGACCGAA TACCTTTATT
301 TGTTTGATCA GTAGGAATTA CAGCTCTTTT ACTATTATTA TCATTGCCAG
351 TATTAGCAGG GGCTATTACT ATACTTTTAA CTGATCGAAA TTTAAATACT
401 TCATTCTTTG ATCCAGCCGG AGGGGGGGAT CCTATTTTAT ACCAACATTT
451 ATTTTGATTT TTTGGACACC CAGAAGTTTA TATTTTAATT CTTCCAGGGT
501 TTGGAATAAT TTCTCACATT ATTAGACAAG AAAGAGGAAA AAAGGAAACT
551 TTTGGTTCTC TAGGTATAAT TTATGCTATA TTAGCAATTG GTCTATTAGG
601 ATTTGTTGTA TGAGCACATC ATATATTTAC TGTAGGAATA GATGTAGACA
651 CACGGGCATA TTTTACTTCT GCTACTATAA TTATTGCTGT ACCCACAGGA
701 ATTAAAATTT TTTCTTGGTT AGCAACTCTT CATGGATCTC AAATTAGATA
751 TAGTCCTTCT TTACTATGAG CATTAGGGTT TGTATTTTTA TTTACTGTAG
801 GGGGTTTAAC AGGAGTAGTA TTAGCTAACT CTTCGATTGA TATTATTCTT
851 CATGATACAT ACTATGTAGT TGCCCATTTT CATTATGTAT TATCTATAGG
901 AGCAGTATTT GCAATTTTAG CTGGATTTAT TCAATGATTC CCCTTATTTA
951 CAGGATTGAC TTTAAATTCT AATTTATTAA AAATTCAATT TGTAGTAATA
1001 TTTATTGGGG TTAATTTAAC TTTCTTTCCT CAACACTTCT TAGGTTTAAG
1051 AGGAATACCT CGTCGATATT CAGATTATCC TGATGCTTAT ACTTCATGAA
1101 ATGTAGTATC TTCAATTGGA TCTACTATTT CATTTATTGG AGTAATATTA
1151 TTAATTTATA TTATCTGAGA AGCTTTTATT TCTCAACGAT TAGTAATTTT
1201 TTCAAATCAA ATACCAACTT CTATTGAATG ATTCCAATCC CATCCCCCAG
1251 CTGAACACAG ATATTCTGAA CTTCCAATAT TATCTAATTT CAAA

Appendix D1: Mitochondrial DNA sequences for parts of COI gene in D. marginalis and D. dimidiatus

D1(xi): *D. dimidiatus* (Germany)

Species: D. dimidiatus Specimen ID: 'Isolate MB244'

Sequence Length: 1381 BP

Location: Brandenburg, Germany

Source: GenBank Published: Balke et al (2009)

GenBank Accession No: FN263065

1 ATTAGACAAG AAAGAGGAAA AAAGGAAACT TTTGGTTCTT TAGGAATAAT
51 TTATGCTATA CTAGCAATTG GTTTATTAGG GTTTGTTGTA TGAGCACATC
101 ATATATTTAC TGTAGGGATA GATGTAGACA CACGAGCATA TTTTACTTCT
151 GCTACTATAA TTATTGCCGT ACCCACAGGA ATTAAAATTT TTTCTTGATT
201 AGCAACTCTT CATGGATCTC AAATTAGTTA TAGCCCATCT TTATTATGAG
251 CATTAGGATT TGTATTTTTA TTTACTGTAG GGGGTTTAAC AGGAGTAGTA
301 TTAGCTAATT CATCAATTGA TATTATTCTT CATGATACAT ACTATGTAGT
351 TGCCCATTTT CATTATGTAT TATCTATAGG AGCAGTATTT GCAATTTTAG
401 CTGGATTTAT TCAATGATTT CCCTTATTTA CAGGATTAAC CTTAAATTCT
451 AATTTATTAA AAATTCAATT TGTAGTAATA TTTGTAGGGG TAAATTTAAC
501 TTTCTTTCCT CAACACTTCT TAGGTTTAAG AGGAATACCT CGTCGATATT
551 CAGATTATCC TGATGCTTAT ACTTCATGAA ATGTAGTATC TTCAATTGGA
601 TCGACTATCT CATTTATTGG GGTAATATTA TTAATTTATA TTATCTGAGA
651 AGCTTTTATT TCTCAACGAT TAGTAATTTT TTCTAATCAA ATACCAACTT
701 CTATTGAATG ATTCCAATCC CATCCCCCAG CTGAACATAG ATATTCCGAA
751 CTTCCAATAT TATCTAATTT CTGATATGGC AGATTAGTGC AATGAATTTA
801 AGCTTCATAT ATAAAGTAAT TAACTTTTAT TAGAAAATGG CAACATGATC
851 TAATTTAAAC CTTCAAGATA GAGCCTCCCC CTTAATAGAA CAACTAACAT
901 TTTTCCATGA TCACACATTA ATAATTTTAA CTATAATTAC TATTTTAGTA
951 GGGTATTTAA TATTTTCACT TTTTTTTAAT AGATATATTA ATCGATATTT
1001 ATTAGAAGGG CAAACTATTG AAGTAATTTG AACAATTTTA CCAGCAATTA
1051 TTTTAGTTTT TATTGCCCTA CCTTCTCTCC GATTATTATA TTTATTAGAT
1101 GAAATTAGAA ATCCTTGATT AACCCTTAAA TCAATTGGAC ATCAATGATA
1151 TTGAAGATAT GAATATTCAG ATTTTAAAAA ATTAGAATTT GATTCTTACA
1201 TAACCCCAAC TAATGAATTA ATAAATAACG GATTTCGATT GTTAGATGTA
1251 GATAATCGAA TTGTTTTACC ATATAATTCC CAAATTCGAA TTTTAGTATC
1301 TGCTATAGAT GTATTACACT CCTGAACAAT CCCTGCCTTA GGGGTTAAAA
1351 TTGATGCTAC CCCTGGTCGA TTAAATCAAA C

Appendix D2: Origins of material providing template DNA for molecular ecology experiments

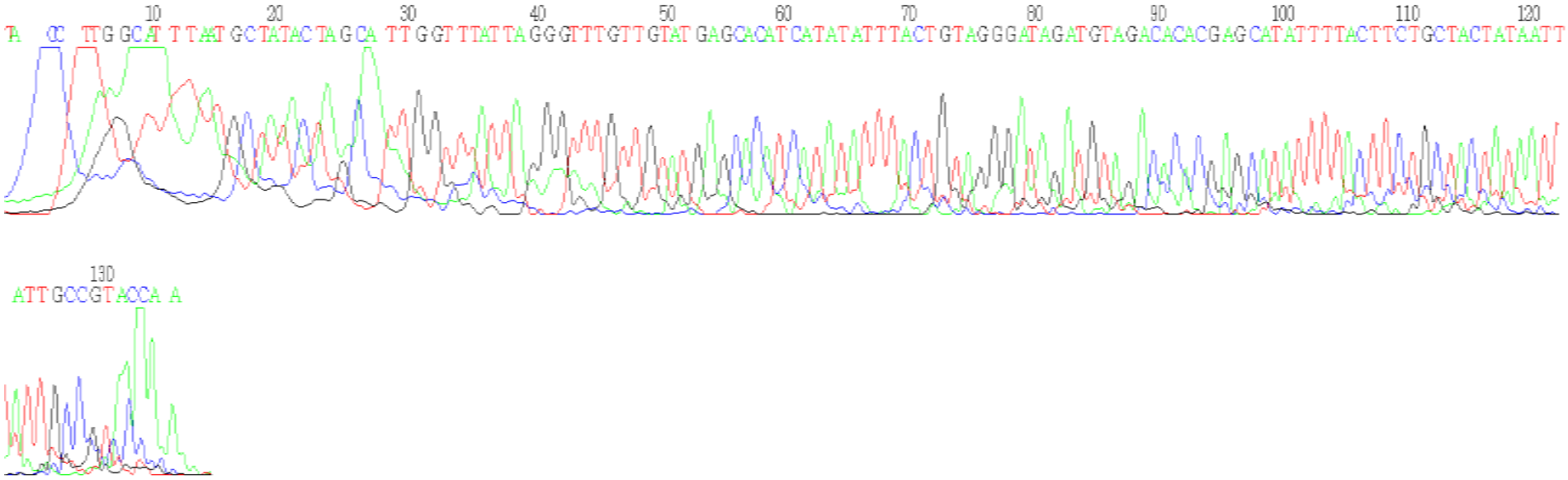
All beetles were adults caught at Shapwick Heath

D.marginalis template DNA	DM1	♂ Middle Leg. Beetle caught 16/7/06. DNA extraction 18/3/09
	DM2	♂ Middle Leg. Beetle caught 16/7/06. DNA extraction 18/3/09
	DM3	♂ Middle Leg. Beetle caught 16/7/06. DNA extraction 18/3/09
	DM4	♂ Middle Leg. Beetle caught 20/5/07. DNA extraction 18/3/09
	DM5	♂ Middle Leg. Beetle caught 11/2/07. DNA extraction 18/3/09
	DM6	♂ Middle Leg. Beetle caught 11/3/07. DNA extraction 18/3/09
	DM7	♀. Middle Leg. Beetle caught 24/6/06. DNA extraction 10/3/09
	DM8	♂ Middle Leg. Beetle caught 25/2/07. DNA extraction 10/3/09
	DM9	♂ Middle Leg. Beetle caught 25/2/07. DNA extraction 10/3/09
	DM10	♀. Middle Leg. Beetle caught 29/1/06. DNA extraction 10/3/09
D.dimidiatus template DNA	DD1	'Beetle 1' Leg DNA Extraction 10/7/09
	DD2	'Beetle 2' Leg DNA Extraction 10/7/09
	DD3	'Beetle 3' Leg DNA Extraction 10/7/09
	DD4	'Beetle 4' Leg DNA Extraction 10/7/09
	DD5	'Beetle 5' Leg DNA Extraction 10/7/09
	DD6	'Beetle 6' Leg DNA Extraction 10/7/09
	DD7	♂. Middle Leg. Beetle caught 22/9/07. DNA Extraction 16/4/09
	DD8	♀. Middle Leg. Beetle caught 8/4/2006. DNA Extraction 16/4/09
	DD9	♂. Middle Leg. Beetle caught 22/9/07 DNA Extraction 16/4/09
	DD10	♂. Hind Leg. Beetle caught 22/9/07 DNA Extraction 16/4/09

Appendix D3: Some examples of CO1 sequence results

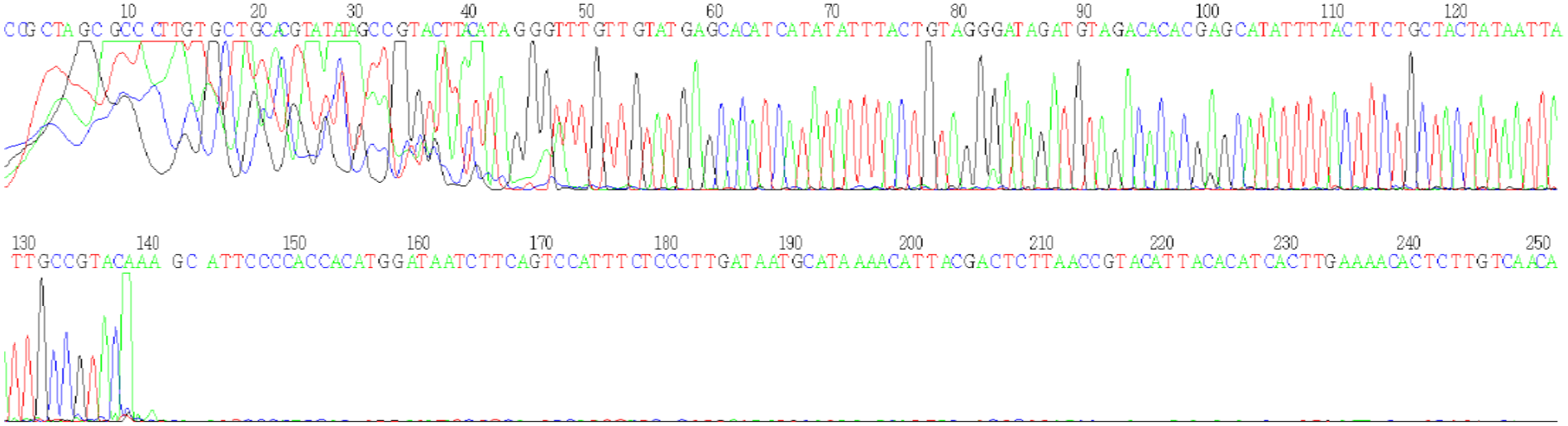
D3a: Sequence from Larva caught in Trap 11 at Shapwick Heath on 17 June 2007 determined as *Dytiscus dimidiatus.*
(Identifier sequences are highlighted in green.)

TACCTTGGCATTTAATGCTATACTAGCATTGGTTTATTAGGGTTTGTTGTATGAGCACATCATATATTTACTGTAGGGATAGATGT
AGACACACGAGCATATTTTACTTCTGCTACTATAATTATTGCCGTACCAA



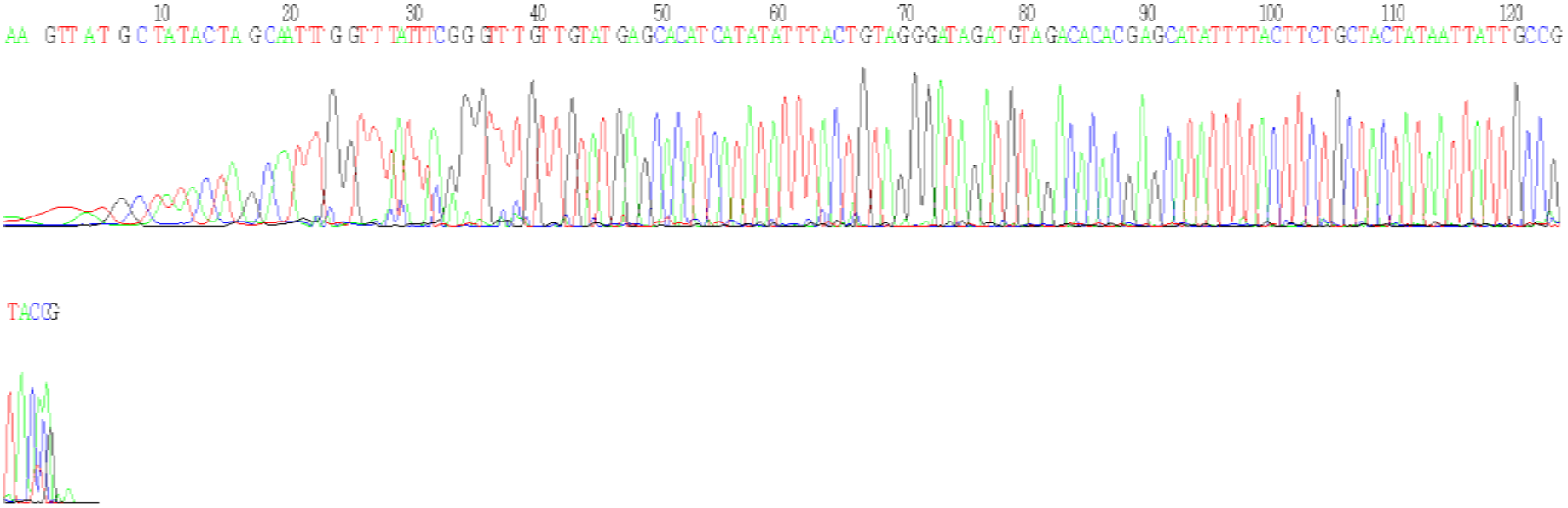
D3b: Sequence from larva caught in Trap 10 at Westhay Heath on 10 May 2008 determined as *Dytiscus dimidiatus*.
(Identifier sequences are highlighted in green.) This is specimen 133 in Tables D4 & D5.

CGCTAGCGCCCTTGTGCTGCACGTATATAGCCGTACTTACATAGGGTTTGTTGTATGAGCACATCATATATTTACTGTAGGGATA
GATGTAGACACACGAGCATATTTTACTTCTGCTACTATAATTATTGCCGTACAAA



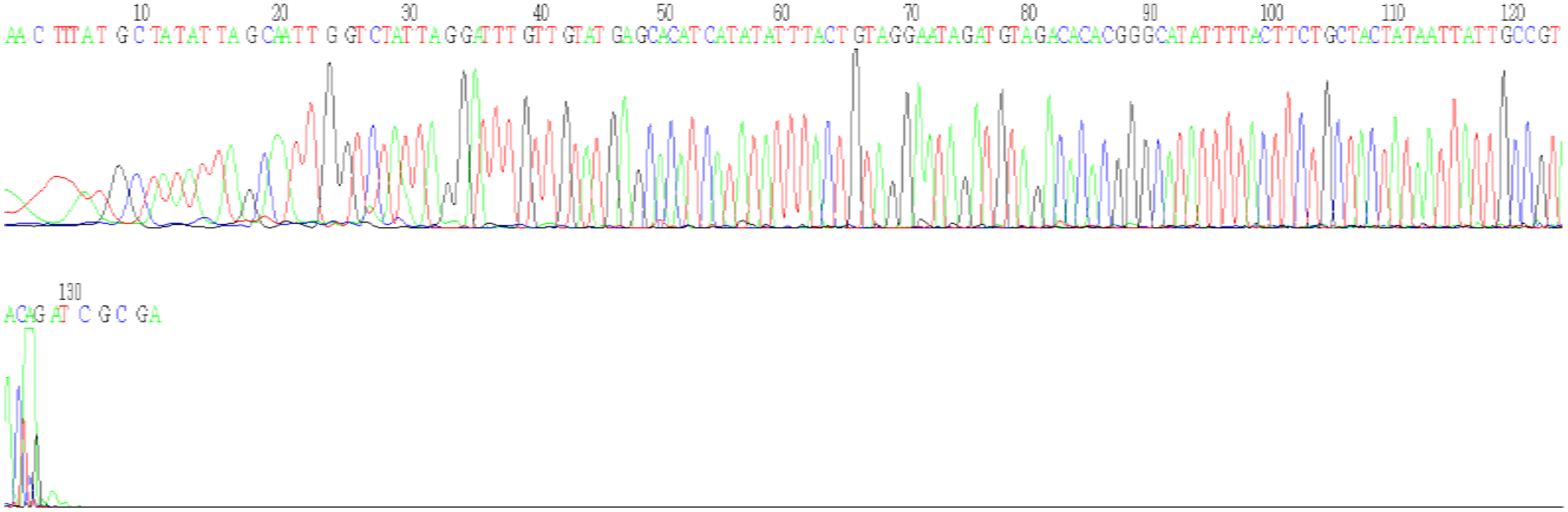
D3c: Sequence from larva caught in Trap 8 at Westhay Heath on 2 June 2008 determined as *Dytiscus dimidiatus*. (Identifier sequences are highlighted in green.) This is specimen 74 in Tables D4 & D5.

AAGTTATGCTATACTAGCAATTTGGTTTATTTCGGGTTTGTTGTATGAGCACATCATATATTTACTGTAGGGATAGATGTAGACAC
ACGAGCATATTTTACTTCTGCTACTATAATTATTGCCGTACCG



D3d: Sequence from larva caught in Trap 14 at Westhay Moor on 23 July 2008 determined as *Dytiscus marginalis.*
(Identifier sequences are highlighted in pink.) This is specimen 123 in Tables D4 & D5.

AACTTTATGCTATATTAGCAATTGGTCTATTAGGATTTGTTGTATGAGCACATCATATATTTACTGTAGGAATAGATGTAGACACA
CGGGCATATTTTACTTCTGCTACTATAATTATTGCCGTACAGATCGCGA



Appendix D4: Determinations of species identity from sequence data

Specimen	Dd				Dm				DETERMINATION	Site	Caught
Number	GTTTA	GGTTT	GGATA	GAGCAT	GTCTA	GATTT	GAATA	GGGCAT			
1	Not sent for sequencing									SH	20/05/2007
2	Not sent for sequencing									SH	20/05/2007
3	Not sent for sequencing									SH	20/05/2007
4	Not sent for sequencing									SH	20/05/2007
5	Not sent for sequencing									SH	20/05/2007
6	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	20/05/2007
7	N	N	N	N	N	N	Y	Y	**D. marginalis**	SH	20/05/2007
8	Y	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	20/05/2007
9	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
10	N	N	Y	N	N	Y	N	Y	**D. marginalis**	SH	29/05/2006
11	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	29/05/2006
12	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	29/05/2006
13	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	29/05/2006
14	Not sent for sequencing									SH	29/05/2006
15	Y	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	16/07/2006
16	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	16/07/2006
17	Small specimen comprising of exoskeleton only. No leg taken for DNA Extraction.									SH	16/07/2006
18	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
19	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
20	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
21	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
22	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
23	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
24	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
25	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
26	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006
27	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2006

Appendix D4: Determinations of species identity from sequence data (Continued)

Specimen	Dd				Dm				DETERMINATION	Site	Caught
Number	GTTTA	GGTTT	GGATA	GAGCAT	GTCTA	GATTT	GAATA	GGGCAT			
28	N	N	N	N	N	N	Y	Y	**D. marginalis**	SH	24/06/2006
29	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	24/06/2006
30	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	24/06/2006
31	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	24/06/2006
32	Not sent for sequencing									SH	24/06/2006
33	Larva not *Dytiscus* sp. Probably *Acilius* sp.									SH	24/06/2006
34	Not sent for sequencing									SH	05/06/2011
35	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	05/06/2011
36	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
37										SH	05/06/2011
38	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
39	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	05/06/2011
40	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
41	N	Y	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
42	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
43	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
44	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
45	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/06/2011
46	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	05/06/2011
47	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	05/06/2011
48	Specimen fragmentary. No legs.									WM	23/07/2008
49	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	WM	23/07/2008
50	Condition of larva very poor. No leg taken.									WM	23/07/2008
51	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	WH	17/07/2008
52	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	13/07/2008
53	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	13/07/2008
54	N	N	N	N	N	N	Y	Y	**D. marginalis**	SH	13/07/2008

Appendix D4: Determinations of species identity from sequence data (Continued)

Specimen	Dd				Dm				DETERMINATION	Site	Caught
Number	GTTTA	GGTTT	GGATA	GAGCAT	GTCTA	GATTT	GAATA	GGGCAT			
55	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	13/07/2008
56	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	05/05/2007
57	N	N	N	N	N	N	Y	Y	**D. marginalis**	SH	20/05/2007
58	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	20/05/2007
59	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	20/05/2007
60	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	17/06/2007
61	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	02/07/2007
62	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	17/06/2007
63	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	17/06/2007
64	N	Y	Y	N	N	N	N	N	**D. dimidiatus**	**TM**	**27/06/2008**
65	Not sent for sequencing									TM	08/06/2008
66	Not sent for sequencing									SH	03/03/2008
67	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	03/03/2008
68	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	17/04/2011
69	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	17/06/2007
70	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	?	?
71	N	N	N	N	Y	N	Y	Y	**D. marginalis**	WM	23/07/2008
72	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	17/06/2007
73	N	N	N	N	N	N	N	Y		SH	17/06/2007
74	Y	Y	Y	Y	N	N	N	N	**D. dimidiatus**	**WH**	**02/06/2008**
75	Y	Y	Y	Y	N	N	N	N	**D. dimidiatus**	**SH**	**08/06/2008**
76	N	N	N	Y	N	N	N	N		WH	09/05/2008
77	N	N	Y	Y	N	N	N	N	**D. dimidiatus**	**SH**	**05/05/2007**
78	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	02/07/2007
79	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	05/05/2007
80	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	05/05/2007
81	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007

Appendix D4: Determinations of species identity from sequence data (Continued)

Specimen	Dd				Dm				DETERMINATION	Site	Caught
Number	GTTTA	GGTTT	GGATA	GAGCAT	GTCTA	GATTT	GAATA	GGGCAT			
82	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
83	Condition of larva very poor. No leg taken.									SH	02/07/2007
84	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	05/05/2006
85	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	20/05/2007
86	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	20/05/2007
87	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	16/07/2007
88	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
89	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	17/06/2007
90	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
91	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	17/06/2007
92	Specimen fragmentary. No legs.									SH	17/06/2007
93	N	N	Y	N	N	Y	Y	Y	**D. marginalis**	SH	17/06/2007
94	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	17/06/2007
95	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
96	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	17/06/2007
97	Condition of larva very poor. No leg taken.									SH	16/07/2007
98	N	N	Y	N	N	N	Y	Y	**D. marginalis**	SH	03/06/2007
99	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
100	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
101	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
102	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
103	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
104	N	Y	N	N	N	N	Y	N		SH	03/06/2007
105	N	Y	N	N	N	N	N	Y		SH	03/06/2007
106	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
107	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
108	N	N	N	N	N	N	Y	N		SH	03/06/2007

Appendix D4: Determinations of species identity from sequence data (Continued)

Specimen	Dd				Dm				DETERMINATION	Site	Caught
Number	GTTTA	GGTTT	GGATA	GAGCAT	GTCTA	GATTT	GAATA	GGGCAT			
109	N	N	Y	N	N	N	N	Y		SH	03/06/2007
110	N	N	N	N	N	N	Y	Y	**D. marginalis**	SH	03/06/2007
111	N	Y	N	N	N	Y	Y	Y	**D. marginalis**	SH	03/06/2007
112	N	Y	N	N	N	N	N	N		SH	03/06/2007
113	N	N	N	N	N	Y	Y	N	**D. marginalis**	SH	03/06/2007
114	N	N	N	N	N	N	Y	Y	**D. marginalis**	WH	29/06/2008
115	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	CN	25/05/2008
116	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	13/07/2008
117	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	WH	17/07/2008
118	N	N	N	N	Y	N	Y	Y	**D. marginalis**	SH	08/06/2008
119	N	N	N	N	N	N	Y	Y	**D. marginalis**	SH	13/07/2008
120	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	13/07/2008
121	N	N	N	N	N	Y	Y	Y	**D. marginalis**	SH	13/07/2008
122	N	N	Y	N	N	N	Y	Y		WM	15/06/2008
123	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	WM	23/07/2008
124	Y	Y	Y	Y	N	N	N	N	**D. dimidiatus**	**WH**	**29/06/2008**
125	Small specimen. No leg taken for DNA Extraction.									WH	29/06/2008
126	N	N	N	N	N	Y	Y	Y	**D. marginalis**	WM	15/06/2008
127	N	N	N	N	N	Y	Y	Y	**D. marginalis**	CN	17/07/2008
128	N	N	N	N	N	N	Y	Y	**D. marginalis**	SH	08/06/2008
129	N	N	N	N	Y	Y	Y	Y	**D. marginalis**	SH	13/07/2008
130	N	N	Y	N	Y	Y	Y	Y	**D. marginalis**	SH	13/07/2008
131	N	N	Y	N	N	N	Y	Y		SH	08/06/2008
132	N	N	Y	N	N	Y	Y	Y	**D. marginalis**	WM	15/06/2008
133	N	Y	Y	Y	N	N	N	N	**D. dimidiatus**	**WH**	**10/05/2008**
134	Small specimen. No leg taken for DNA Extraction.									SH	05/05/2008
135	Specimen fragmentary. No legs.									?	08/05/2008

Appendix D4: Determinations of species identity from sequence data (Continued)

Specimen Number							Site	Caught
136	Specimen fragmentary. No legs.						CN	07/05/2008
137	Larva not *Dytiscus* sp. *Colymbetes* sp.						SH	31/12/2007
138	Larva not *Dytiscus* sp. *Colymbetes* sp.						SH	28/10/2007
139	Larva not *Dytiscus* sp. *Colymbetes* sp.						SH	20/01/2008
140	Larva not *Dytiscus* sp. *Colymbetes* sp.						SH	20/05/2007
141	Specimen fragmentary. No legs.						?	08/05/2008
142	Specimen fragmentary. No legs.						SH	22/04/2007
143	Larva not *Dytiscus* sp. *Colymbetes* sp.						SH	31/12/2007

CN = Catcott North

SH = Shapwick Heath

TM = Tadham Moor

WH = Westhay Heath

WM = Westhay Moor

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae

	A	B	C		D		E	F	G	H	I	J	K	
Specimen Number	L Antenna	L Hd Cp	W Hd Cp	B/C	W Neck	C/D	L T1	L T2 - T3	L A1 - A3	L A4 - A6	L A7	L A8	L Uro	J/K
1	5.44	6.30	6.90	0.91	3.60	1.92	7.50	6.00	9.75	11.40	4.80	6.15	4.80	1.28
2	3.95	5.70	6.75	0.84	3.60	1.88	6.75	5.55	8.40	9.75	4.50	6.45		
3	5.10	6.15	7.05	0.87	3.75	1.88	7.65	7.35	11.85	13.50	4.65	7.50	3.90	1.92
4	6.00	6.75	8.10	0.83	4.05	2.00	7.50	9.60	10.80	12.30	4.65	7.65	5.10	1.50
5	4.80	5.70	6.60	0.86	3.60	1.83	7.05	6.00	8.85	11.85	3.60	6.90	3.75	1.84
6	4.95	5.55	6.90	0.80	3.45	2.00	7.20	6.15	7.50	7.50	4.05	7.35	3.15	2.33
7	5.00	5.70	6.20	0.92	3.50	1.77	6.60	4.00	9.20	13.80	4.95	6.30	4.50	1.40
8	5.17	6.46	6.50	0.99	3.30	1.97	6.10	4.50	7.30	8.00	3.10	6.00	4.20	1.43
9	4.90	6.10	6.30	0.97	3.80	1.66	6.10	4.50	10.80	11.55	3.45	6.45	4.20	1.54
10	3.94	4.10	4.15	0.99	2.10	1.98	3.15	2.75	3.90	5.98	2.99	4.62	2.58	1.79
11	5.10	5.90	6.60	0.89	3.40	1.94	6.00	4.60	6.40	9.75	4.95	7.05	4.05	1.74
12	3.80	5.30	6.40	0.83	3.40	1.88	5.50	2.80	4.80	5.40	2.90	4.70	3.50	1.34
13	4.20	3.95	4.30	0.92	2.15	2.00	3.85	2.45	5.10	6.32	2.86	5.17	3.20	1.62
14	4.20	3.80	4.30	0.88	2.00	2.15	3.70	3.00	3.40	5.70	2.40	4.90	2.70	1.81
15	4.75	5.80	6.30	0.92	3.50	1.80	5.90	4.00	6.30	6.80	3.50	6.60	4.20	1.57
16	4.60	5.70	6.10	0.93	3.20	1.91	6.50	4.30	6.90	9.50	3.70	6.80	3.80	1.79
17	2.90	2.15	2.65	0.81	1.15	2.30	2.25	2.85	4.00	4.90	1.90	3.10	1.65	1.88
18	4.25	5.50	6.40	0.86	3.20	2.00	5.70	3.00	7.30	9.00	3.10	6.00	4.00	1.50
19	4.80	5.20	6.40	0.81	3.50	1.83	6.30	4.50	7.50	9.60	3.50	7.00	4.30	1.63
20	4.50	5.90	6.70	0.88	3.60	1.86	6.30	5.40	5.40	9.50	5.10	6.90	3.70	1.86
21	4.50	5.60	6.60	0.85	3.50	1.89	6.50	5.00	6.20	9.80	4.70	6.70	5.00	1.34
22	3.76	4.85	4.50	1.08	2.10	2.14	4.10	3.65	5.00	5.00	2.50	4.00	2.71	1.48
23	4.06	7.20	6.60	1.09	3.81	1.73	6.50	3.80	5.40	10.70	5.00	6.70	4.00	1.68
24	5.37	6.80	5.70	1.19	3.40	1.68	6.32	4.76	6.12	6.12	3.67	6.66	3.69	1.80
25	4.69	5.44	6.46	0.84	3.20	2.02	6.26	3.81	5.00	8.00	3.90	6.30	4.85	1.30
26	3.20	2.58	2.62	0.98	1.15	2.28	1.80	1.48	2.26	2.75	1.39	3.24	2.09	1.55

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	A	B	C		D		E	F	G	H	I	J	K	
Specimen Number	L Antenna	L Hd Cp	W Hd Cp	B/C	W Neck	C/D	L T1	L T2 - T3	L A1 - A3	L A4 - A6	L A7	L A8	L Uro	J/K
27	Fragments only. No head						3.50	3.30	5.30					
28	5.10	5.71	6.60	0.87	3.54	1.86	6.80	6.80	11.25	12.15	4.35	7.65	4.22	1.81
29	5.05	6.30	6.60	0.95	3.50	1.89	6.70	4.30	6.00	8.50	4.00	7.40	4.69	1.58
30	5.10	5.92	6.73	0.88	3.40	1.98	6.46	3.88	5.44	8.30	4.60	6.60	4.70	1.40
31	4.70	6.12	6.46	0.95	3.54	1.82	7.00	4.10	7.00	9.10	3.80	6.80	4.70	1.45
32	4.70	6.20	6.50	0.95	3.20	2.03	5.70	3.70	8.40	11.20	5.10	7.20	4.10	1.76
33	Larva not *Dytiscus* sp. Probably *Acilius sulcatus*.													
34	Fragments only. No head.						6.50	5.80	7.30	10.00	4.00	6.50	4.10	1.59
35	5.40	6.10	6.60	0.92	3.40	1.94	6.50	4.30	6.60	10.00	4.10	6.70	4.90	1.37
36	3.30	5.85	6.30	0.93	3.30	1.91	7.20	7.20	9.80	12.90	4.65	7.20	4.35	1.66
37	4.10	5.90	6.20	0.95	3.15	1.97	7.50	6.90	8.10	10.95	4.80	7.05	3.90	1.81
38	4.80	5.80	6.33	0.92	3.20	1.98	6.00	4.90	7.20	12.45	5.25	6.90	3.90	1.77
39	5.00	6.40	6.80	0.94	3.70	1.84	6.70	5.00	8.60	12.00	3.80	6.70	4.40	1.52
40	4.20	5.50	6.80	0.81	3.40	2.00	6.50	9.00	9.20	8.30	3.40	4.80	5.10	0.94
41	5.30	6.00	7.00	0.86	3.60	1.94	6.60	4.80	7.70	14.25	3.60	6.60	3.50	1.89
42	5.20	5.60	6.20	0.90	3.00	2.07	6.66	5.30	9.00	9.60	3.90	6.30	3.90	1.62
43	4.80	5.50	6.30	0.87	3.40	1.85	6.00	7.80	12.60	15.00	3.90	6.00	4.65	1.29
44	Fragments only. No head.						4.00	3.20						
45	4.60	5.30	6.40	0.83	3.60	1.78	6.30	3.90	5.50	8.10	2.90	6.10	4.00	1.53
46	4.10	5.20	6.10	0.85	3.50	1.74	6.00	3.90	5.50	8.00	4.60	6.60	4.10	1.61
47	4.80	6.00	6.50	0.92	3.60	1.81	6.30	4.40	5.90	8.50	4.20	7.40	3.80	1.95
48	Fragments only. No head.										6.80	3.20		
49	3.90	4.05	4.60	0.88	2.25	2.04	3.90	3.20	4.10	5.00	1.95	4.30	2.70	1.59
50	3.15	2.40	2.75	0.87	1.50	1.83	2.00	1.15				3.00	2.20	1.36
51	4.20	5.50	6.10	0.90	3.20	1.91	6.20	4.50	7.20	9.30	3.30	5.20	3.30	1.58
52	4.55	5.60	6.10	0.92	3.10	1.97	5.50	4.40	6.20	9.60	4.40	5.80	3.40	1.71
53	5.90	5.40	6.30	0.86	3.50	1.80	6.00	4.50	6.00	9.45	4.65	7.35	4.20	1.75

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	A	B	C		D		E	F	G	H	I	J	K	
Specimen Number	L Antenna	L Hd Cp	W Hd Cp	B/C	W Neck	C/D	L T1	L T2 - T3	L A1 - A3	L A4 - A6	L A7	L A8	L Uro	J/K
54	4.60	5.40	6.10	0.89	3.30	1.85	5.90	5.20	9.10	11.10	4.05	5.70	3.30	1.73
55	4.50	5.50	6.40	0.86	3.40	1.88	6.50	6.20	8.40	12.30	4.50	6.60	4.05	1.63
56	4.60	5.70	6.60	0.86	3.70	1.78	6.70	3.20	4.70	7.50	3.80	6.20	3.70	1.68
57	5.00	5.10	6.40	0.80	3.30	1.94	6.50	3.20	7.50	8.00	4.70	6.90	4.50	1.53
58	5.50	6.50	6.80	0.96	3.60	1.89	6.70	5.70	10.00	12.50	4.70	6.50	3.80	1.71
59	4.90	6.00	6.40	0.94	3.50	1.83	7.00	6.30	7.00	10.00	3.60	7.10	4.00	1.78
60	5.00	5.60	6.70	0.84	3.40	1.97	6.40	5.00	6.50	8.70	4.80	6.50	3.60	1.81
61	4.40	5.20	6.20	0.84	2.90	2.14	6.00	5.30	7.00	9.00	4.00	6.40	3.50	1.83
62	4.60	6.00	6.70	0.90	3.50	1.91	6.30	5.10	6.80	11.70	4.40	6.70	4.30	1.56
63	4.70	6.00	6.40	0.94	3.40	1.88	6.60	4.80	6.60	9.60	4.00	6.50	3.20	2.03
64	5.10	5.50	6.40	0.86	3.30	1.94	6.40	7.00	7.70	9.80	3.50	6.50	3.20	2.03
65	4.80	5.10	6.50	0.78	3.40	1.91	Fragments only.							
66	4.60	5.50	6.10	0.90	3.10	1.97	5.80	4.70	6.30	9.10	3.70	6.20	3.20	1.94
67	5.20	5.50	6.20	0.89	3.50	1.77	6.50	6.00	9.75	10.95	4.20	6.40	3.50	1.83
68	3.20	3.60	3.80	0.95	2.10	1.81	3.70	4.30	5.40	5.70	2.30	3.50	2.80	1.25
69	4.60	5.80	6.40	0.91	3.20	2.00	5.60	4.50	5.60	8.30	3.50	5.20	4.30	1.21
70	4.45	5.20	6.40	0.81	3.30	1.94	6.50	4.00	8.20	13.65	3.80	6.30	3.30	1.91
71	4.80	5.30	6.30	0.84	3.30	1.91	5.90	5.90	8.50	9.50	3.50	6.10	3.40	1.79
72	4.70	5.60	6.60	0.85	3.40	1.94	7.00	6.60	11.40	15.00	5.40	7.50	4.70	1.60
73	4.80	5.50	6.00	0.92	3.20	1.88	6.00	5.00	7.30	10.20	3.50	6.20	4.20	1.48
74	5.00	6.10	7.20	0.85	3.40	2.12	7.10	4.00	6.00	8.70	3.90	7.10	4.00	1.78
75	6.00	6.60	7.60	0.87	4.20	1.81	7.00	4.90	4.80	9.80	4.80	7.70	4.00	1.93
76	5.70	4.30	5.10	0.84	2.50	2.04	5.40	5.00	5.50	7.00	3.20	5.70	3.60	1.58
77	3.80	3.30	3.60	0.92	1.60	2.25	3.60	3.40	3.50	4.20	2.20	4.30	2.30	1.87
78	4.95	5.80	6.20	0.94	3.30	1.88	6.30	3.90	5.50	7.50	3.70	6.00	4.20	1.43
79		6.20	7.00	0.89	3.80	1.84							4.50	
80	4.50	5.80	6.70	0.87	4.00	1.68	6.50					6.50	3.90	1.67

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	A	B	C		D		E	F	G	H	I	J	K	
Specimen Number	L Antenna	L Hd Cp	W Hd Cp	B/C	W Neck	C/D	L T1	L T2 - T3	L A1 - A3	L A4 - A6	L A7	L A8	L Uro	J/K
81	4.90	6.10	6.60	0.92	3.40	1.94	6.30	6.00	7.90	9.00	3.90	5.40	4.20	1.29
82	5.00	5.80	6.40	0.91	3.30	1.94	6.80	5.10	9.75	12.30	4.30	6.60	4.20	1.57
83	3.70	4.10	4.40	0.93	2.30	1.91	4.00					4.80	3.20	1.50
84	4.80	6.00	6.60	0.91	3.40	1.94	Fragments only.							
85	4.80	5.90	6.70	0.88	3.60	1.86	6.50	4.60	8.80	8.30	3.50	6.40	4.30	1.49
86	4.70	5.80	6.90	0.84	4.20	1.64	6.30	7.00	7.80	10.80	4.00	6.60	4.70	1.40
87	3.70	4.90	6.20	0.79	3.40	1.82	6.00	4.40	8.00	9.80	3.70	6.10	3.50	1.74
88		3.30	4.00	0.83	2.10	1.90	3.20	2.40	3.00	3.70	2.00	4.00	2.60	1.54
89	5.30	6.00	6.80	0.88	3.50	1.94	6.80	5.00	8.30	11.25	4.10	7.20	4.70	1.53
90	4.40	5.30	6.40	0.83	3.40	1.88	6.50	4.00	6.50	7.90	3.40	6.30	3.00	2.10
91	4.90	5.10	6.10	0.84	3.40	1.79	6.10	5.00	9.00	12.00	4.70	6.60	4.00	1.65
92		5.50	6.50	0.85	3.70	1.76	Head only.							
92a		5.50	6.50	0.85	3.80	1.71	Head only.							
93	Fragments only. No head.						6.40	5.50	7.00	10.00	3.60	6.00	3.50	1.71
94	Fragments only. No head.						6.50	4.60	6.20	6.90	3.50	7.00	4.50	1.56
95	4.90	5.70	6.50	0.88	3.40	1.91	6.50	6.00	10.80	14.70	3.80	6.50	4.20	1.55
96	4.30	5.00	6.10	0.82	3.20	1.91	5.30	2.80	2.90	6.70	2.60	6.00	3.80	1.58
97	3.90	3.90	4.10	0.95	2.20	1.86	3.50					4.60	2.70	1.70
98	4.60	5.30	6.50	0.82	3.70	1.76	6.50	4.30	6.80	10.10	3.80	6.70	3.80	1.76
99	4.70	5.70	6.60	0.86	3.70	1.78	6.80	5.40	9.10	10.20	4.60	7.30	4.80	1.52
100	4.20	5.70	6.90	0.83	3.90	1.77	6.80	6.20	9.20	11.80	3.30	6.70	5.10	1.31
101	5.40	5.30	6.50	0.82	3.80	1.71	6.40	5.20	8.40	10.30	4.40	6.50	4.00	1.63
102	4.40	4.80	6.30	0.76	3.70	1.70	6.30	5.50	8.80	9.80	3.60	5.70	4.40	1.30
103	4.20	5.40	6.10	0.89	3.40	1.79	6.20	3.40	5.70	6.50	4.00	5.80	4.30	1.35
104	5.20	5.80	6.60	0.88	3.50	1.89	6.60	7.70	9.00	11.80	4.40	6.80	4.90	1.39
105	4.60	5.40	6.50	0.83	3.40	1.91	6.60	5.20	8.30	11.70	4.20	6.50	4.40	1.48
106	4.90	5.30	6.60	0.80	3.90	1.69	6.70	5.00	8.60	12.00	4.20	6.70	4.20	1.60

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	A	B	C		D		E	F	G	H	I	J	K	
Specimen Number	L Antenna	L Hd Cp	W Hd Cp	B/C	W Neck	C/D	L T1	L T2 - T3	L A1 - A3	L A4 - A6	L A7	L A8	L Uro	J/K
107	5.20	5.70	6.60	0.86	3.90	1.69	6.50	6.20	8.30	10.65	4.50	7.30	4.40	1.66
108	6.00	5.70	7.60	0.75	3.80	2.00	6.70	8.40	10.00	14.80	4.90	7.10	4.20	1.69
109	5.40	5.60	6.80	0.82	3.80	1.79	6.70	7.50	9.75	12.60	4.90	7.10	4.20	1.69
110	5.00	4.90	6.30	0.78	3.40	1.85	6.30	5.80	8.80	11.10	4.00	7.00	3.40	2.06
111	4.70	5.30	6.60	0.80	3.70	1.78	6.70	5.00	11.00	12.50	5.00	7.40	5.00	1.48
112	4.50	5.20	6.60	0.79	3.50	1.89	6.80	5.70	10.00	10.90	4.90	6.90	4.50	1.53
113	4.70	5.40	6.40	0.84	3.40	1.88	5.50	3.50	6.00	7.50	3.70	6.50	4.20	1.55
114	4.10	5.00	6.20	0.81	3.50	1.77	6.40	4.50	8.00	12.75	5.00	6.90	4.40	1.57
115	4.60	5.00	6.60	0.76	3.80	1.74	5.90	2.50	4.80	7.60	3.70	6.30	4.00	1.58
116	3.70	4.10	4.20	0.98	2.10	2.00	Fragments only.							
117	4.70	5.30	6.10	0.87	3.40	1.79	6.30	4.40	6.70	9.60	3.60	5.40	4.00	1.35
118	5.50	6.00	7.60	0.79	4.00	1.90	7.30	4.60	11.10	11.25	4.50	7.50	4.70	1.60
119	4.60	5.20	6.10	0.85	3.20	1.91	6.20	4.30	5.50	8.60	3.80	6.50	4.50	1.44
120	4.60	5.40	6.20	0.87	3.40	1.82	6.00	3.60	6.30	8.60	4.00	6.50	4.20	1.55
121	5.50	5.30	6.70	0.79	3.50	1.91	5.80	5.80	8.00	11.80	4.60	6.80	4.80	1.42
122	4.10	5.10	6.30	0.81	3.60	1.75	5.60	5.30	8.40	9.00	3.60	6.30	4.00	1.58
123	4.00	5.60	6.20	0.90	3.10	2.00	6.00	3.80	7.20	10.80	4.20	6.40	3.60	1.78
124	5.40	5.20	7.00	0.74	3.70	1.89	6.70	3.10	5.40	9.20	3.80	7.20	4.40	1.64
125	2.25	2.30	2.70	0.85	1.35	2.00	2.75	1.50	2.75	4.00	2.25	2.25	1.85	1.22
126	5.00	5.10	6.20	0.82	3.30	1.88	6.00	3.70	6.30	11.00	4.20	5.90	4.00	1.48
127	5.00	5.80	6.30	0.92	3.40	1.85	5.70	3.50	4.00	7.80	3.30	6.50	4.00	1.63
128	6.20	6.30	7.30	0.86	3.70	1.97	7.30	5.10	7.10	13.40	4.40	7.40	4.50	1.64
129	5.00	5.00	6.70	0.75	3.50	1.91	6.00	7.50	11.40	14.70	4.10	6.20	3.60	1.72
130	4.90	5.30	6.60	0.80	3.40	1.94	6.20	7.00	8.70	12.60	4.30	6.60	3.00	2.20
131	3.55	3.60	4.30	0.84	2.30	1.87	3.40	2.35	2.95	5.10	2.50	4.60	2.75	1.67
132	4.60	5.50	6.20	0.89	3.30	1.88	5.50	2.50	6.50	8.50	4.00	6.70	4.40	1.52
133	4.50	5.50	7.40	0.74	3.80	1.95	6.20				4.60	6.70	4.70	1.43

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	A	B	C		D		E	F	G	H	I	J	K	
Specimen Number	L Antenna	L Hd Cp	W Hd Cp	B/C	W Neck	C/D	L T1	L T2 - T3	L A1 - A3	L A4 - A6	L A7	L A8	L Uro	J/K
134	3.50	3.15	3.45	0.91	1.70	2.03	2.05	1.25	2.00	2.55	1.25	3.70	2.35	1.57
135		4.50	4.75	0.95	2.50	1.90	Head only							
136	Fragments only. No head.													
137	Larva not *Dytiscus* sp. *Colymbetes* sp.													
138	Larva not *Dytiscus* sp. *Colymbetes* sp.													
139	Larva not *Dytiscus* sp. *Colymbetes* sp.													
140	Larva not *Dytiscus* sp. *Colymbetes* sp.													
141		3.20	4.10	0.78	2.10	1.95	4.10					4.30	2.80	1.54
142	3.70	3.90	4.50	0.87	2.20	2.05	3.80							
143	Larva not *Dytiscus* sp. *Colymbetes* sp.													

Specimens marked in red = *D. dimidiatus* according to CO1 sequence

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	L	M	N	O	P		L1 Larvae	Body Length	Leg Length
Specimen Number	L Claw	L Tarsus	L Tibia	L Femur	L Coxa	P/M			
1	1.00	2.41	3.73	4.90	3.60	1.49		45.60	14.64
2	0.78	2.31	3.81	4.65	3.65	1.58		41.40	14.42
3	0.95	2.44	3.53	5.44	4.35	1.78		52.50	15.76
4	0.88	2.70	4.30	5.64	4.62	1.71		52.50	17.26
5	0.90	2.30	3.81	4.65	3.95	1.72		44.25	14.71
6	0.50	2.21	3.60	4.65	3.95	1.79		39.75	14.41
7	0.76	2.31	3.50	4.75	3.88	1.68		44.85	14.44
8	0.89	2.71	3.80	4.96	3.94	1.45		35.00	15.41
9	0.66	2.51	3.69	4.75	4.15	1.65		42.85	15.10
10	0.69	1.91	2.74	3.36	2.67	1.40	L1	23.39	10.68
11	0.89	2.31	3.80	4.85	3.85	1.67		38.75	14.81
12	0.89	2.40	3.70	4.85	3.80	1.58		26.10	14.75
13	0.66	1.98	2.85	3.35	2.65	1.34	L1	25.75	10.83
14	0.76	1.95	2.74	3.50	2.85	1.46	L1	23.10	11.04
15	0.83	2.48	3.35	3.95	3.60	1.45		33.10	13.38
16	0.85	2.35	3.25	4.55	4.10	1.74		37.70	14.25
17	0.50	1.25	1.85	2.35	1.75	1.40	L1	19.00	7.20
18	0.86	2.44	3.60	4.50	3.75	1.54		34.10	14.29
19	0.73	2.07	3.70	4.75	3.90	1.88		38.40	14.42
20	0.76	2.38	3.75	4.65	2.90	1.22		38.60	13.68
21	0.73	2.34	3.65	4.80	3.65	1.56		38.90	14.44
22	0.73	1.98	2.74	3.30	1.78	0.90		24.25	9.80
23	0.73	2.50	3.60	4.60	3.90	1.56		38.10	14.60
24	0.76	2.30	3.60	4.60	4.10	1.78		33.65	14.60
25	0.79	2.45	3.55	4.60	4.00	1.63		33.27	14.60
26	0.62	1.39	2.01	2.46	1.68	1.21	L1	12.92	7.54

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	L	M	N	O	P		L1 Larvae	Body Length	Leg Length
Specimen Number	L Claw	L Tarsus	L Tibia	L Femur	L Coxa	P/M			
27	0.75	1.90	2.70	3.40	2.90	1.53			10.90
28	0.95	2.72	3.81	5.17	4.08	1.50		49.00	15.78
29	0.85	2.72	4.42	5.45	4.76	1.75		36.90	17.35
30	0.80	2.55	3.75	4.65	3.60	1.41		35.28	14.55
31	1.05	2.30	3.40	5.20	3.40	1.48		37.80	14.30
32	0.95	2.50	3.50	4.90	3.30	1.32		41.30	14.20
33	Larva not *Dytiscus* sp. Probably *Acilius sulcatus*.								
34	1.03	2.51	3.77	4.80	3.90	1.55		40.10	14.98
35	0.90	2.70	4.10	5.30	4.28	1.59		38.20	16.38
36	0.85	2.50	3.70	4.75	3.80	1.52		48.95	14.75
37	0.90	2.75	3.50	5.00	3.80	1.38		45.30	15.05
38	0.95	2.50	3.55	4.60	2.90	1.16		42.70	13.55
39	0.95	2.50	3.80	4.80	4.10	1.64		42.80	15.20
40	0.80	2.55	3.65	4.75	4.40	1.73		41.20	15.35
41	0.85	2.60	3.75	4.85	3.50	1.35		43.55	14.70
42	1.00	2.65	3.80	5.00	3.55	1.34		40.76	15.00
43	0.80	2.35	3.65	4.75	4.00	1.70		51.30	14.75
44	Fragments only. Only front legs available.								
45	0.80	2.10	3.45	4.40	3.65	1.74		32.80	13.60
46	0.60	2.25	3.50	4.55	3.15	1.40		34.60	13.45
47	0.80	2.40	3.60	4.60	3.25	1.35		36.70	13.85
48	Fragments only. No legs.								
49	0.70	1.80	2.75	3.50	2.90	1.61		22.45	10.95
50	0.55	1.50	2.00	2.20	1.50	1.00	L1		7.20
51	0.90	2.40	3.50	4.75	4.10	1.71		35.70	14.75
52	0.90	2.40	3.50	4.60	4.00	1.67		35.90	14.50
53	0.80	2.50	3.75	4.50	3.20	1.28		37.95	13.95

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	L	M	N	O	P		L1 Larvae	Body Length	Leg Length
Specimen Number	L Claw	L Tarsus	L Tibia	L Femur	L Coxa	P/M			
54	0.80	2.35	3.40	4.50	3.50	1.49		41.05	13.75
55	0.95	2.70	3.80	4.90	2.75	1.02		44.50	14.15
56	0.80	2.50	3.90	4.95	3.50	1.40		32.10	14.85
57	0.75	2.30	3.70	5.00	3.10	1.35		36.80	14.10
58	0.90	2.65	3.80	5.00	4.00	1.51		46.10	15.45
59	0.80	2.70	4.25	5.00	3.65	1.35		41.00	15.60
60	0.90	2.55	3.75	4.80	3.50	1.37		37.90	14.60
61	0.85	2.50	3.75	4.65	4.00	1.60		37.70	14.90
62	0.80	2.35	3.70	4.70	4.20	1.79		41.00	14.95
63	0.80	2.65	3.85	5.00	3.90	1.47		38.10	15.40
64	0.90	2.30	3.50	4.50	3.25	1.41		40.90	13.55
65	Fragments only. No legs.								
66	0.80	2.60	3.70	4.50	3.40	1.31		35.80	14.20
67	0.75	2.55	3.60	4.60	3.75	1.47		43.80	14.50
68	0.80	2.50	3.70	4.60	4.40	1.76	Very pale	24.90	15.20
69	0.90	2.50	3.75	5.00	4.20	1.68		32.70	15.45
70	0.80	2.40	3.75	4.80	4.00	1.67		42.45	14.95
71	0.80	2.55	3.55	5.00	4.10	1.61		39.40	15.20
72	0.90	2.60	3.65	4.90	3.70	1.42		52.90	14.85
73	0.70	2.35	3.65	4.65	3.30	1.40		38.20	13.95
74	0.80	2.55	4.15	5.17	3.94	1.55		36.80	15.81
75	0.85	2.60	3.65	5.00	3.50	1.35		39.00	14.75
76	0.80	2.25	3.45	4.15	3.20	1.42		31.80	13.05
77	0.65	1.55	2.35	2.75	2.05	1.32	L1	21.20	8.70
78	1.00	2.70	3.80	4.85	3.70	1.37		32.90	15.05
79	Fragments only.								
80	0.80	2.80	4.10	5.10	4.50	1.61	Fragments only.		16.50

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	L	M	N	O	P		L1 Larvae	Body Length	Leg Length
Specimen Number	L Claw	L Tarsus	L Tibia	L Femur	L Coxa	P/M			
81	0.90	2.60	3.70	4.90	4.15	1.60		38.50	15.35
82	0.80	2.35	3.65	4.75	4.00	1.70		44.85	14.75
83	Fragments only. No legs.								
84	0.90	2.50	3.70	4.65	4.10	1.64	Fragments only.		14.95
85	0.80	2.55	4.00	4.35	3.45	1.35		38.10	14.35
86	0.75	2.65	3.85	4.60	3.55	1.34		42.50	14.65
87	0.75	2.40	3.60	4.70	3.50	1.46		38.00	14.20
88	0.80	1.95	2.75	3.25	3.00	1.54		18.30	10.95
89	1.00	2.50	3.70	4.75	3.00	1.20		42.65	13.95
90	0.90	2.40	3.75	4.85	3.15	1.31		34.60	14.15
91	0.80	2.65	3.95	4.65	4.00	1.51		43.40	15.25
92	Head only.								
92a	Head only.								
93	0.80	2.50	3.65	4.55	3.25	1.30		38.50	13.95
94	0.95	2.65	3.85	4.70	3.35	1.26		34.70	14.55
95	0.80	2.75	4.10	5.00	3.75	1.36		48.30	15.60
96	0.85	2.50	3.50	4.80	3.60	1.44	L1	26.30	14.40
97	0.80	2.05	3.00	3.35			Fragments only.		
98	0.80	2.30	3.60	4.35	4.00	1.74		38.20	14.25
99	0.90	2.75	3.95	4.80	3.50	1.27		43.40	15.00
100	0.85	2.70	3.95	5.00	3.60	1.33		44.00	15.25
101	0.85	2.60	4.00	4.75	3.60	1.38		41.20	14.95
102	0.80	2.60	3.85	4.60	3.80	1.46		39.70	14.85
103	0.90	2.30	3.40	4.65	4.25	1.85		31.60	14.60
104	0.90	2.10	3.35	4.65	3.75	1.79		46.30	13.85
105	0.80	2.50	3.95	4.85	3.95	1.58		42.50	15.25
106	0.85	2.55	3.95	4.60	3.50	1.37		43.20	14.60

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	L	M	N	O	P		L1 Larvae	Body Length	Leg Length
Specimen Number	L Claw	L Tarsus	L Tibia	L Femur	L Coxa	P/M			
107	0.80	2.40	3.65	4.20	3.25	1.35		43.45	13.50
108	0.80	2.80	4.25	5.58	3.35	1.20		51.90	15.98
109	0.85	2.35	3.95	4.85	3.70	1.57		48.55	14.85
110	0.80	2.45	3.75	4.45	2.75	1.12		43.00	13.40
111	0.80	2.60	3.90	5.05	4.10	1.58		47.60	15.65
112	1.00	2.70	4.15	5.00	4.35	1.61		45.20	16.20
113	1.00	2.35	3.70	3.70	3.85	1.64		32.70	13.60
114	0.85	2.40	3.65	4.60	4.00	1.67		43.55	14.65
115	0.90	2.40	3.75	4.95	3.50	1.46		30.80	14.60
116	0.70	1.80	2.60	2.60	2.20	1.22	L1		9.20
117	0.85	2.50	3.85	4.75	3.85	1.54		36.00	14.95
118	0.85	2.55	4.05	5.00	3.95	1.55		46.25	15.55
119	0.90	2.50	3.85	4.80	4.15	1.66		34.90	15.30
120	0.85	2.25	3.50	4.50	3.60	1.60		35.00	13.85
121	0.80	2.50	3.85	4.75	3.50	1.40		42.80	14.60
122	0.80	2.45	3.60	4.50	3.50	1.43		38.20	14.05
123	0.80	2.10	3.55	4.50	3.95	1.88		38.40	14.10
124	0.95	2.85	4.00	5.00	4.50	1.58		35.40	16.35
125	Legs fragmentary.							15.50	
126	0.80	2.15	3.90	4.55	4.25	1.98		37.10	14.85
127	0.80	2.40	3.85	4.80	3.90	1.63		30.80	14.95
128	0.80	2.80	4.50	5.24	4.10	1.46		44.70	16.64
129	0.85	2.50	3.85	4.80	3.70	1.48		49.90	14.85
130	0.75	2.55	3.80	4.50	3.35	1.31		45.40	14.20
131	0.60	1.90	2.65	3.30	3.10	1.63		20.90	10.95

Appendix D5: Measurements (in mm) of and ratios calculated for *Dytiscus* larvae (continued)

	L	M	N	O	P		L1 Larvae	Body Length	Leg Length
Specimen Number	L Claw	L Tarsus	L Tibia	L Femur	L Coxa	P/M			
132	0.80	2.65	3.85	5.00	3.45	1.30		33.70	14.95
133	0.95	2.35	3.85	4.50	3.55	1.51	Fragments only.		14.25
134	0.70	1.50	2.30	3.00	2.00	1.33	L1	12.80	8.80
135	Head only.								
136	Fragments only.								
137	Larva not *Dytiscus* sp. *Colymbetes* sp.								
138	Larva not *Dytiscus* sp. *Colymbetes* sp.								
139	Larva not *Dytiscus* sp. *Colymbetes* sp.								
140	Larva not *Dytiscus* sp. *Colymbetes* sp.								
141	Fragments only.								
142	Fragments only.								
143	Larva not *Dytiscus* sp. *Colymbetes* sp.								

Specimens marked in red = *D. dimidiatus* according to CO1 sequence

Appendix D6: Larval Key [Klausnitzer (1991)]

Die L_1 hat keine Schwimmhaare an den Seiten des 7. As. L_2 können durch Vergleich der in der Tabelle angegebenen Maße erkannt werden, eine Bestimmungstabelle für dieses Stadium kann aber vorläufig nicht gegeben werden.

Tabelle für die Arten (L_1) (nach BLUNCK 1923a)

Es fehlen: *D. circumflexus* F. und *D. lapponicus* GYLLENHAL

1 Urogomphi länger als 8. As (Abb. 85). Kopfkapsel, Stipes und Labialpalpen ohne Schuppen (Abb. 80). *semisulcatus* MÜLLER

1+ Urogomphi nur reichlich halb so lang wie das 8. As (Abb. 81–84). Wenigstens die Kopfkapsel ist beschuppt. 2

2 Kopfkapsel in Höhe der Stemmata 1,5 mal so breit wie der Hals (Abb. 73). Labialpalpen ohne Schuppen (Abb. 78). *latissimus* L.

2+ Kopfkapsel in Höhe der Stemmata mehr als doppelt so breit wie der Hals (Abb. 71, 72, 74). Labialpalpen mit Schuppen (Abb. 76, 77, 79). . . . 3

3 Körper bis 27 mm lang, graubraun. Schläfenecken deutlich vorgewölbt (Abb. 72). *dimidiatus* BERGSTRÄSSER

3+ Körper höchstens 23 mm lang, braun oder gelbbraun. Schläfenecken nicht oder nur wenig vorgewölbt (Abb. 71, 74). 4

4 Maxillen verhältnismäßig lang. Hintertarsus etwa 1/5 länger als das 3. Antennenglied. *circumcinctus* AHRENS

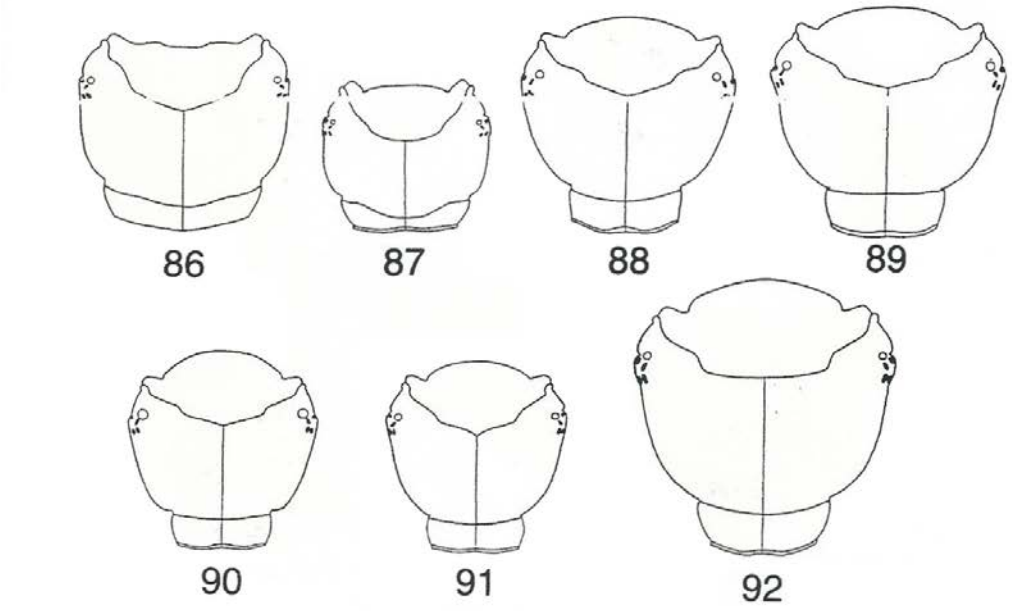


Abb. 86: *Dytiscus latissimus*, Kopf (aus BLUNCK & KLYNSTRA 1929).
Abb. 87: *Dytiscus semisulcatus*, Kopf (aus BLUNCK & KLYNSTRA 1929).
Abb. 88: *Dytiscus marginalis*, Kopf (aus BLUNCK & KLYNSTRA 1929).
Abb. 89: *Dytiscus circumcinctus*, Kopf (aus BLUNCK & KLYNSTRA 1929).
Abb. 90: *Dytiscus circumflexus*, Kopf (aus BLUNCK & KLYNSTRA 1929).
Abb. 91: *Dytiscus lapponicus*, Kopf (aus BLUNCK & KLYNSTRA 1929).
Abb. 92: *Dytiscus dimidiatus*, Kopf (aus BLUNCK & KLYNSTRA 1929).

4+ Maxillen (besonders der Stipes) kürzer. Hintertarsus etwa 2/5 länger als das 3. Antennenglied (Beschuppung der Kopfkapsel und Mundwerkzeuge sowie Bedornung aller Körperteile kräftiger als bei den anderen 4 Arten). *marginalis* L.

Tabelle für die Arten (L_3) (nach BLUNCK & KLYNSTRA 1929)

1 Kopfkapsel quer, die Breite des Halses beträgt mehr als 3/4 der Kopfkapselbreite (Abb. 86, 87). Vorderrand des Frontoclypeus konkav oder schwach konvex. Ventralseite der Vordertarsen mit Schwimmhaaren, die bis zur distalen Hälfte reichen (Abb. 94, 96). Hintercoxa mehr als doppelt so lang wie der Tarsus. 2

1+ Kopfkapsel länger als breit, Hals nicht breiter als 0,58 der Kopfkapselbreite (Abb. 88–92). Vorderrand des Frontoclypeus sehr deutlich konvex. Ventralseite der Vordertarsen nur in der proximalen Hälfte mit Schwimmhaaren besetzt (Abb. 93, 95, 97–99). Hintercoxa höchstens 1,75 mal so lang wie der Tarsus. 3

2 Vorderrand des Frontoclypeus konkav (Abb. 86). Kopfkapsel größer, 5,9 mm lang. 8. As länger (7,3 mm), mehr als doppelt so lang wie die Urogomphi (3,58 mm) (Abb. 101). *latissimus* L.

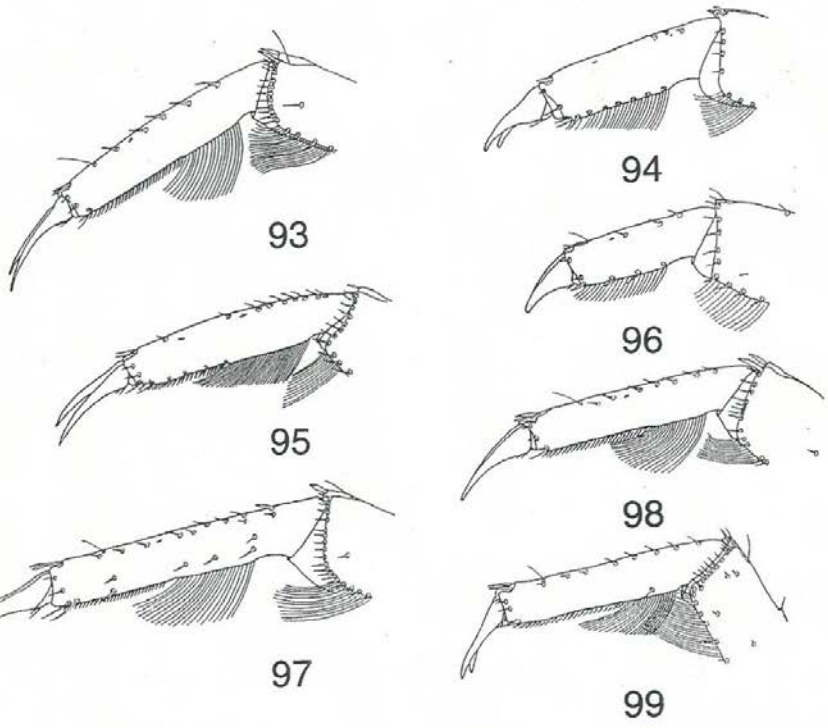


Abb. 93: *Dytiscus circumflexus*, Vordertarsus (aus BLUNCK & KLYNSTRA 1929).
Abb. 94: *Dytiscus latissimus*, Vordertarsus (aus BLUNCK & KLYNSTRA 1929).
Abb. 95: *Dytiscus lapponicus*, Vordertarsus (aus BLUNCK & KLYNSTRA 1929).
Abb. 96: *Dytiscus semisulcatus*, Vordertarsus (aus BLUNCK & KLYNSTRA 1929).
Abb. 97: *Dytiscus dimidiatus*, Vordertarsus (aus BLUNCK & KLYNSTRA 1929).
Abb. 98: *Dytiscus marginalis*, Vordertarsus (aus BLUNCK & KLYNSTRA 1929).
Abb. 99: *Dytiscus circumcinctus*, Vordertarsus (aus BLUNCK & KLYNSTRA 1929).

2+ Vorderrand des Frontoclypeus schwach konvex (Abb. 87). Kopfkapsel kleiner, etwa 4,7 mm lang. 8. As kürzer (5,24 mm), nicht länger als 1,5 mal der Länge der Urogomphi (3,57 mm) (Abb. 103).
semisulcatus MÜLLER

3 Kopfkapsel 8,6 mm lang und 8,3 mm breit (Abb. 92). Frontoclypeus vorn stark konvex. Antenne länger als 6,5 mm. Beine länger (Hinterbeine ohne Klauen etwa 19,5 mm). *dimidiatus* BERGSTRÄSSER

3+ Kopfkapsel kleiner (bis zu 7,6×7,3 mm) (Abb. 88–91). Frontoclypeus vorn sehr stark konvex. Antennen höchstens 5,5 mm lang. Beine kürzer (Hinterbeine ohne Klauen etwa 17,4 mm). 4

4 Kopfkapsel länger als 7,0 mm und bei den meisten Exemplaren mehr als 6,3 mm breit (Abb. 88, 89). Maxille länger als 5 mm. Hinterfemur länger als 4,5 mm. 8. As länger als 5,5 mm. 5

4+ Kopfkapsel bis zu 6,7 mm lang und bis zu 6,4 mm breit (Abb. 90, 91). Maxille maximal 5 mm lang. Hinterfemur höchstens 4,5 mm lang. 8. As kürzer als 5,5 mm. 6

5 Kopfkapsel schmaler (Breite über den Stemmata 6,4–7,0 mm) (Abb. 88). Körper mit einer deutlichen gelbbraunen Mittellinie, die Seiten sind dunkler. *marginalis* L.

5+ Kopfkapsel breiter (über den Stemmata 7,1–7,4 mm) (Abb. 89). Körper höchstens mit einer sehr schwach sichtbaren hellen Mittellinie.
circumcinctus AHRENS

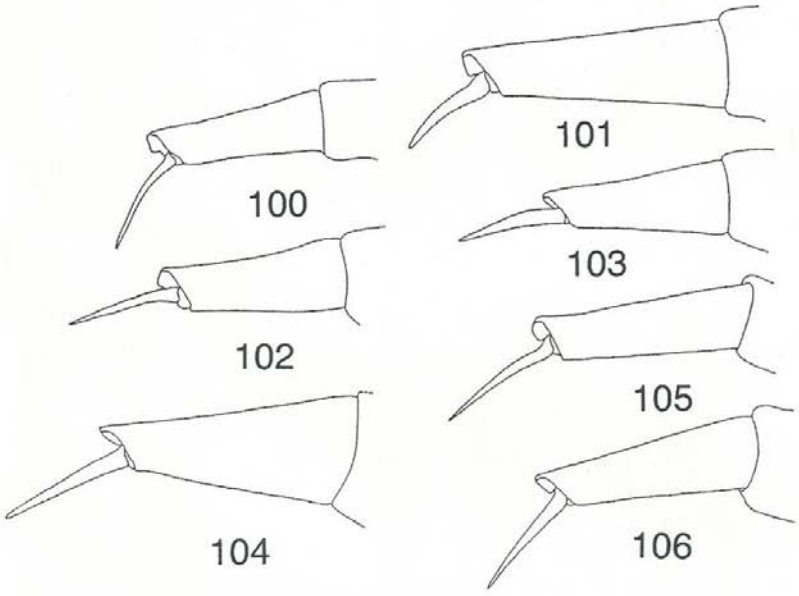


Abb. 100: *Dytiscus circumflexus*, 8. As, lateral (aus BLUNCK & KLYNSTRA 1929).
Abb. 101: *Dytiscus latissimus*, 8. As, lateral (aus BLUNCK & KLYNSTRA 1929).
Abb. 102: *Dytiscus lapponicus*, 8. As, lateral (aus BLUNCK & KLYNSTRA 1929).
Abb. 103: *Dytiscus semisulcatus*, 8. As, lateral (aus BLUNCK & KLYNSTRA 1929).
Abb. 104: *Dytiscus dimidiatus*, 8. As, lateral (aus BLUNCK & KLYNSTRA 1929).
Abb. 105: *Dytiscus marginalis*, 8. As, lateral (aus BLUNCK & KLYNSTRA 1929).
Abb. 106: *Dytiscus circumcinctus*, 8. As, lateral (aus BLUNCK & KLYNSTRA 1929).
Die Abb. 86–106 stellen die L_3 dar.

6 Hintercoxen (3,8 mm) etwa 1,6 mal so lang wie die Tarsen (2,39 mm). Körper mit einer deutlichen gelbbraunen Mittellinie, die Seiten sind dunkler. Kopfkapsel siehe Abb. 90. Urogomphi knapp 3,5 mm lang.
circumflexus F.

6+ Hintercoxen (3,78 mm) mehr als 1,75 mal so lang wie die Tarsen (2,15 mm). Körper höchstens mit einer sehr diffusen Mittellinie. Kopfkapsel siehe Abb. 91. Urogomphi 3,75–4 mm lang.
lapponicus GYLLENHAL

Körpermaße (mm) der Gattung *Dytiscus* (nach BLUNCK 1923a und BLUNCK & KLYNSTRA 1929)

		Länge[1] Kopfkapsel	Breite Kopfkapsel	Länge Antenne	Länge[2] Hintertarsus	Länge Kralle	Länge[3] 8. As	Länge[2] Urogomphus
circumcinctus	L_1	n. a.	3,1	3,5	n. a.	n. a.	n. a.	2,0
	L_3	7,42	7,22	5,51	2,42	0,64	5,68	3,87
circumflexus	L_3	6,58	6,25	4,95	2,39	0,63	4,9	3,48
dimidiatus	L_1	n. a.	3,2	3,7	n. a.	n. a.	n. a.	2,1
	L_3	8,63	8,3	6,8	2,64	0,78	6,4	4,45
lapponicus	L_3	6,5	6,0	5,05	2,15	0,64	5,2	3,9
latissimus	L_1	n. a.	2,6	2,3	n. a.	n. a.	n. a.	1,7
	L_3	5,5	6,6	4,32	2,05	0,55	7,3	3,58
marginalis	L_1	n. a.	3,0	3,1	n. a.	n. a.	n. a.	2,1
	L_3	7,3	6,72	5,31	2,48	0,74	5,63	4,05
semisulcatus	L_1	n. a.	2,4	2,0	n. a.	n. a.	n. a.	2,8
	L_3	4,72	5,6	3,55	1,6	0,53	5,24	3,57

Anmerkungen: [1] einschließlich Hals [2] Außenkante
[3] ventrale Länge n. a. = Maß nicht angegeben

Literatur

BLUNCK, H. (1923 a): Zur Kenntnis des „Breitrands" *Dytiscus latissimus* L. und seiner Junglarve. – Zool. Anz. 57, 157–168.

BLUNCK, H. (1923 b): Die Entwicklung des *Dytiscus marginalis* L. vom Ei bis zur Imago. – Z. wiss. Zool. 121, 172–392.

BLUNCK, H. & B. H. KLYNSTRA (1929): Die Kennzeichen der Jugendstände in Deutschland und Holland vorkommender *Dytiscus*-Arten. – Zool. Anz. 81, 114–140.

5. Gattung: *Cybister* CURTIS

In Mitteleuropa nur *C. lateralimarginalis* (DEGEER), dessen Larve (Abb. 26) durch einige Besonderheiten auffällt:
– Vorderrand des Frontoclypeus dreispitzig (Abb. 35, 66)

Appendix D6 (continued)

Translation of Klausnitzer's Key by Inga Zeisset (Part A – L_1 Larvae)

4.) Genus *Dytiscus* L.

p.265 key for the species (L1)

1) Urogomphi longer than 8th segment (fig 85). head capsule, stipes and labialpalps without flakes (fig80) *semisulcatus*

1+) Urogomphi only (liberally) half as long as teh 8th segment (fig 81-84). At least the head capsule is flaked. 2

2) Head capsule at the height of the Stemmata 1,5 times as wide as the neck (fig 73). Labialpalps without flakes (fig 78). *latissumus*

2+) Head capsule at the height of the Stemmata more than twice as wide as the neck (fig 71,72,74). Labialpalps with flakes (fig 76,77,79) 3

3) Body up to 27 mm long, grey brown. Temples bulge clearly forward (fig 72). *dimidiatus*

3+) Body maximal 23mm long, brown or yellowish brown. Temples little or not bulged forward (fig 71, 74). 4

4) Maxillen relatively long. Back tarsus (Hintertarsus) roughly 1/5 longer than the 3rd antenna segment. *circumcinctus*

4+) Maxillen (especially the Stipes) shorter. Back tarsus roughly 2/5 longer than the 3rd antenna segment (Head capsule and mouthparts are flaked and the thorniness of all bodyparts is stronger than in the other 4 species) *marginalis*

Appendix D6 (continued)

Translation of Klausnitzer's Key by Inga Zeisset (Part A – L_1 Larvae)

p.266-268 key for the species (L3)

1) Head capsule horizontal (diagonally?), the width of the neck is more than ¾ of the head capsule width (fig 86, 87). Front edge of the fronotclypeus is concave or slightly concave. Ventral side of the front tarsel (Vordertarse) with swimming hairs, reaching up to the distal half (fig 94, 96). Back coxa more than twice as long as the tarsus. 2

1+) Head capsule longer than wide, neck not wider than 0.58 of the head capsule width (fig 88-92). Front edge of the fronotclypeus clearly convex. Ventral side of the front tarsel only in the proximal half covered in Swimming hairs (fig. 93, 95, 97-99). Back coxa not more than 1.75 times as long as the tarsus. 3

2) Front edge of the frontoclypeus concave (fig 86). Head capsule bigger, 5.9mm long. 8th segment is longer (7.3mm), more than double the length of the Urogomphi (3.58mm) (fig 101). *latissumus*

2+) Front edge of the frontoclypeus weakly convex (fig 87). Head capsule smaller, roughly 4.7 mm long. 8th segment is shorter (5.24mm), not longer than 1.5 times the length of the urogomphi (3.57mm) (fig 103). *semisulcatus*

3) Head capsule 8.6 mm long and 8.3 mm wide (fig 92). Frontoclypeus is very convex at the front. Antennae longer than 6.5 mm. Legs longer (back legs without claws roughly 19.5mm). *dimidiatus*

3+) Head capsule smaller (up to 7.6x7.3mm) (fig 88-91). Frontoclypeus is extremely convex at the front. Antennae maximal 5.5mm long. Legs shorter (back legs without claws roughly 17.4mm). 4

4) Head capsule longer than 7.0 mm and in most animals more than 6.3 mm wide (fig 88, 89). Maxille longer than 5 mm. Back femur longer than 4.5mm. 8th segment longer than 5.5mm. 5

4+) Head capsule up to 6.7 mm long an up to 6.4mm wide (fig 90, 91). Maxille maximal 5 mm long. Back femur not more than 4.5 mm long. 8th segment shorter than 5.5mm. 6

5) Head capsule more narrow (width over the Stemmata 6.4-7.0 mm) (fig88). Body with a clear brown-yellow line (stripe?) down the middle, the sides are darker. *marginalis*

5+) Head capsule wider (width over the Stemmata 7.1-7.4 mm) (fig 89). Body may have a very weak lighter coloured line down the middle. *circumcinctus*

6) Back coxen (3.8mm) roughly 1.6 times as long as the tarsa (2.39mm). Body with a clear brown-yellow middle line, the sides are darker. Head capsule see fig 90. Urogomphi just about 3.5mm long. *circumflexus*

6+) Back coxen (3.78mm) more than 1.75 times as long as the tarsa (2.15mm). Body with not more than a very diffuse middle line. Head capsule see fig 91. Urogomphi 3.75-4mm long. *lapponicus*

Simplified Key to distinguish larvae of Dytiscus recorded from Somerset Levels and Moors [modified from Zeisset's translation of Klausnitzer (1991)]

1. Swimming hairs NOT present on side of 7th segment 2

   Swimming hairs present on side of 7th segment 4

2. Urogomphi viewed from side longer than the segment to which they are attached - semisulcatus

   Urogomphi viewed from side not longer than the segment to which they are attached 3

3. Total length of body more than 27mm – dimidiatus

   Total length of body less than 27mm – marginalis

4. Head capsule more than 7.6mm long and 7.3mm wide – dimidiatus

   Head capsule less than 7.6mm long and 7.3mm wide 5

5. The width of the neck is more than three quarters the width of the head capsule (measured at its widest point). The leading edge of the frontoclypeus is concave or slightly concave. The ventral side of the last (i.e. claw-bearing) tarsal segment on the tarsus of the front leg has swimming hairs on the distal half only (i.e. on the half closest to the claws). The coxae on hind legs are more than twice as long as the tarsi. – semisulcatus

   The width of the neck is less than three quarters the width of the head capsule (measured at its widest point). The leading edge of the frontoclypeus is clearly convex. The ventral side of the last (i.e. claw-bearing) tarsal segment on the tarsus of the front leg has swimming hairs on the proximal half only (i.e. on the half furthest from the claws and closest to the body). The coxae on hind legs are less than twice as long as the tarsi - marginalis

Simplified Key to distinguish larvae of Dytiscus recorded from Somerset Levels and Moors [modified from Zeisset's translation of Klausnitzer (1991)] (continued)

Body measurements in mm of Dytiscus larvae (after Blunck 1923, Blunck & Kynstra 1929 cited in Klausnitzer 1991)								
		A	B	N	I	M	G	H
D. circumflexus	L3	6.58	6.25	4.95	2.39	0.64	4.9	3.48
D. dimidiatus	L1		3.2	3.7				2.1
	L3	8.63	8.3	6.8	2.64	0.78	6.4	4.45
D. marginalis	L1		3.0	3.1				2.1
	L3	7.3	6.72	5.31	2.48	0.74	5.63	4.05
D. semisulcatus	L1		2.4	2.0				2.8
	L3	4.72	5.6	3.55	1.6	0.53	5.24	3.57

Definition of terms used [modified after Zombori & Steinmann (1999)]

Urogomphi – Projections on the terminal abdominal segment of immature stages in various coleopteran families

Stemmata – Eyes of larvae of several orders of insects. Similar to ommatidia (units in compound eyes), they have lenses and photo-receptors

Frontoclypeus – The unified plate at the front of the head to which the labrum is attached.

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Appendix E1: Photographs of some trapping locations, Shapwick Heath, 2007- 8 Taken 6 April 2007

Photographs of trapping locations, Shapwick Heath, 2007- 8 (continued)
Taken 6 April 2007

9.

10.

15.

16.

Photographs of trapping locations, Shapwick Heath, 2007- 8 (continued)

Above: Open area with Bog Myrtle and wet woodland (Taken 6 April 2007)

Above: Contrasting areas - A. Heavily shaded (Typical of trap locations 1 – 15);
B. More open ditch (Typical of locations 16 – 20). (Taken in September 2007)

Appendix E2: Water chemistry results from 2006

Date given is date of measurement, Field/lab indicates where measurement was made (some water samples were tested in SCC laboratories c. 24 hours after collection).

SITE	pH	Date	Field/Lab	Conductivity	Date	Field/Lab	Dissolved Oxygen	Date	Field/Lab
Shapwick Heath	6.17	12/04/2006	Lab	404	11/04/2006	Field	2.52	28/04/2006	Field
	6.19	17/05/2006	Lab	407	11/04/2006	Field	1.31	28/04/2006	Field
	7.17	30/05/2006	Lab	303	28/04/2006	Field	0.86	28/04/2006	Field
	6.42	05/06/2006	Lab	818	28/04/2006	Field	0.46	14/05/2006	Field
	6.52	05/06/2006	Lab	779	28/04/2006	Field	0.33	14/05/2006	Field
				409	14/05/2006	Field	2.06	14/05/2006	Field
				412	14/05/2006	Field			
				430	14/05/2006	Field	0.882602213		
				411	17/05/2006	Lab			
				712	30/05/2006	Lab			
Catcott North	6.95	18/04/2004	Lab	911	17/04/2006	Field	0.09	17/04/2006	Field
	7.55	18/04/2004	Lab	899	18/04/2006	Lab	0.15	17/04/2006	Field
				769	17/04/2006	Field			
				768	18/04/2006	Lab	0.042426407		
Westhay Moor	6.55	24/04/2006	Lab	302	22/04/2006	Field	1.15	22/04/2006	Field
	6.56	24/04/2006	Lab	327	22/04/2006	Field	1.24	22/04/2006	Field
	6.35	24/04/2006	Lab	307	22/04/2006	Field	0.66	22/04/2006	Field
				291	24/04/2006	Lab			
				289	24/04/2006	Lab	0.31214313		
				284	24/04/2006	Lab			

Appendix E3: Environmental parameters measured at Shapwick Heath 2007

8th April 2007	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10
Width of Waterbody (m)	2.0	2.3	2.0	2.2	1.8	1.8	1.5	2.0	2.2	2.0
Shade by Trees & Shrubs (%)	30	30	50	50	40	40	50	60	40	50
Glyceria Cover (%)	30	40	10	5	10	10	5	0	0	40
Duckweed Cover (%)	80	95	100	100	100	100	98	20	100	50
Nearside Gradient	1	2	2	3	2	3	3	3	4	4
Farside Gradient	2	1	1	3	4	4	4	1	4	1
Combined Gradient	3	3	3	6	6	7	7	4	8	5

17 June 2007	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10
Width of Waterbody (m)	2.0	2.0	2.0	2.0	1.5	1.5	1.3	1.2	2.3	1.0
Depth of Water (cm)	30	50	45	25	45	30	35	35	45	35
Shade by Trees & Shrubs (%)	90	50	100	100	80	75	90	90	90	90
Glyceria Cover (%)	5	10	5	0	5	5	0	0	0	70
Duckweed Cover (%)	100	95	100	100	100	100	100	100	100	100

9 September 2007	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10
Width of Waterbody (m)	2.0	1.6	1.6	1.8	1.5	1.5	1.3	1.5	1.4	0.6
Depth of Water (cm)	55	45	55	50	50	45	45	45	40	40
Shade by Trees & Shrubs (%)	90	50	95	100	90	80	80	95	80	90
Glyceria Cover (%)	5	50	5	0	0	0	0	0	0	80
Duckweed Cover (%)	100	100	100	100	100	100	100	50	100	100

AVERAGES	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10
Width of Waterbody (m)	2.0	2.0	1.9	2.0	1.6	1.6	1.4	1.6	2.0	1.2
Depth of Water (cm)	42.5	47.5	50	37.5	47.5	37.5	40	40	42.5	37.5
Shade by Trees & Shrubs (%)	70.0	43.0	82.0	83.0	70.0	65.0	73.0	82.0	70.0	77.0
Glyceria Cover (%)	13.0	33.0	7.0	2.0	5.0	5.0	2.0	0.0	0.0	63.0
Duckweed Cover (%)	93.0	97.0	100.0	100.0	100.0	100.0	99.0	57.0	100.0	83.0

Appendix E3: Environmental parameters measured at Shapwick Heath 2007 (continued)

8th April 2007	Trap 11	Trap 12	Trap 13	Trap 14	Trap 15	Trap 16	Trap 17	Trap 18	Trap 19	Trap 20
Width of Waterbody (m)	1.7	2.0	1.7	2.0	2.5	2.0	2.3	2.8	2.8	2.2
Shade by Trees & Shrubs (%)	30	40	30	30	15	0	40	0	0	0
Glyceria Cover (%)	10	10	5	2	0	15	0	10	10	10
Duckweed Cover (%)	40	100	100	80	100	100	100	90	100	100
Nearside Gradient	3	3	3	3	3	2	2	1	2	1
Farside Gradient	3	3	3	3	3	2	2	2	2	2
Combined Gradient	6	6	6	6	6	4	4	3	4	3

17-Jun-07	Trap 11	Trap 12	Trap 13	Trap 14	Trap 15	Trap 16	Trap 17	Trap 18	Trap 19	Trap 20
Width of Waterbody (m)	1.4	1.8	1.3	1.6	2.0	2.0	2.5	2.5	2.2	2.2
Depth of Water (cm)	50	55	45	40	50	55	50	40	45	50
Shade by Trees & Shrubs (%)	30	50	90	90	70	0	0	0	0	0
Glyceria Cover (%)	5	5	5	5	5	0	0	0	0	0
Duckweed Cover (%)	100	100	100	100	100	100	100	100	100	100

09-Sep-07	Trap 11	Trap 12	Trap 13	Trap 14	Trap 15	Trap 16	Trap 17	Trap 18	Trap 19	Trap 20
Width of Waterbody (m)	1.2	1.1	1.0	1.0	1.2	1.6	1.6	1.65	1.5	1.6
Depth of Water (cm)	50	50	45	45	65	65	60	85	75	75
Shade by Trees & Shrubs (%)	60	50	90	90	80	0	0	0	0	0
Glyceria Cover (%)	5	10	10	0	5	5	0	10	0	5
Duckweed Cover (%)	100	100	100	100	100	100	100	100	100	100

AVERAGES										
Width of Waterbody (m)	1.4	1.6	1.3	1.5	1.9	1.9	2.1	2.3	2.2	2.0
Depth of Water (cm)	50	52.5	45	42.5	57.5	60	55	62.5	60	62.5
Shade by Trees & Shrubs (%)	40.0	47.0	70.0	70.0	55.0	0.0	13.0	0.0	0.0	0.0
Glyceria Cover (%)	7.0	8.0	7.0	2.0	3.0	7.0	0.0	7.0	3.0	5.0
Duckweed Cover (%)	80.0	100.0	100.0	93.0	100.0	100.0	100.0	97.0	100.0	100.0

Appendix E4: Sample scores, etc connected with CCA Plot in Figures 5.6a - c

Sample Scores	Sample	Can. Axis 1	Can. Axis 2	Can. Axis 3
Scores derived from the species scores.	Trap 1	-0.0695033	-0.328846	3.42497
	Trap 2	0.328873	0.333638	4.60492
	Trap 3	-0.223024	0.390799	-2.92368
	Trap 4	0.976834	3.83341	-0.134491
	Trap 5	0.537602	1.51748	-5.92036
	Trap 6	-0.238015	4.14934	1.48155
	Trap 7	0.581284	4.17502	-4.35998
	Trap 8	-2.92387	-2.46236	0.609961
	Trap 9	-2.25138	-0.842645	-10.4209
	Trap 10	-1.10469	2.33953	1.22351
	Trap 11	-1.53318	1.16502	-3.37106
	Trap 12	1.02891	-1.499	-1.68354
	Trap 13	1.35952	-1.01743	3.35176
	Trap 14	0.530095	0.408308	8.44645
	Trap 15	0.779641	-0.935093	-1.9004
	Trap 16	0.166723	-4.76117	-2.49361
	Trap 17	0.321076	1.9675	-0.730729
	Trap 18	0.2658	-4.45381	1.72032
	Trap 19	1.0425	-0.481462	3.45772
	Trap 20	1.61176	-1.18614	1.33773

Sample Scores	Sample	Can. Axis 1	Can. Axis 2	Can. Axis 3	Row sum (weights)
Scores that are linear combinations of environmental variables and weights	Trap 1	-0.137558	0.770193	-0.203146	33
	Trap 2	0.285488	0.531784	1.51171	27
	Trap 3	0.355594	1.27967	-0.877637	24
	Trap 4	0.346336	1.22213	-1.22289	14
	Trap 5	0.41839	0.883936	-0.774749	14
	Trap 6	0.445341	0.729891	-0.685578	11
	Trap 7	0.344596	0.884516	-1.03998	18
	Trap 8	-3.26138	-0.784451	-1.00119	28
	Trap 9	0.418116	0.775048	-1.09028	15
	Trap 10	-1.00535	1.62164	2.90187	31
	Trap 11	-1.08901	-0.889702	0.00260287	51
	Trap 12	0.544347	0.237653	-0.148263	35
	Trap 13	0.418481	0.920232	-0.669571	24
	Trap 14	-0.138161	0.530639	-0.897317	20
	Trap 15	0.499153	0.385505	-0.612414	20
	Trap 16	0.795804	-1.2364	0.578823	21
	Trap 17	0.723568	-0.970796	-0.0796772	36
	Trap 18	0.517606	-1.38401	0.601681	19
	Trap 19	0.795622	-1.30899	0.368467	30
	Trap 20	0.795713	-1.27269	0.473645	32

Appendix E4: Sample scores, etc connected with CCA Plot in Figures 5.6a – c (continued)

Species Scores	**Can. Axis 1**	**Can. Axis 2**	**Can. Axis 3**
D. marginalis Fem	0.0495406	0.111263	-0.0813395
D. marginalis Male	0.176359	0.117719	0.0572813
D. dimidiatus Fem	0.272784	-0.0793965	0.002162
D. dimidiatus Male	0.290208	-0.197276	-0.0061942
Dytiscus larvae	-0.518318	-0.0257493	-0.0230242
D. dimidiatus larvae	-1.13603	-0.165507	0.302593

Biplot Scores	**Can. Axis 1**	**Can. Axis 2**	**Can. Axis 3**
Shade	-0.467195	0.835912	-0.288061
Glyceria Cover	-0.178036	0.469462	0.864817
Duckweed Cover	0.988332	0.143713	-0.050453

Regression	**Can. Axis 1**	**Can. Axis 2**	**Can. Axis 3**
Shade	-0.161342	0.922168	-0.533809
Glyceria Cover	0.0008464	0.336128	0.974023
Duckweed Cover	0.935689	0.496468	-0.0768795

Correlations	**Shade**	***Glyceria* Cover**	**Duckweed Cover**
Shade	1	0.132687	-0.265282
Glyceria Cover	0.132687	1	-0.177055
Duckweed Cover	-0.26528	-0.177055	1

Multicolinearity

Dependent variable	R-squared	VIF
Shade	0.209945	1.26573
Glyceria Cover	0.055581	1.05885
Duckweed Cover	0.18937	1.23361

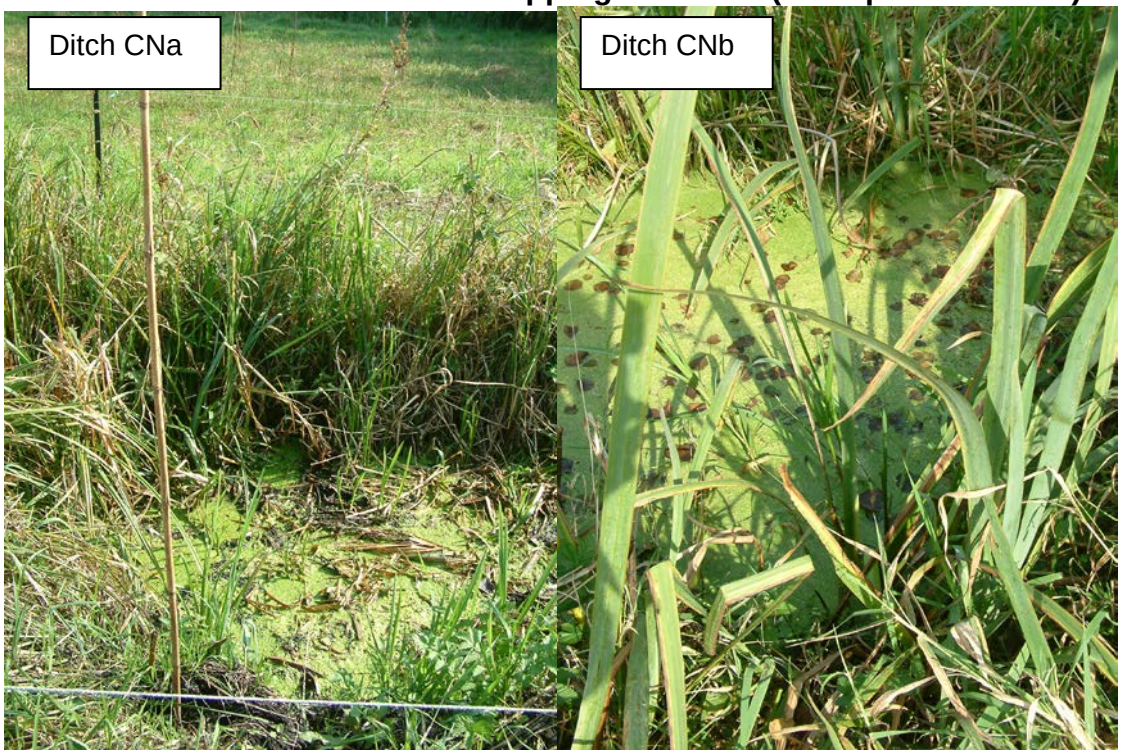
Monte Carlo Simulation

Axis	1	2	3
Actual Eigenvalues	0.115078	0.014339	0.003486
Eigenvalue results from simulation			
Mean	0.040879	0.012467	0.003301
Maximum	0.119712	0.040361	0.017104
Minimum	0.006129	0.00104	6.43E-05
Probability	0.004995	0.338661	0.368631
Number of trials	1000		

Appendix F1: Photographs of two sites sampled in 2008

An example of an open site, Catcott North: Habitat (28 September 2008)

Catcott North: Some unshaded trapping locations (28 September 2008)



An example of a shaded site - Westhay Heath – with some trapping locations and a photo of the wet woodland habitat (28 September 2008)

Appendix F2: *Dytiscus* spp. caught in 2008

Table F2a: Summary of captures of *Dytiscus marginalis* in 2008								
Site (No. of visits)	Males		Females		All Beetles			
	Total	Av.	Total	Av.	Total	Av.	Max	Date
Shapwick Heath (8)	29	3.63	28	3.50	57	7.13	17	5 May
Westhay Heath (7)	10	1.43	3	0.43	13	1.86	6	27 April
Westhay Moor (7)	49	7.00	16	2.29	65	9.29	24	13 April
All Shaded (22)	88	4.00	47		135			
Catcott North (7)	22	3.14	10	1.43	32	4.57	13	12 May
Tealham Moor (8)	19	2.38	10	1.25	29	3.63	15	27 April
East Waste (6)	6	1.00	4	0.67	10	1.67	3	12 May
All Unshaded (21)	47		24		71			

Table F2b: Summary of captures of *Dytiscus dimidiatus* in 2008								
Site (No. of visits)	Males		Females		All Beetles			
	Total	Av.	Total	Av.	Total	Av.	Max	Date
Shapwick Heath (8)	13	1.63	7	0.88	20	2.50	9	18 May
Westhay Heath (7)	5	0.71	3	0.43	8	1.14	4	27 April
Westhay Moor (7)	14	2.00	11	1.57	25	3.57	9	13 April
All Shaded (22)	32		21		53			
Catcott North (7)	0	0	2	0.29	2	0.29	1	N/A
Tealham Moor (8)	2	0.25	0	0	2	0.25	2	27 April
East Waste (6)	2	0.33	4	0.67	6	1.00	4	2 June
All Unshaded (21)	4		6		10			

Table F2c: Summary of captures of *Dytiscus* larvae in 2008								
Site (No. of visits)	D. marginalis/NK		D. dimidiatus		All Larvae			
	Total	Av.	Total	Av.	Total	Av.	Max	Date
Shapwick Heath (8)	23	2.88	1	0.13	24	3.00	14	13 July
Westhay Heath (7)	9	1.29	8	1.14	17	2.43	6	29 June
Westhay Moor (7)	17	2.43	0	0.00	17	2.43	8	23 July
All Shaded (22)	49		9		58			
Catcott North (7)	11	1.57	0	0.00	11	1.57	4	11 Aug
Tealham Moor (8)	5	0.63	1	0.13	6	0.75	4	8 June
East Waste (6)	0	0.00	0	0.00	0	0.00	N/A	N/A
All Unshaded (21)	16		1		17			

Appendix F3: Physicochemical data from study sites August 2008

Table F3a: Physicochemical data from Catcott North 28/8/08. SD = Standard Deviation, n = number of observations

	Ditch CNa		Ditch CNb		Ditch CNc		All Ditches	
	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]
Width of Waterbody (m)	1.5	(0.00) [5]	1.8	(0.27) [5]	1.5	(0.00) [5]	1.6	(0.21) [15]
Depth of Water (cm)	73.0	(22.25) [5]	77.0	(7.58) [5]	78.0	(8.37) [5]	76.0	(13.52) [15]
Poaching Near Bank	0.2	(0.45) [5]	0.0	(0.00) [5]	0.6	(0.55) [5]	0.3	(0.46) [15]
Poaching Far Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Gradient Near Bank	2.4	(0.55) [5]	2.2	(0.84) [5]	2.2	(0.45) [5]	2.3	(0.59) [15]
Gradient Far Bank	3.6	(0.89) [5]	2.0	(0.71) [5]	3.0	(1.00) [5]	2.9	(1.06) [15]
Summed Gradients	6.0		4.2		5.2		5.1	(0.90) [3]
Shade by Trees & Shrubs (%)	0.0	(0.00) [5]	10.0	(22.36) [5]	4.0	(8.94) [5]	4.7	(13.56) [15]
Glyceria Cover (%)	54.0	(27.02) [5]	15.0	(8.66) [5]	45.0	(20.62) [5]	38.0	(25.48) [15]
Duckweed Cover (%)	100.0	(0.00) [5]	100.0	(0.00) [5]	100.0	(0.00) [5]	100.0	(0.00) [15]
Oxygen (mg/l)	1.9	(1.70) [2]	3.4	(3.75) [2]	1.9	(0.64) [2]	2.4	(2.01) [6]
Temperature (ºC)	15.1	(0.50) [2]	15.9	(0.71) [2]	18.9	(3.54) [2]	16.6	(2.43) [6]
Pressure (mB)	1015.5	(0.71) [2]	1016.0	(0.00) [2]	1016.5	(0.71) [2]	1016.0	(0.63) [6]
Conductivity (µS)	511.5	(72.83) [2]	516.0	(39.60) [2]	569.5	(183.14) [2]	532.3	(94.42) [6]
Temperature (ºC)	17.0	(0.50) [2]	16.6	(1.13) [2]	18.7	(2.97) [2]	17.4	(1.76) [6]
pH	7.3	(0.00) [2]	7.3	(0.14) [2]	7.3	(0.14) [2]	7.3	(0.09) [6]
Temperature (ºC)	16.8	(0.28) [2]	16.5	(0.35) [2]	18.4	(3.75) [2]	17.2	(1.92) [6]

Table F3b: Physicochemical data from Westhay Heath 28/8/08. SD = Standard Deviation, n = number of observations

	Ditch WHa		Ditch WHb		Ditch WHc		All Ditches	
	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]
Width of Waterbody (m)	2.9	(0.54) [5]	4.5	(2.40) [5]	4.0	(1.17) [5]	3.8	(1.61) [15]
Depth of Water (cm)	61.6	(23.24) [5]	66.0	(30.90) [5]	73.0	(25.15) [5]	66.9	(25.13) [15]
Poaching Near Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Poaching Far Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Gradient Near Bank	1.0	(0.00) [5]	1.0	(0.00) [5]	1.0	(0.00) [5]	1.0	(0.00) [15]
Gradient Far Bank	1.0	(0.00) [5]	1.0	(0.00) [5]	1.0	(0.00) [5]	1.0	(0.00) [15]
Summed Gradients	2.0		2.0		2.0		2.0	(0.00) [3]
Shade by Trees & Shrubs (%)	84.0	(19.17) [5]	94.0	(2.24) [5]	92.0	(4.47) [5]	90.0	(11.50) [15]
Glyceria Cover (%)	2.0	(2.74) [5]	1.0	(2.24) [5]	22.0	(10.37) [5]	8.3	(11.60) [15]
Duckweed Cover (%)	100.0	(0.00) [5]	89.0	(13.42) [5]	100.0	(0.00) [5]	96.3	(8.96) [15]
Oxygen (mg/l)	0.7	(0.14) [2]	1.8	(1.49) [2]	3.7	(4.53) [2]	2.1	(2.53) [6]
Temperature (ºC)	14.7	(0.42) [2]	15.5	(0.57) [2]	14.9	(0.28) [2]	15.0	(0.50) [6]
Pressure (mB)	1015.0	(0.00) [2]	1015.0	(0.00) [2]	1015.0	(0.00) [2]	1015.0	(0.00) [6]
Conductivity (µS)	258.0	(5.66) [2]	287.5	(9.19) [2]	277.0	(5.66) [2]	274.2	(14.44) [6]
Temperature (ºC)	15.3	(0.28) [2]	16.0	(0.07) [2]	15.2	(0.28) [2]	15.5	(0.41) [6]
pH	7.2	(0.14) [2]	7.5	(0.00) [2]	7.6	(0.21) [2]	7.4	(0.20) [6]
Temperature (ºC)	14.8	(0.57) [2]	15.8	(0.14) [2]	14.9	(0.35) [2]	15.2	(0.59) [6]

Table F3c: Physicochemical data from Westhay Moor 30/8/08. SD = Standard Deviation, n = number of observations

	Ditch WMa		Ditch WMb		Ditch WMc		All Ditches	
	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]
Width of Waterbody (m)	2.8	(0.27) [5]	3.0	(0.71) [5]	1.6	(0.22) [5]	2.5	(0.77) [15]
Depth of Water (cm)	36.0	(11.40) [5]	48.0	(8.37) [5]	30.0	(7.91) [5]	38.0	(11.62) [15]
Poaching Near Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Poaching Far Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Gradient Near Bank	3.4	(0.89) [5]	3.4	(0.89) [5]	3.6	(0.55) [5]	3.5	(0.74) [15]
Gradient Far Bank	2.4	(0.89) [5]	2.8	(1.30) [5]	4.0	(0.00) [5]	3.1	(1.10) [15]
Sum of Average Gradients	5.8		6.2		7.6		6.5	(0.95) [3]
Shade by Trees & Shrubs (%)	57.0	(26.83) [5]	38.0	(31.74) [5]	56.0	(39.59) [5]	50.3	(31.99) [15]
Glyceria Cover (%)	10.0	(5.00) [5]	6.2	(6.30) [5]	4.0	(2.24) [5]	6.7	(5.15) [15]
Duckweed Cover (%)	100.0	(0.00) [5]	99.2	(1.10) [5]	100.0	(0.00) [5]	99.7	(0.70) [15]
Oxygen (mg/l)	0.6	(0.71) [2]	0.7	(0.07) [2]	2.4	(0.42) [2]	1.2	(0.99) [6]
Temperature (ºC)	18.0	(0.99) [2]	17.7	(0.85) [2]	18.2	(0.14) [2]	18.0	(0.63) [6]
Pressure (mB)	1008.0	(0.00) [2]	1008.0	(0.00) [2]	1008.0	(0.00) [2]	1008.0	(0.00) [6]
Conductivity (µS)	273.0	(35.36) [2]	202.5	(3.54) [2]	196.0	(2.83) [2]	223.8	(41.39) [6]
Temperature (ºC)	20.0	(0.07) [2]	19.9	(0.07) [2]	19.6	(0.35) [2]	19.8	(0.25) [6]
pH	7.2	(0.14) [2]	6.8	(0.07) [2]	6.6	(0.07) [2]	6.8	(0.31) [6]
Temperature (ºC)	19.7	(0.21) [2]	19.6	(0.14) [2]	19.3	(0.28) [2]	19.5	(0.24) [6]

Table F3d: Physicochemical data from East Waste 30/8/08. SD = Standard Deviation, n = number of observations

	Ditch EWa		Ditch EWb		Ditch EWc		All Ditches	
	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]
Width of Waterbody (m)	2.9	(0.22) [5]	2.7	(0.27) [5]	3.5	(0.00) [5]	3.0	(0.40) [15]
Depth of Water (cm)	87.0	(9.08) [5]	47.0	(34.57) [5]	76.0	(2.24) [5]	70.0	(25.91) [15]
Poaching Near Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Poaching Far Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Gradient Near Bank	1.6	(0.55) [5]	1.8	(0.45) [5]	4.0	(0.00) [5]	2.5	(1.19) [15]
Gradient Far Bank	3.2	(0.45) [5]	2.4	(0.89) [5]	4.0	(0.00) [5]	3.2	(0.86) [15]
Summed Gradients	4.8		4.2		8.0		5.7	(2.04) [3]
Shade by Trees & Shrubs (%)	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Glyceria Cover (%)	10.0	(8.66) [5]	65.0	(30.82) [5]	7.0	(2.74) [5]	27.3	(32.51) [15]
Duckweed Cover (%)	100.0	(0.00) [5]	60.0	(54.77) [5]	100.0	(0.00) [5]	86.7	(35.19) [15]
Oxygen (mg/l)	2.2	(2.05) [2]	0.4	(0.00) [2]	0.6	(0.28) [2]	1.3	(1.56) [6]
Temperature (ºC)	15.6	(0.64) [2]	16.1	(0.14) [2]	17.4	(0.35) [2]	16.1	(0.82) [6]
Pressure (mB)	1010.0	(0.00) [2]	1009.5	(0.71) [2]	1008.5	(0.71) [2]	1009.8	(0.50) [6]
Conductivity (µS)	442.0	(45.26) [2]	454.5	(6.36) [2]	441.5	(0.71) [2]	443.8	(26.46) [6]
Temperature (ºC)	19.5	(0.64) [2]	21.2	(0.92) [2]	22.4	(0.14) [2]	20.5	(1.47) [6]
pH	7.5	(0.00) [2]	7.2	(0.14) [2]	7.6	(0.00) [2]	7.4	(0.22) [6]
Temperature (ºC)	19.1	(0.07) [2]	21.3	(1.27) [2]	21.3	(0.14) [2]	19.9	(1.06) [6]

Table F3e: Physicochemical data from Tealham Moor 31/8/08. SD = Standard Deviation, n = number of observations

	Ditch TMa		Ditch TMb		Ditch TMc		All Ditches	
	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]
Width of Waterbody (m)	3.0	(0.00) [5]	2.5	(0.00) [5]	2.5	(0.00) [5]	2.7	(0.24) [15]
Depth of Water (cm)	68.0	(5.70) [5]	10.0	(0.00) [5]	52.0	(4.47) [5]	43.3	(25.61) [15]
Poaching Near Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Poaching Far Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Gradient Near Bank	4.0	(0.00) [5]	3.4	(0.55) [5]	3.6	(0.55) [5]	3.7	(0.49) [15]
Gradient Far Bank	4.0	(0.00) [5]	4.0	(0.00) [5]	3.8	(0.45) [5]	3.9	(0.26) [15]
Summed Gradients	8.0		7.4		7.4		7.6	(0.35) [3]
Shade by Trees & Shrubs (%)	0.0	((0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Glyceria Cover (%)	63.0	(29.92) [5]	0.0	(0.00) [5]	4.0	(5.48) [5]	22.3	(33.96) [15]
Duckweed Cover (%)	63.0	(36.67) [5]	78.6	(30.98) [5]	100.0	(0.00) [5]	80.5	(30.08) [15]
Oxygen (mg/l)	4.2	(2.62) [2]	3.3	(2.05) [2]	4.0	(3.39) [2]	3.8	(2.17) [6]
Temperature (ºC)	17.2	(1.20) [2]	17.7	(0.07) [2]	16.4	(1.27) [2]	17.1	(0.96) [6]
Pressure (mB)	1007.0	(0.00) [2]	1007.0	(0.00) [2]	1007.0	(0.00) [2]	1007.0	(0.00) [6]
Conductivity (µS)	881.5	(9.19) [2]	713.5	(30.41) [2]	681.5	(24.75) [2]	758.8	(97.76) [6]
Temperature (ºC)	17.7	(0.71) [2]	17.7	(0.14) [2]	17.5	(0.21) [2]	17.6	(0.36) [6]
pH	8.2	(0.71) [2]	8.1	(0.14) [2]	8.0	(0.21) [2]	8.1	(0.15) [6]
Temperature (ºC)	17.8	(0.64) [2]	17.8	(0.07) [2]	17.4	(0.28) [2]	17.6	(0.36) [6]

Table F3f: Physicochemical data from Shapwick Heath 31/8/08. SD = Standard Deviation, n = number of observations

	Ditch SHa		Ditch SHb		Ditch SHc		All Ditches	
	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]	Average	(SD) [n]
Width of Waterbody (m)	2.7	(0.27) [5]	1.9	(0.22) [5]	1.6	(0.22) [5]	2.1	(0.54) [15]
Depth of Water (cm)	43.0	(0.27) [5]	33.0	(12.04) [5]	42.0	(10.96) [5]	39.3	(9.98) [15]
Poaching Near Bank	1.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.3	(0.49) [15]
Poaching Far Bank	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [5]	0.0	(0.00) [15]
Gradient Near Bank	2.8	(0.45) [5]	3.4	(0.55) [5]	3.0	(0.00) [5]	3.1	(0.46) [15]
Gradient Far Bank	4.0	(0.00) [5]	2.8	(0.84) [5]	3.0	(0.00) [5]	3.3	(0.70) [15]
Sum of Average Gradients	6.8		6.2		6.0		6.3	(0.42) [3]
Shade by Trees & Shrubs (%)	89.0	(8.22) [5]	88.6	(8.36) [5]	75.0	(22.91) [5]	84.2	(15.32) [15]
Glyceria Cover (%)	2.0	(2.74) [5]	15.0	(20.62) [5]	3.0	(4.47) [5]	6.7	(12.91) [15]
Duckweed Cover (%)	59.0	(40.22) [5]	89.0	(17.47) [5]	80.6	(26.21) [5]	76.2	(30.28) [15]
Oxygen (mg/l)	5.4	(4.53) [2]	4.8	(4.17) [2]	0.7	(N/A) [1]	4.2	(3.66) [5]
Temperature (ºC)	16.6	(0.35) [2]	16.6	(0.35) [2]	16.6	(N/A) [1]	16.6	(0.25) [5]
Pressure (mB)	1007.0	(0.00) [2]	1007.0	(0.00) [2]	1007.0	(N/A) [1]	1007.0	(0.00) [5]
Conductivity (µS)	563.0	(113.14) [2]	433.5	(57.28) [2]	387.0	(0.00) [2]	461.2	(99.35) [6]
Temperature (ºC)	16.4	(0.14) [2]	16.7	(0.21) [2]	17.2	(0.35) [2]	16.7	(0.39) [6]
pH	7.8	(0.28) [2]	7.4	(0.21) [2]	7.5	(0.00) [2]	7.6	(0.26) [6]
Temperature (ºC)	16.4	(0.14) [2]	16.6	(0.28) [2]	16.9	(0.07) [2]	16.6	(0.25) [6]

Appendix G1: Predator/prey experiments using aquaria

Figure G1a: Configuration of aquaria 10 October 2010 to 17 October 2010

1	2 t	3	4 t	5	6	7	8 t	9	10	11 t
12	13	14	15	16	17	18	19	20	21	22

Potential prey + No Beetle

Potential prey + *Dytiscus marginalis* adult

Potential Prey + *Hydrophilus piceus* adult

Not set up

t = Aquarium with maximum and minimum thermometer

Potential prey at start (numbers in each aquarium): *Notonecta glauca* (2); *Ilyocoris cimicoides* (2); *Lymnaea stagnalis* (3); Planorbid snail (1); *Sphaerium* sp. (4).

Figure G1b: Configuration of aquaria 17 October 2010 to 23 October 2010

1	2 t	3	4 t	5	6	7	8 t	9	10	11 t
12 t	13	14	15	16	17	18 t	19	20	21	22 t

Potential prey + No Beetle

Potential prey + *Dytiscus marginalis* adult

Potential Prey + *Hydrophilus piceus* adult

Not set up

t = Aquarium with maximum and minimum thermometer

Potential prey at start (numbers in each aquarium): *Notonecta glauca* (2); *Ilyocoris cimicoides* (2); *Lymnaea stagnalis* (6) Planorbid snail (2) *Sphaerium* sp. (8).

Figure G1c: Configuration of aquaria 17 April 2011 to 1 May 2011

1	2 t	3	4	5	6	7	8 t	9	10	11
12	13	14	15	16	17	18	19	20	21	22

Potential prey + No Beetle

Potential prey + *Dytiscus marginalis* adult

Potential prey + *Dytiscus marginalis* larva

Potential Prey + *Dytiscus sulcatus* adult

Not set up

t = Aquarium with maximum and minimum thermometer

Potential prey at start (numbers in each aquarium): *Lymnaea stagnalis* (6); Planorbid snail (2); *Bithynia* sp. (2); *Sphaerium* sp. (1), *Asellus* sp. (7).

Figure G1d: Configuration of aquaria 1 May 2011 to 15 May 2011

1 t	2	3	4	5	6 t	7	8	9	10	11
12 t	13	14	15	16	17	18	19	20	21	22 t

Potential prey + No Beetle

Potential prey + *Dytiscus marginalis* adult

Potential prey + *Dytiscus dimidiatus* adult

Not set up

t = Aquarium with maximum and minimum thermometer

Potential prey at start (numbers in each aquarium): *Lymnaea stagnalis* (6); Planorbid snail (2); *Bithynia* sp. (2); *Sphaerium* sp. (1).

Figure G1e: Configuration of aquaria 5 June 2011 to 19 June 2011

1	2	3	4	5	6	7	8	9	10	11
12	13	14	15	16	17 t	18	19	20	21	22 t

Potential prey + No Beetle

Potential prey + *Dytiscus marginalis* larva

Not set up

t = Aquarium with maximum and minimum thermometer

Potential prey at start (numbers in each aquarium): *Lymnaea stagnalis* (8); Planorbid snail (2); *Bithynia* sp. (1).

Appendix G2: RTUs netted during May 2008

Table G2a: Recognisable Taxonomic Units (RTUs) recorded at Shapwick Heath during 15 minute timed netting and sorting at each of three ditches on 5 May 2008

Category	**RTU**	SH a	SH b	SH c	SH Total
Dytiscus	*Dytiscus marginalis*	1			1
	Dytiscus dimidiatus				
	Dytiscus larva	1			1
Other beetles (Coleoptera)	*Acilius sulcatus*		1		1
	Agabus sp.		1		1
	Agabus sturmii		1		1
	Hydaticus seminiger		1		1
	Hydaticus transversalis				
	Hydrochara caraboides				
	Hydrobius fuscipes	1	1		2
	Hydrophilus piceus				
	Ilybius ater		1		1
	Unidentified larva				
Water Bugs (Hemiptera)	*Notonecta* sp				
	Velia sp				
	Ilyocoris cimicoides				
	Hydrometra sp.				
Other insects	Chironomid larva		1		1
	Anisopteran larva				
	Zygopteran larva				
	Cased Caddisfly larva				
	Dipteran Larva				
	Mayfly Larva				
Other Arthropods	*Argyroneta aquatica*				
	Asellus sp	C	C	D	✓
	Gammarid shrimp	C	C	C	✓
Molluscs	Planorbid Snail	1			1
	Lymnaeid Snail				
	Bithynia sp				
	Valvata macrostoma				
	Physa sp				
	Pea Mussel				
	Succineid snail				
Other macro-invertebrates	Oligochaete worm	B		B	✓
	Leech				
	Flatworm				
Vertebrates	Stickleback				
	Bufo bufo				
	Trituris vulgaris				
RTU Count (Total RTUs less *Dytiscus* & vertebrates)		5	9	3	13 Taxa

Where numbers estimated: B = > 10 < 100; C = > 100 < 1000; D = > 1000

Table G2b: Recognisable Taxonomic Units (RTUs) recorded at Westhay Moor during 15 minute timed netting and sorting at each of three ditches on 7 May 2008

Category	**RTU**	WM a	WM b	WM c	WM Total
Dytiscus	*Dytiscus marginalis*				
	Dytiscus dimidiatus				
	Dytiscus larva				
Other beetles (Coleoptera)	*Acilius sulcatus*			1	1
	Agabus sp.				
	Agabus sturmii				
	Hydaticus seminiger				
	Hydaticus transversalis				
	Hydrochara caraboides				
	Hydrobius fuscipes		3		3
	Hydrophilus piceus				
	Ilybius ater				
	Unidentified larva		1		1
Water Bugs (Hemiptera)	*Notonecta* sp		2		2
	Velia sp		2		2
	Ilyocoris cimicoides				
	Hydrometra sp.				
Other insects	Chironomid larva				
	Anisopteran larva		1		1
	Zygopteran larva				
	Cased Caddisfly larva				
	Dipteran Larva				
	Mayfly Larva				
Other Arthropods	*Argyroneta aquatica*				
	Asellus sp	C	C	C	✓
	Gammarid shrimp	C	C	C	✓
Molluscs	Planorbid Snail				
	Lymnaeid Snail				
	Bithynia sp				
	Valvata macrostoma				
	Physa sp				
	Pea Mussel	1		1	1
	Succineid snail				
Other macro-invertebrates	Oligochaete worm				
	Leech				
	Flatworm		2		2
Vertebrates	Stickleback		3		3
	Bufo bufo				
	Trituris vulgaris				
RTU Count (Total RTUs less *Dytiscus* & vertebrates)		3	8	4	10 Taxa

Where numbers estimated: B = > 10 < 100; C = > 100 < 1000; D = > 1000

Table G2c: Recognisable Taxonomic Units (RTUs) recorded at Westhay Heath during 15 minute timed netting and sorting at each of three ditches on 9 May 2008

Category	**RTU**	WH a	WH b	WH c	WH Total
Dytiscus	*Dytiscus marginalis*				
	Dytiscus dimidiatus				
	Dytiscus larva	1	1		2
Other beetles (Coleoptera)	*Acilius sulcatus*		1		1
	Agabus sp.				
	Agabus sturmii	3	4	5	12
	Hydaticus seminiger		1		
	Hydaticus transversalis	1	3	1	5
	Hydrochara caraboides				
	Hydrobius fuscipes	1			1
	Hydrophilus piceus				
	Ilybius ater				
	Unidentified larva				
Water Bugs (Hemiptera)	*Notonecta* sp		2		2
	Velia sp	C		B	✓
	Ilyocoris cimicoides		1		1
	Hydrometra sp.		3	1	4
Other insects	Chironomid larva				
	Anisopteran larva				
	Zygopteran larva				
	Cased Caddisfly larva		4	1	5
	Dipteran Larva			1	1
	Mayfly Larva				
Other Arthropods	*Argyroneta aquatica*				
	Asellus sp	C	C	C	✓
	Gammarid shrimp	C	B	C	✓
Molluscs	Planorbid Snail	6	7	2	15
	Lymnaeid Snail				
	Bithynia sp				
	Valvata macrostoma				
	Physa sp				
	Pea Mussel	16	5	11	32
	Succineid snail			1	1
Other macro-invertebrates	Oligochaete worm	1			1
	Leech		4	2	6
	Flatworm	1			1
Vertebrates	Stickleback		1	2	3
	Bufo bufo				
	Trituris vulgaris				
RTU Count (Total RTUs less *Dytiscus* & vertebrates)		10	13	12	20 Taxa

Where numbers estimated: B = > 10 < 100; C = > 100 < 1000; D = > 1000

Table G2d: Recognisable Taxonomic Units (RTUs) recorded at Catcott North during 15 minute timed netting and sorting at each of three ditches on 11 May 2008

Category	**RTU**	CN a	CN b	CN c	CN Total
Dytiscus	*Dytiscus marginalis*				
	Dytiscus dimidiatus				
	Dytiscus larva				
Other beetles (Coleoptera)	*Acilius sulcatus*				
	Agabus sp.				
	Agabus sturmii				
	Hydaticus seminiger				
	Hydaticus transversalis				
	Hydrochara caraboides				
	Hydrobius fuscipes	1			1
	Hydrophilus piceus				
	Ilybius ater				
	Unidentified larva	2			2
Water Bugs (Hemiptera)	*Notonecta* sp				
	Velia sp				
	Ilyocoris cimicoides				
	Hydrometra sp.				
Other insects	Chironomid larva				
	Anisopteran larva				
	Zygopteran larva				
	Cased Caddisfly larva				
	Dipteran Larva				
	Mayfly Larva				
Other Arthropods	*Argyroneta aquatica*				
	Asellus sp	C	C	C	✓
	Gammarid shrimp	C	C	C	✓
Molluscs	Planorbid Snail	2	1		3
	Lymnaeid Snail				
	Bithynia sp				
	Valvata macrostoma				
	Physa sp				
	Pea Mussel	2			2
	Succineid snail	1	1		2
Other macro-invertebrates	Oligochaete worm				
	Leech		1		1
	Flatworm				
Vertebrates	Stickleback				
	Bufo bufo				
	Trituris vulgaris				
RTU Count (Total RTUs less *Dytiscus* & vertebrates)		7	5	2	8 Taxa

Where numbers estimated: B = > 10 < 100; C = > 100 < 1000; D = > 1000

Table G2e: Recognisable Taxonomic Units (RTUs) recorded at Tadham Moor during 15 minute timed netting and sorting at each of three ditches on 10 May 2008

Category	**RTU**	TM a	TM b	TM c	TM Total
Dytiscus	*Dytiscus marginalis*				
	Dytiscus dimidiatus				
	Dytiscus larva				
Other beetles (Coleoptera)	*Acilius sulcatus*				
	Agabus sp.				
	Agabus sturmii			1	1
	Hydaticus seminiger				
	Hydaticus transversalis				
	Hydrochara caraboides				
	Hydrobius fuscipes			1	1
	Hydrophilus piceus				
	Ilybius ater				
	Unidentified larva	1			1
Water Bugs (Hemiptera)	*Notonecta* sp	1	3		4
	Velia sp				
	Ilyocoris cimicoides	2			2
	Hydrometra sp.				
Other insects	Chironomid larva	B			✓
	Anisopteran larva				
	Zygopteran larva				
	Cased Caddisfly larva	1			1
	Dipteran Larva				
	Mayfly Larva				
Other Arthropods	*Argyroneta aquatica*				
	Asellus sp	C	C	C	✓
	Gammarid shrimp	B			✓
Molluscs	Planorbid Snail	1		1	2
	Lymnaeid Snail	1		1	2
	Bithynia sp	1	1	3	5
	Valvata macrostoma		6		6
	Physa sp				
	Pea Mussel		1	3	4
	Succineid snail		1		1
Other macro-invertebrates	Oligochaete worm				
	Leech	3	3	8	14
	Flatworm				
Vertebrates	Stickleback		1		1
	Bufo bufo				
	Trituris vulgaris				
RTU Count (Total RTUs less *Dytiscus* & vertebrates)		11	7	8	16 Taxa

Where numbers estimated: B = > 10 < 100; C = > 100 < 1000; D = > 1000

Table G2f: Recognisable Taxonomic Units (RTUs) recorded at East Waste during 15 minute timed netting and sorting at each of three ditches on 11 May 2008

Category	**RTU**	EW a	EW b	EW c	EW Total
Dytiscus	*Dytiscus marginalis*				
	Dytiscus dimidiatus				
	Dytiscus larva				
Other beetles (Coleoptera)	*Acilius sulcatus*	1	1		2
	Agabus sp.				
	Agabus sturmii	1			1
	Hydaticus seminiger				
	Hydaticus transversalis				
	Hydrochara caraboides		2		2
	Hydrobius fuscipes	1			1
	Hydrophilus piceus			1	1
	Ilybius ater				
	Unidentified larva		3		3
Water Bugs (Hemiptera)	*Notonecta* sp	2		1	3
	Velia sp				
	Ilyocoris cimicoides	3		1	4
	Hydrometra sp.				
Other insects	Chironomid larva			1	1
	Anisopteran larva	1			1
	Zygopteran larva	1	1	2	4
	Cased Caddisfly larva	4	4		8
	Dipteran Larva				
	Mayfly Larva				1
Other Arthropods	*Argyroneta aquatica*				1
	Asellus sp	B	B		✓
	Gammarid shrimp	B	B		✓
Molluscs	Planorbid Snail	C	C	C	✓
	Lymnaeid Snail	C	C	C	✓
	Bithynia sp	2	1	1	4
	Valvata macrostoma				
	Physa sp			1	1
	Pea Mussel	1	5		6
	Succineid snail				
Other macro-invertebrates	Oligochaete worm				
	Leech	5	2	7	14
	Flatworm				
Vertebrates	Stickleback	2	4	1	7
	Bufo bufo	2			2
	Trituris vulgaris			1	1
RTU Count (Total RTUs less *Dytiscus* & vertebrates)		15	12	10	22 Taxa

Where numbers estimated: B = > 10 < 100; C = > 100 < 1000; D = > 1000

Appendix G3: Records of traps with evidence of predation of *Dytiscus* larvae

KEY TO ABBREVIATIONS

DD = *Dytiscus dimidiatus* adult
DM = *Dytiscus marginalis* adult
Dyt = *Dytiscus* larva(e)

Ab = *Agabus bipustulatus* (Linnaeus, 1767)
As = *Agabus sturmii* (Gyllenhal, 1808)
Hs = *Hydaticus seminiger* (Degeer, 1774)
Ht = *Hydaticus transversalis* (Pontoppidan, 1763)
Ia = *Ilybius ater* (Degeer, 1774)

Table G3: Records of Traps with evidence of predation of *Dytiscus* larvae.

Site Caught	Trap No	Date Caught	Damaged			Other Dytiscus Records	Other Records
Site Code	Number	Date	Category 2 Injured	Category 3 Exoskeleton	Category 4 Fragments		
SH	10b	05/06/2006	1			1 DM fem	1 Horse Leech
SH	19	22/04/2007			1	1 DM male, 1 DM fem, 2 DD male,1 Dyt larva	1 Horse Leech
SH	10	05/05/2007			1	3 DM male, 1 DM fem, 1 DD male	
SH	12	05/05/2007			1	None	
SH	15	05/05/2007			1	3 Dm male, 3 DM fem, 3 DD male	1 *Hydaticus transversalis*
SH	1	20/05/2007		2		2 DM males, 3 Dyt larvae	
SH	8	20/05/2007		1		3 Dyt larvae	1 *Hydrochara caraboides*
SH	10	20/05/2007		1		2 Dyt larvae	
SH	11	20/05/2007	2	2		1 DM male, 1 DM fem, 3 Dyt larvae	
SH	1	03/06/2007		1		1 DM male, 1 Dyt larva	
SH	8	03/06/2007		1		6 Dyt larvae	
SH	11	03/06/2007	2			3 Dyt larvae	
SH	14	03/06/2007		1		1 DM fem, 4 Dyt larvae	
SH	1	17/06/2007			1	1 DD male, 1 Dyt larva	1 *Acilus sulcatus* male
SH	9	17/06/2007		1		1 DM Fem, 1DD male, 2 Dyt larvae	
SH	10	17/06/2007	1			4 Dyt larvae	
SH	11	17/06/2007			2	8 Dyt larvae	1 Dipteran larva
SH	17	17/06/2007			2	3 DM male, 2 DM fem, 3 Dyt larvae	1 Horse Leech
SH	2	02/07/2007	1			1 DM male, 1 DM fem, 2 Dyt larvae	
SH	5	02/07/2007		1		1 DM male, 1 Dyt larva	1 *Ilybius ater*
SH	12	02/07/2007			1	1 DM Fem	1 *Ilybius* sp., 1 Stickleback fry
SH	11	16/07/2007			1	1 DD fem	

Table G3: Records of Traps with evidence of predation of *Dytiscus* larvae (Continued)**.**

Site Caught	**Trap No**	**Date Caught**	**Damaged**			**Other Dytiscus Records**	**Other Records**
Site Code	Number	Date	Category 2 Injured	Category 3 Exoskeleton	Category 4 Fragments		
WM	11	08/05/2008			1	1 Dyt larva	1 *Agabus sturmii*
WM	15	08/05/2008			1	None	1 Newt male, 2 As, 1 Hs
WH	10	10/05/2008			1	1 DM male	1 Ht inside dead Stickleback, 3 Hs
SH	10	08/06/2008	1			2 DM fem, 1 Dyt larva	3 Ia, 1 Ab, 3 As, 1Hs, 1Hc
TM	11	08/06/2008		1		None	5 Sticklebacks
WM	15	15/06/2008			1	1 DD fem	
WH	10	29/06/2008		1		1 Dyt larva	1 *Hydaticus transversalis*
SH	1	13/07/2008		1		3 Dyt larvae	1 *Agabus sturmii*
SH	4	13/07/2008			1	1 Dyt larva	1 *Ilybius ater*
SH	12	13/07/2008		1		1 DM male, 3 Dyt larva	4 *Ilybius ater,* 1 *Agabus sturmii*
WM	5	23/07/2008		1		1 Dyt larva	1 *Hydaticus transversalis*
WM	14	23/07/2008	1			1 Dyt larva	
WM	15	23/07/2008			1	None	1 *Hydrochara caraboides*

Appendix G4: Photographs of aquaria at the Peat Moors Centre, Shapwick, 2010-11 (Photos taken December 2010)

G4a
Left: The Peat Moors Centre, Shapwick

G4b
Left: The aquaria in the right hand row are set up as they would be in use with transparent plastic

G4c
Left: The table is set up for sorting samples. Material can be emptied onto the plastic sheet on the floor for checking through.