# Insect Migration in a Changing Climate

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# Abstract

This thesis studies the responses of migratory insects to climate warming in terms of changes in their distribution and abundance using data from a number of recording schemes, while examining different aspects of their migratory behaviour, namely directionality and spring arrival patterns in the UK.

Migrants from three taxonomic groups have shown increases in their distribution extent over the past 40 years, coupled with northward shifts at their range margins at comparable rates to shifting isotherms. These responses by migrants were generally greater than those of generalist and specialist resident species.

Migrant butterflies have shown increases in their abundance as a result of both increased immigration and increased reproductive success of migrants in Britain, resulting in larger summer populations, with the later of these increases correlated with British summer temperatures in *Vanessa atalanta.* The analyses suggest that butterflies are not restricted to their flight boundary layer, with no evidence indicating that coastal sites are colonised before inland sites.

Hydrogen isotopes appear to be a reliable tool for distinguishing between individuals from different locations in Europe. Comparisons between wing and abdomen tissue provided some evidence of the migratory status of an individual, although this technique requires further study to increase its reliability.

Migratory insects appear to be responding positively to climate warming, increasing their distribution and abundance, efficiently ‘tracking’ shifting isotherms northwards. These responses have significant implications not only on species assemblages, but on human welfare, with a vast proportion of migrants being agricultural pests. Further understanding of the driving forces behind these changes are needed in order to predict the full impact of increased migration.

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# Declaration

Some of the material in this thesis was produced in collaboration with other workers:

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# Chapter 1

# General Introduction

## 1.1 Thesis Introduction:

Since the end of the 19th century, the global mean surface temperature has increased by 0.6°C, with 0.2-0.3°C of this increase occurring since the 1960s (IPCC, 2001; 2007). Globally, 1998 was the hottest year ever recorded with seven of the 10 hottest years recorded occurring during the 1990s, with temperatures exceeding the 1961-1990 mean by 0.546°C (Jones, 2008; www.cru.uea.ac.uk). Not only are temperatures increasing, but there is mounting evidence showing long term increases in winter rainfall intensity (Maraun *et al.*, 2008). Temperatures are predicted to continue to rise by another 1.4 to 5.8°C by 2100 (UNFCCC, 2005), and an increase within this range, especially above a 2° change, is likely to result in significant ecological changes (Root & Schneider, 2002). Figure 1.1 shows how the Central England Temperature (CET) has undergone a sharp increase the late 1980s. The CET represents roughly a triangular area in England with the three apexes occurring at Bristol, London and Lancashire (Met Office, 2004).

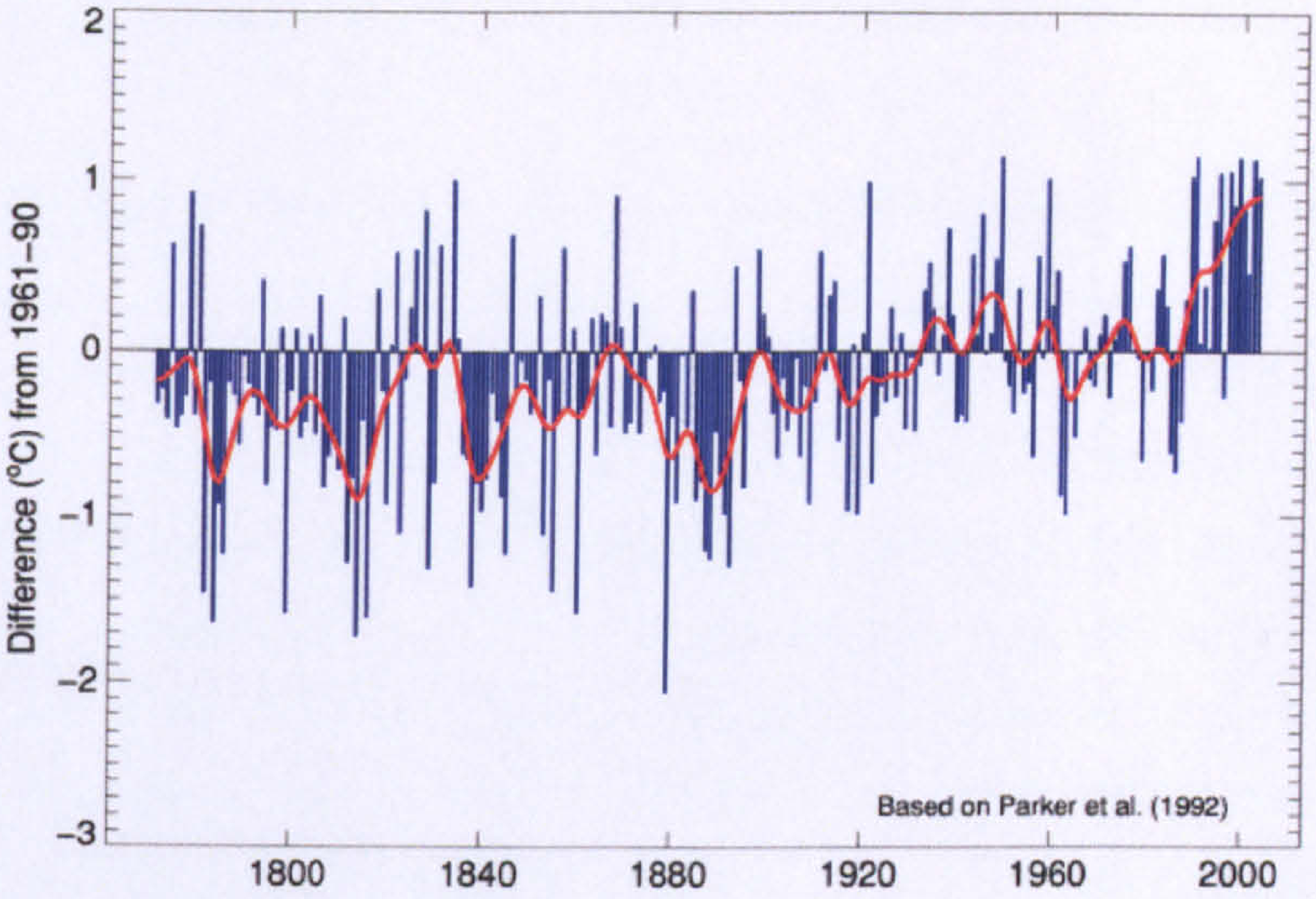


Figure 1.1: Central England Temperature, annual anomalies, 1772-2003 (Met Office, 2004).

The effects of fluctuations in the climate on biological systems have been widely recognised over geological time scales, for example a 'runaway greenhouse' effect, as described by Benton & Twitchett (2003) has been named responsible for the mass extinction (up to 95% of all species lost) at the end of the Permian. In an analysis of the fossil record over the last 520 million years, Mayhew *et al.* (2008) found that global climate has historically been correlated with biodiversity and origination rates, as well as with extinction events. The impacts of recent climate warming have already been observed in a variety of species and ecosystems, but as yet it is unclear what biological consequences will occur with future warming events. To get a full understanding of the consequences of 21st century warming it is important to continually monitor biological systems and to evaluate any changes that may be occurring.

This thesis will focus on how migratory insects, with the emphasis on Lepidoptera species, have responded to climate warming over the past 40 years in terms of changes in their distribution and abundance. It also explores methods for determining a greater understanding of migratory behaviour which will enable better predictions to be made as to how migratory species are likely to respond to continued warming in the future.

## 1.2 Causes of climate change:

There are a number of trace gases, carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$) and ozone ($O_3$), which are collectively known as greenhouse gases, that absorb and emit infrared radiation (IPCC, 2001). The atmosphere is made up of about 1% of these greenhouse gases, which act as a blanket, making the earth about 30°C warmer than it otherwise would be (UNFCCC, 2005). Throughout history, the climate has varied naturally, as components of the system are never in equilibrium, thus constantly changing; an example of this is the El Nino Southern Oscillation (ENSO) which is the interaction between the atmosphere and the tropical Pacific Ocean (IPCC 2001). The climate also varies due to external forces acting at different time-points, for example solar radiation or by volcanoes emitting large quantities of aerosols (IPCC, 2001; Root & Schneider, 2002). However since the industrial revolution there has been a steady increase in $CO_2$ emissions (>30% increase) from the burning of the fossil fuels (coal, oil and natural gas), with increases in farming activity and changes in land use leading to increased concentrations in $CH_4$ and $N_2O$ (IPCC 2001; UNFCC, 2005). The rapid climate warming since the 1970s has been attributed to these increased concentrations of the greenhouse gases caused by human activities (Dennis & Shreeve, 1991).

Anthropogenic causes being responsible for climate warming has been questioned, with suggestions that factors such as increased solar irradiance or reduced volcanic action are behind observed changes in climate (Crowley, 2000). However, the rate of warming over the 20th Century greatly exceeds that seen over the past 1000 years, and secondly a climate model successfully fitted to Northern hemisphere temperatures between 1000-1850 was only able to attribute 25% of recent warming to natural variability with the majority of warming being consistent with increases in greenhouse gases (Crowley, 2000).

Fig 1.2 shows the output from models of 20th century warming based on a) natural forcings, encompassing solar variation and volcanic activity, b) anthropogenic forcings, including greenhouse gases and sulphate aerosols, whilst c) combines both forcings. (IPCC, 2001). From the model outputs the consensus is that early 20th century warming was a result of natural forcings, however more recent warming (post 1970) is primarily human induced.

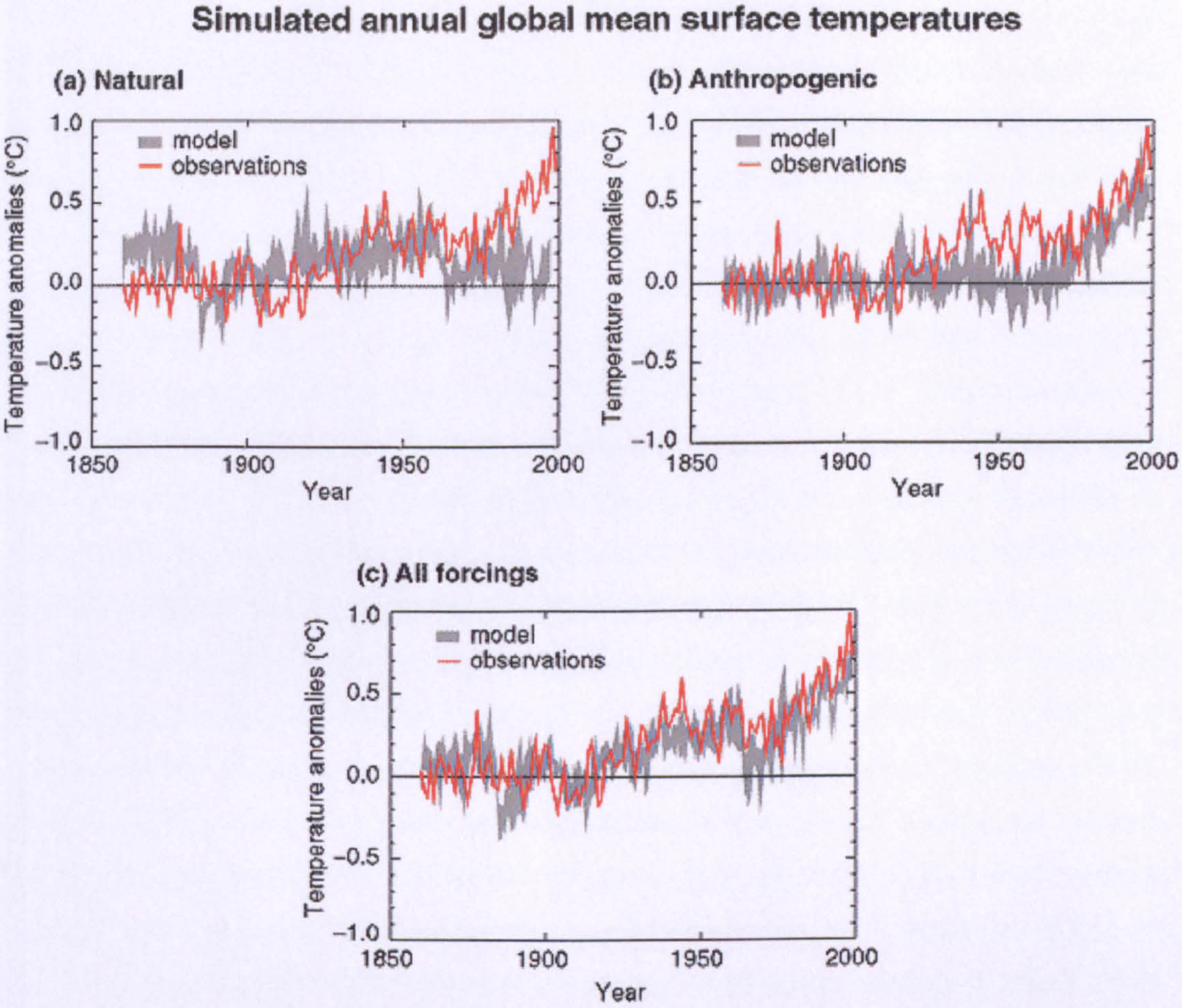


Figure 1.2: A climate model is used to simulate the Earth's temperature variations that results from both natural and anthropogenic causes. Simulations in (a) were done with natural forcings: solar variation and volcanic activity, (b) were done with anthropogenic forcings: greenhouse gases and an estimate of sulphate aerosols, while (c) were done with both natural and anthropogenic forcings (IPCC, 2001).

## 1.3 Effects of Climate Change:

### 1.3.1 **Effects on Physiology:**

Changes in temperature, precipitation and $CO_2$ concentrations in the atmosphere are likely to have a considerable impact on animal and plant physiology, for example metabolic and developmental rates (Hughes, 2000; Bale *et al.*, 2002; Walther *et al.*, 2002; Parmesan, 2006). Increased temperatures are predicted to initiate changes in development time, voltinism, population density, genetic composition and the extent of host plant exploitation (Hughes, 2000, Bale *et al.*, 2002). The pros and cons of increased temperatures have been examined for butterflies, for which many species reach their range limits in Britain. Higher ambient temperatures may be beneficial for many species because they will decrease the time required to increase body temperature to enable flight dependent activities, for example, mate-location, egg-laying, predator evasion, dispersal and migration (Dennis & Shreeve, 1991). However excessively high temperatures may cause heat stress at all developmental stages, in particular to early life cycle stages where larval movement is greatly restricted compared to adults which can fly to a cooler micro-habitat. High temperatures can lead to an associated decrease in survival and fecundity, especially in northern and montane species (Dennis & Shreeve, 1991). Elevated $CO_2$ concentrations are predicted to affect not only plants but also animals through herbivore-plant interactions. For example, a meta-analysis by Curtis & Wang (1998) found that increased $CO_2$ levels caused increases in net $CO_2$ assimilated and leaf starch content, with decreases in leaf dark respiration and leaf nitrogen content. The effects of these climate-mediated changes in host-plants on different herbivore guilds vary considerably. While external feeders, for example leaf chewers, phloem feeders and whole cell eaters, have responded to decreased nitrogen levels in plant material by increasing their consumption rate, leaf miners are failing to follow suit with negative effects being observed on pupal weight (Bezemer & Jones, 1998; Bezemer *et al.*, 1999). The effects of increased $CO_2$ levels throughout food webs have also been investigated. For example, studies on soil biota found that elevated $CO_2$ resulted in changes in fungal assemblages and increases in the biomass of cellulose decomposers, driving changes in the abundance and composition of their predators, Collembola (Jones *et al.*, 1998; Hartley & Jones, 2003).

### 1.3.2. Effects on Distribution:

Most species' distributions and/or abundances have been affected in some way by human activities, mostly due to change or loss of habitat (Hughes, 2000; Millennium Ecosystem Assessment, 2005). It is widely accepted that species' ranges are also influenced by climatic variables through species-specific physiological thresholds of temperature and precipitation (Walther *et al*, 2002). A 3°C increase in mean annual temperature correlates with a shift in isotherms of approximately 300-400 km in latitude or 500 m in elevation (Hughes, 2000). Species are therefore expected to shift their ranges upwards in elevation or towards higher latitude in response to climate warming, although shifts will be limited by species' dispersal abilities and availability of habitat (Hughes, 2000; Warren *et al.*, 2001; Walther *et al*, 2002; Franco *et al.*, 2006; Hickling *et al.*, 2006). There has been a wealth of observations in Northern European countries revealing species shifting their ranges over a period of time associated with climate warming (Parmesan, 1996; Parmesan *et al.*, 1999; Warren *et al.*, 2001; Hill *et al.*, 2002; Kullman, 2002; Konvicka *et al.*, 2003; Perry *et al.*, 2005; Wilson *et al.*, 2005; Hickling *et al.*, 2006; Franco *et al.*, 2006; Wilson *et al.*, 2007) with an average northwards expansion of northern boundaries of 6.1 km per decade, and upward shifts in elevation of 6.1 m per decade (Parmesan & Yohe, 2003, Parmesan, 2006). A similar change in distributions of insect species occurred in the Quaternary period due to glacial/interglacial climatic oscillations, which demonstrated the ability of species to track climate (Lawton, 1995). Examples of such range changes have been demonstrated in several Coleoptera species for which sub-fossil records have been found in locations quite remote from their current distributions (Coope, 1993). These responses are not limited to insects, with evidence of range shifts to higher latitudes and elevations in a number of tree taxa during a period of climate warming at the end of the last glaciations (Davis & Shaw, 2001). Further effects of climate warming on species' distributions are discussed in more detail in subsequent chapters.

### 1.3.3. Effects on Phenology:

Phenology is the timing of seasonal activities of plants and animals, and has been used to track species responses to climate change (Walther *et al.*, 2002; Parmesan, 2007). Spring activities, for example arrival of migrant birds, first appearance of butterflies, spawning in amphibians and leaf unfolding in plants, have been occurring earlier since the 1960s (Forchhammer *et al.*, 1998; Menzel & Fabian, 1999; Roy & Sparks, 2000; Walther *et al.*, 2002; Cotton *et al.*, 2003). There is also some evidence of a later onset of autumnal phenological events, but these time shifts are less pronounced and are more varied among species (Menzel & Fabian, 1999; Walther *et al*, 2002; Cotton., 2003). Butterflies are good indicator organisms to use when studying the effect of climate on phenology, because not only are they poikilothermic but they also have been extensively recorded on monitoring schemes over the last 30 years in several countries.

Roy and Sparks (2000) looked at the effect of temperature on the phenology of 35 British butterflies from 1976 to 1998. They found that most species showed advancement in emergence, peak appearance and had longer flight periods over the study period, and that these variables showed a strong relationship with temperature, especially with mean February temperatures. The advancement of first appearance ranged from 1 to 10 days per °C for British butterflies over the past 30 years (Roy & Sparks, 2000). Phenological advances have also been recorded at mid-latitudes. For example, Forister and Shapiro (2003) found an average advance of 24 days in the first flight of 23 butterfly species in the Central valley of California, an area subjected to a Mediterranean climate. It was found that higher winter temperatures were responsible for increasing larval growth rate and thus promoting earlier adult emergence, which also benefited from drier winter conditions (Forister & Shapiro, 2003). Similar observations for butterflies have also been recorded in Spain (Stefanescu *et al.*, 2003) where the relationship of phenological parameters with temperature and precipitation between 1988 and 2002 revealed an earlier first appearance of 17 species.

### 1.3.4. **Adaptation:**

Although phenological and distributional changes have been shown to be common responses of species to climate warming, it is also possible that species will undergo evolutionary changes in response to climate. For example, the butterfly *Polygonia c*-album has apparently incorporated a wider range of larval host plants into its diet which may have increased its ability to expand its distribution during recent climate warming (Braschler & Hill, 2007). Evolutionary changes in dispersal as a consequence of climate-driven range changes have also been observed in a number of species. For example, *Pararge aegeria* (speckled wood butterfly) individuals at expanding range boundaries have increased dispersal ability, which is associated with decreased allocation of resources to reproduction (Hughes *et al.*, 2003). This phenomenon has also been observed in two bush cricket species, *Conocephalus discolor* (long-winged conehead) and *Metrioptera roeselii* (Roesel's bush cricket), which have increased frequency of longer winged, more dispersive individuals at range margins (Thomas *et al.*, 2001).

## 1.4 Migration:

Animals that embark on seasonal migrations are found within all major branches of the animal kingdom, moving considerable distances through flying, swimming, walking or drifting (Alerstam *et al.*, 2003; Dingle & Drake, 2007) to exploit seasonally available habitats at high latitudes. Dingle (1996) suggested that migration generally involves a number of characteristics which are derived from Kennedy's (1985) definition of migration:

> *"Migratory behaviour is persistent and straightened-out movements effected by the animal's own locomotion exertions or by its active embarkation on a vehicle. It depends on some temporary inhibition of station-keeping responses, but promotes their eventual disinhibition and recurrence."*

The first characteristic involves persistent movement, which has been observed in a number of species from a variety of taxa. For example, aphids migrate distances greater than 1000 km to reach suitable habitats (Johnson, 1969) and *Danaus plexippus* (Monarch butterfly) can exceed 3000 km during their seasonal migration from Mexico to North America and Canada (Dingle *et al.*, 2005; Cardé, 2008). *Autographa gamma* (silver Y moth) migrates more than 1000 km between overwintering sites in the Mediterranean region to summer breeding grounds in the UK, covering distances of up to 650 km in one night (Chapman *et al.*, 2008a). In addition, a number of birds species, including *Phylloscopus nitidus* (garden warbler), *Phoenicurus phoenicurus* (common redstart) and *Ficedula hypoleuca* (pied flycatcher) cover distances of thousands of kilometres from overwintering sites in central and southern Africa to summer breeding territories throughout Europe (Coppack *et al.*, 2003; Ahola *et al.*, 2004).

The second characteristic exhibited by migrants is that of a straightened out movement, requiring orientation and navigation using a variety of cues, for example sun, stars and the Earth's magnetic field (Dingle, 1996, 2006). Mechanisms by which migrants orient will be discussed in more depth in Chapter 6 of this thesis.

Migrant species exhibit directed undistracted flight, which comprises the third characteristic of migratory taxa. As such, behavioural studies on migrants have demonstrated individuals continuing to pass over suitable habitat without terminating their migration (Kennedy, 1985). Nonetheless, some migrant species are forced to interrupt their migration to re-fuel, for example birds and butterflies (Dingle 1996) or to shelter, for example nocturnal moths (Gatehouse, 1997). However, these generally are short-lived stops and do not result in the termination of migration.

The fourth characteristic is the distinct behaviour of leaving and arriving. The onset of migration is thought to be initiated by several factors including habitat quality and availability, competition, temperature, wind speed and direction and day length (photoperiod) (Baker, 1978). Decreasing nocturnal temperatures are used as a cue for *Anax junius* (common green darner dragonfly) to initiate migration (Holland *et al.*, 2006). Photoperiod as a cue for the onset of migration has been demonstrated in a number of insect species, for example *Oncopellus fasciatus* (Large milkweed bug) and *Danaus plexippus* (Dingle 1996), while Berthold (1996) discusses the role of photoperiod in bird migration. Temperature as an important cue in migration has also been observed in the moth *Agrotis ipsilon*, with individuals becoming absent from their southern ranges during periods of sustained hot temperatures and then disappearing from their northern limits under sustained cold temperatures (Showers, 1997). The role of photoperiod in regulating insect migrations is discussed in further detail in Chapter 6. Mechanisms controlling the termination of migration are less well understood. Although some species do not return to the same over-wintering locations, some species including birds and the migratory Monarch butterfly, *Danaus plexippus*, return to the same overwintering sites year on year (Holland *et al.*, 2006).

The last characteristic which is exhibited in a wide range of migratory taxa is the relocation of energy reserves to movement during migration (Johnson, 1969; Dingle, 1972; Dingle, 1996; Dingle, 2006; Akesson & Hendenstrom, 2007; Ramenofsky & Wingfield, 2007). There is usually a trade off between reproduction and migration, such that reproduction is delayed during migration and resources diverted to the wings and flight muscles (Dingle, 1972). This tendency for migration to occur prior to reproduction was coined by Johnson (1969) as the "oogenesis-flight syndrome". This syndrome is evident in insect taxa where long distance migrations are principally undertaken by young adults in a pre-reproductive stage (Johnson, 1969; Dingle, 1978). However, this is not always the case, for example *Agrotis ipsilon* (Dark Swordgrass moth), migrates in both the pre-reproductive and sexually mature stages (Showers, 1997). In *Dysdercus* bugs, wings are maintained during migration but they are subsequently discarded and the wing muscle broken down through histolysis once migration is terminated (Ramenofsky & Wingfield, 2007). As discussed by Dingle (2006), many migratory taxa increase deposition of fat, an important fuel for flight, prior to migration.

### 1.4.1. **Insect Migration:**

Migratory activity has been documented in a wide range of insect taxa and occurs regularly in locusts, butterflies, moths, dragonflies, hoverflies and beetles, with numbers of individuals partaking in migratory movements ranging from a few hundred to thousands of millions individuals (Williams, 1957). Migration is usually directional, in which individuals either fly under their own power within the flight boundary layer or climb out of their flight boundary layer to altitudes of up to 1500 m to be carried long distances on fast, high-altitude airstreams (Gatehouse & Zhang, 1995; Pedgley *et al.*, 1995; Gatehouse, 1997; Coulson *et* al., 2002; Wood *et al.*, 2006; Stefanescu *et al.*, 2007; Chapman *et al.*, 2008a,b). Migratory flights taken within the flight boundary layer are generally limited to larger insects such as butterflies, for example *Danaus plexippus* and *Vanessa atalanta.* However this type of migration is expensive in terms of energy, with individuals having to stop and re-fuel, often resulting in a series of shorter flights over several days/nights (Gatehouse & Zhang, 1995; Gatehouse, 1997). By contrast, most insect migrants have evolved a wind-bourne migration strategy whereupon individuals fly up to higher altitudes where flight speeds are predominantly determined by the wind. Such flights often result in a single long-distance flight, which takes substantially less time to cover long distances than if insects were under their own power alone (Gatehouse & Zhang, 1995; Gatehouse, 1997; Chapman *et al.*, 2008).

In nearly all insect migrations a trend has been observed of movements away from the tropics and subtropics during the spring, allowing the colonisation of temperate seasonal habitats, with return flights in the autumn towards the equator (Williams, 1957; Pedgley *et* *al.*, 1995). Observation studies on *Vanessa atalanta* and *V.cardui* have shown strong tendencies of flight orientation to be predominantly N, NNW during spring and summer months, and S, SSW towards the end of the summer into autumn in the UK (Baker, 1978). However not all migration routes run south north, for example *Plutella xylostella* (Diamond back moth) and *Nymphalis antiopa* (Camberwell beauty) arrive in the UK from Sweden, Denmark, Finland and the Netherlands (Pedgley *et al.*, 1995). These migrations allow breeding to occur throughout the year, although in some species there may be a period of diapause in the overwintering range (Williams, 1957). Baker (1978) hypothesised that autumn migrations southwards were caused by latitudinal temperature gradients, with migrants moving towards higher temperatures in order to produce offspring with a higher probability of reaching the overwintering stage compared with individuals flying in other directions. However, migratory behaviour is also genetically controlled, with migration switching in orientation by 180°C between autumn and spring (Brower, 1996). Outbreak populations of *Spodoptera exempta* (African armyworm moth), in sub-Saharan Africa differ in the length of their pre-reproductive period (potential for migration), with individuals in regions of high rainfall and plentiful breeding habitat having shorter pre-reproductive periods than those in areas of low rainfall that exhibit longer pre-reproductive periods allowing them to migrate to more suitable breeding habitats (Wilson & Gatehouse, 1993). The lack of any evidence of environmental factors controlling the pre-reproductive period within this species strongly suggests that the difference in these outbreak populations is under genetic control (Wilson & Gatehouse, 1993).

Migratory behaviour in insects is difficult to study, and thus the majority of migratory studies have been carried out on birds where it is possible to mark and recapture individuals and thus trace migration routes; although it is difficult to apply this method reliably to insects. However with the use of stable isotopes (Wassenaar & Hobson 1998) and by examining arrival patterns of migrants associated with wind patterns (Stefanescu *et al.*, 2007) the potential to learn more about overwintering locations and migratory events in insects is increasing.

## 1.5. Thesis outline:

The aim of this study was to investigate the responses of migratory insects to climate change, both in terms of changes in their distribution and abundance, to gain further knowledge into factors initiating migratory events, the natal origins of Lepidoptera arriving in the UK in spring and their pattern of arrival. Migratory insects are poorly studied and this thesis will add to current knowledge by investigating species range changes and methods for determining migration routes that have not been studied previously.

Chapter 2 describes the study organisms and methods used to analyse changes in distribution and abundance in subsequent chapters.

Chapter 3 analyses existing data sets to investigate changes in distributional extent of migratory insects, from three taxa, arriving into Britain each spring over the past 40 years in a cross taxon comparison.

Chapter 4 analyses existing data sets to investigate whether migratory butterflies in Britain are responding to climate change to a similar extent as resident butterflies. This chapter compares distribution and abundance changes in migrants with southerly-distributed generalist and specialist resident species.

Chapter 5 analyses existing data sets to investigate abundance changes in migratory Lepidoptera over recent years, exploring relationships between numbers of spring arrivals and numbers developing in subsequent generations. It examines patterns of arrival of migrant individuals into Britain each spring and the subsequent colonisation of Britain each summer. It investigates factors affecting observed changes in abundance.

Chapter 6 discusses the results of a rearing experiment that investigates the effect of photoperiod on the direction of flight in *Vanessa atalanta* (the red admiral butterfly).

Chapter 7 explores stable isotope techniques to investigate whether the natal origins of migratory butterflies can be determined from oxygen and hydrogen isotopes. It also investigates whether it is possible to determine the migratory status of individuals by comparing isotope ratio values of abdomen and wing tissues.

Chapter 8 brings the results from the previous chapters together to explore their significance in terms of climate change on migrants, and discusses the wider implications of the thesis's findings.

# Chapter 2

# Study Species and Materials and Methods

## 2.1 Abstract:

Within this chapter I will introduce the study species used throughout the thesis, with particular attention to butterflies, as the majority of the following chapters are based on this taxonomic group. I will then describe the two different data sources from which data are analysed throughout the thesis: distribution data, used in Chapters 3-5, and abundance data, used in Chapters 4-5. Within the thesis a number of the dataset analyses are repeated through Chapters 3 and 4, and I will introduce these analyses within this Chapter, along with methods derived for dealing with changes in recorder effort over time, a well known problem associated with distribution data.

## 2.2 Study Species:

### 2.2.1 Species Selection:

I analysed data for species from three taxonomic groups that occur in terrestrial and/or freshwater environments and regularly migrate into Great Britain during the spring. The groups analysed were butterflies (Rhopalocera), hoverflies (Syrphidae) and dragonflies (Odonata). I focussed analyses on these taxonomic groups because of the relatively large number of records for each species available from the Biological Records Centre (BRC) database, with good recording coverage across the UK over a long time span. Within each of these taxonomic groups I selected species that are considered to be migratory. As discussed in Chapter 1, the definition of migration can be much debated, however initially in my choice of species I defined a species as migratory if 1) individuals undertake a seasonal displacement into the UK each spring to breed, and 2) do not overwinter at any stage of development in the UK in significant numbers (Williams, 1965), relative to the number of individuals that arrive through migration. Using this definition resulted in four migrant butterflies, *Vanessa atalanta, Vanessa cardui, Colias croceus* and *Nymphalis* *antiopa* to analyse, but only a few rare hoverfly (2) and dragonfly (8) species, for which there are not sufficient data for comparisons with the butterfly species.

To enable an analysis of migration among taxa, as detailed in Chapter 3, I thus widened my definition of migration to include species 1) that historically have not been able to overwinter in the UK, 2) whose UK populations remain substantially boosted each spring by immigrant arrivals and 3) that have been referred to as 'migratory' at least twice in the literature. These criteria resulted in me analysing four hoverfly species (*Episyrphus balteatus, Scaeva pyrastri, Sphaerophoria scripta* and *Syrphus vitripennis*) and two dragonfly species (*Aeshna mixta* and *Sympetrum striolatum*). Under these criteria it could be argued that the butterflies *Pieris brassicae* (large white) and *P. rapae* (small white) should also be included as migrants as there is evidence of spring arrivals from the continent each year (Baker 1969). However evidence from the UKBMS (see section 2.5) suggests that individuals from Europe do not play a major role in British population dynamics, with year-to-year fluctuations being similar to other resident species (Pollard & Yates 1993), and thus I did not include them in the analyses.

### 2.2.2 Butterflies:

In Britain, ten migrant butterflies have been recorded, however there are only three species that are recorded regularly and occur in substantial numbers each year in the UK: *Vanessa atalanta* (red admiral), *Vanessa cardui* (painted lady) and *Colias croceus* (clouded yellow) and the life cycles of these species are described in detail below. Data for these species are analysed in Chapters 3, 4 and 5, *Nymphalis antiopa* (camberwell beauty) is recorded infrequently in the UK and thus is only included in analyses presented in Chapter 3. Rare migrants that have been recorded relatively infrequently in the UK since 1970 include *Pontia daplidice* (Bath white), *Aporia crataegi* (black-veined white), *Issoria lathonia* (Queen of Spain fritillary), *Lampides boeticus* (long tailed blue), *Danaus plexippus* (monarch) and *Colias hyale* (pale clouded yellow). These rare species are included in analyses presented in Chapter 4.

#### 2.2.2.1 Taxonomy and Distribution:

*Vanessa atalanta, V. cardui* and *Nymphalis antiopa* are all members of the family Nymphalidae, while *Colias croceus* is a member of the family Pieridae. The three most commonly recorded migrants arriving in to the UK each spring, *Vanessa atalanta*, *V. cardui* and *Colias croceus*, have similar distributions within Europe and North Africa, with individuals overwintering in habitats at southerly latitudes (namely North Africa and southern Europe), before migrating northwards during March to June into central and north Europe (Pollard & Yates, 1993; Asher *et al*, 2001; Stefanesecu 2001, Stefanescu *et al.*, 2007; Brattström et al., 2008). By contrast, the fourth most common migrant, *Nymphalis antiopa*, undertakes an east-west migration, arriving into the UK in summer from overwintering habitats in central and northern Europe (Tolman, 1997; Asher *at al.*, 2001).

### *Vanessa atalanta*

*V. atalanta* is a Holarctic species, widely distributed through North Africa, Europe, Asia, Iran and North America (Emmet & Heath, 1990; Tolman, 1997; Asher *et al.*, 2001). Within Western Europe it is particularly widespread, with populations present all year round from the Mediterranean to central Germany, migrating to more northerly latitudes to breed during summer months, see Fig 2.1 (Emmet & Heath, 1990; Asher *et al.*, 2001).

Figure 2.1. European distribution of *Vanessa atalanta*. Black indicates the overwintering range, whilst grey indicates temporary summer breeding habitats (from Tolman 1997).

*Vanessa cardui*

This species is the most geographically widespread of the butterfly study species, occurring worldwide except in South America (Asher et al., 2001). Throughout the majority of Europe, this species occurs only during summer months, returning to overwintering habitats on the edges of desert bands throughout North Africa, across to Arabia and central Asia (Emmet & Heath, 1990).

Figure 2.2. European distribution of *Vanessa cardui*. Black indicates the overwintering range, whilst grey indicates temporary summer breeding habitats (from Tolman 1997).

*Colias croceus*

*C. croceus* has a more restricted geographic range than previous two species, occurring throughout North Africa, southern Europe, and through to the Middle East (Emmet & Heath, 1990; Asher et al., 2001). During summer months, this species is found across much of Europe, but reaches its northern range boundary in the UK, with very few records from Scandinavia and Finland, see Fig 2.3 (Tolman, 1997; Asher *et al.*, 2001).

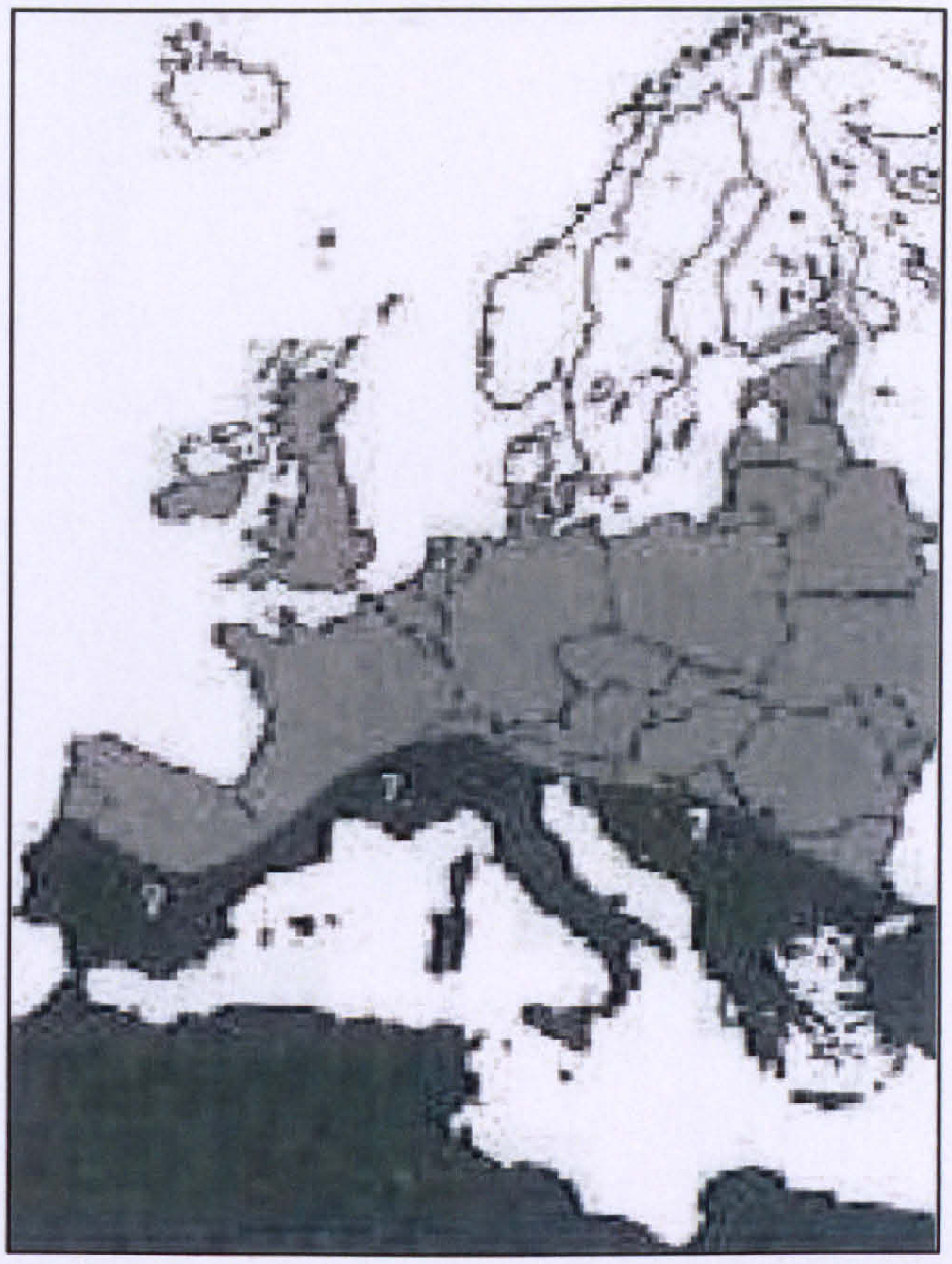


Figure 2.3. European distribution of *Colias croceus*. Black indicates the overwintering range, whilst grey indicates temporary summer breeding habitats (from Tolman 1997).

*Nymphalis antiopa*

This species has a wide geographical range, occurring across Europe eastwards to Turkey, through temperate Asia and across much of North America (Emmet & Heath, 1990; Tolman, 1997; Asher *et al.*, 2001). Although resident across most of central and eastern Europe, this species becomes progressively less common in north west Europe, and is a relatively rare migrant to Britain (Fig 2.4, Tolman, 1997).

Figure 2.4. European distribution of *Nymphalis antiopa*. Black indicates the overwintering range, whilst grey indicates temporary summer breeding habitats (from Tolman 1997).

### 2.2.2.2 **Life History:**

*Vanessa atalanta*

Being such a mobile species, *V. atalanta* occurs in a variety of habitats within the UK from late March onwards, favouring flower-rich habitats in woodland, grasslands, heathland and moors (Emmet & Heath, 1990; Tolman, 1997). During late summer and early autumn adult *V. atalanta* are regularly seen in gardens and orchards feeding on *Buddleja davidii* (butterfly-bush) and rotting fruit (Asher *et al*., 2001). The species can have up to three generations a year, of which two generations occur in Britain (Heath *et al*., 1984), with a further generation occurring during the winter months within the species southern over-wintering range (Stefanescu, 2001). Anecdotal evidence suggest that an increasing number of individuals are overwintering in the UK, especially during milder winters, although very slow larval development rates make larvae vulnerable to disease and predation, with adults of small body mass emerging in the spring (Asher *et al*, 2001). The small numbers of overwintering individuals in the UK are unlikely to contribute significantly to abundance in the UK the following spring (Pollard & Greatorex-Davies, 1998).

This species predominately uses *Urtica dioica* (common nettle) as its larval host plant, although *U. urens* (small nettle), *Parietaria judaica* (Pellitory-of-the-Wall) and *Humulus lupulus* (Hop) may also be used (Asher *et al.*, 2001). Eggs are laid sparsely throughout patches of *U. dioica*, with bright green eggs laid singly on the upper surface of young leaves (Asher *et* al., 2001; Stefanescu, 2001). The eggs hatch within approximately a week (dependant on temperature), with the larvae passing through 5 instars before pupation, with both developmental stages occurring on the host plant (Asher *et* al., 2001; Stefanescu, 2001).

### *Vanessa cardui*

*V. cardui*, like *V. atalanta*, has been recorded from a wide-variety of habitats. Within Europe, adults migrate northwards to Spain in spring from overwintering sites in North Africa, where upon they breed, with the subsequent generation continuing the northward migration during early summer (Pollard *et al.*, 1998; Stefanescu *et al.*, 2007). Small scale breeding has also been recorded within Spain in September during the autumn southward migration, with the subsequent generation, emerging in October, continuing the southward migration to Africa (Stefanescu *et al.*, 2007). There is very little evidence for the ability of this species to overwinter at these northerly latitudes, with only very occasional occurrences of overwintering within the Mediterranean (Stefanescu, 1997; Pollard *et al.*, 1998). However, in recent years a small number of individuals have been recorded overwintering within southern Britain, although the viability of these individuals, in terms of breeding success, in the following spring remains unknown (Asher *et al.*, 2001).

*V. cardui* is multi-voltine (dependent on temperature) and larvae feed on a wide range of foodplants, mainly *Cirsium* spp. and *Carduus* spp. (thistles) in Britain. Larvae have also been recorded on *Malva* spp. (Mallows), *U. dioica* and *Echium vulgare* (Viper's-bugloss), with mallows more commonly used in Spain (Asher *et al.*, 2001; Stefanescu *et al.*, 2007). A single egg is laid on the upper surface of leaves, with larvae and pupae developing within silk tents constructed on the underside of the leaves (Asher et al., 2001).

### *Colias croceus*

*Colias croceus* occurs in a wide range of habitats, including coastal cliffs and open downs, particularly on warm south-facing habitats with an abundance of flowering plants (Tolman, 1997; Asher *et al.*, 2001). There are many discussions as to the ability of *C. croceus* larva to develop during the winter within northerly summer breeding grounds, with larvae observed in winter within frost-free regions in southern Europe (Tolman, 1997). In recent years, larvae have also been recorded in winter in southern Britain, although the viability of these early developmental stages is unknown (Asher *et al.*, 2001).

Following a similar migratory pattern to *V. cardui*, *C. croceus* undertakes northward migrations from overwintering habitats in North Africa and southern Europe during spring to colonise much of Europe (as shown at Fig 2.3). Larvae feed on a range of leguminous plants, including wild and cultivated *Trifolium* spp. (clovers), *Medicago sativa* (Lucerne) and occasionally *Lotus corniculuatus* (Bird's-foot-trefoil) (Emmet & Heath, 1990; Tolman, 1997). The species is multi-voltine with no diapause stage, breeding continuously all year round within its southern overwintering habitats (Tolman, 1997), and migrants achieving up to three generations occurring in Britain during optimal conditions (Asher *et al.*, 2001). Single pale yellow eggs are laid upon the upper surface of larval foodplants, which hatch after approximately 6-10 days (Asher *et al.*, 2001). Larva pass through 4 instars before attaching to stems using silken girdles and pupating after approximately 20 – 40 days depending on climatic conditions (Emmet & Heath, 1990; Asher *et al.*, 2001). Both the larval and pupal stages are susceptible to periods of prolonged damp and frost (Emmet & Heath, 1990).

### *Nymphalis antiopa*

This species is found most commonly in woodland habitats, although it also breeds throughout a number of other habitats, for example river valleys, parks and gardens (Asher *et al.*, 2001). Adults enter diapause during the winter months, however conditions within

Britain are thought to be too mild and damp to allow successful hibernation by this species, and as such there are very few records of overwintering individuals in Britain.

*N. antiopa* larvae feed on a range of tree species, including *Salix* spp. (willows), *Ulmus* spp. (elms) and *Populus* spp. (poplars) (Asher *et al.*, 2001). Unlike the other three migrant species discussed above, *N. antiopa* only has one generation per year with flight periods in southern Europe occurring from June-July to August-September at higher latitudes (Asher *et al.*, 2001). Eggs are laid in large batches in a spiralling cluster formation around the stems of their food plant, with the larvae quickly dispersing on hatching, feeding on young leaves before pupating.

### 2.2.2.3 Changes in Abundance:

Migrant butterflies arriving in Britain each spring exhibit annual fluctuations in their abundance from year to year, with *Vanessa cardui* displaying the most extreme fluctuations (see Fig 2.5). Both *V. atalanta* and *V. cardui* have increased in abundance in the UK over time, increasing by 340.5% and 433% respectively since 1976 when the Butterfly Monitoring Scheme (BMS) began (see below for details of BMS methodology; Greatorex-Davies *et al.*, 2006). This increase in abundance of *V. atalanta* in Britain has been attributed primarily to increased influx in spring, rather than to increased breeding success in the UK in summer (Pollard & Greatorex-Davis, 1998). There are insufficient data available for *C. croceus* to determine the extent to which this species has increased its abundance in Britain. However compared to earlier time periods, relatively high counts have been recorded in most years since 1992 suggesting that this species is following trends exhibited by other migrants with more individuals arriving in Britain each spring (Greatorex-Davies *et al.*, 2006). Very little is known about the changes in the abundance of *N. antiopa* in Britain, with individuals only having been recorded twice within the BMS, and as such information for this species is limited to analysis of distribution records (www.ukbms.org).

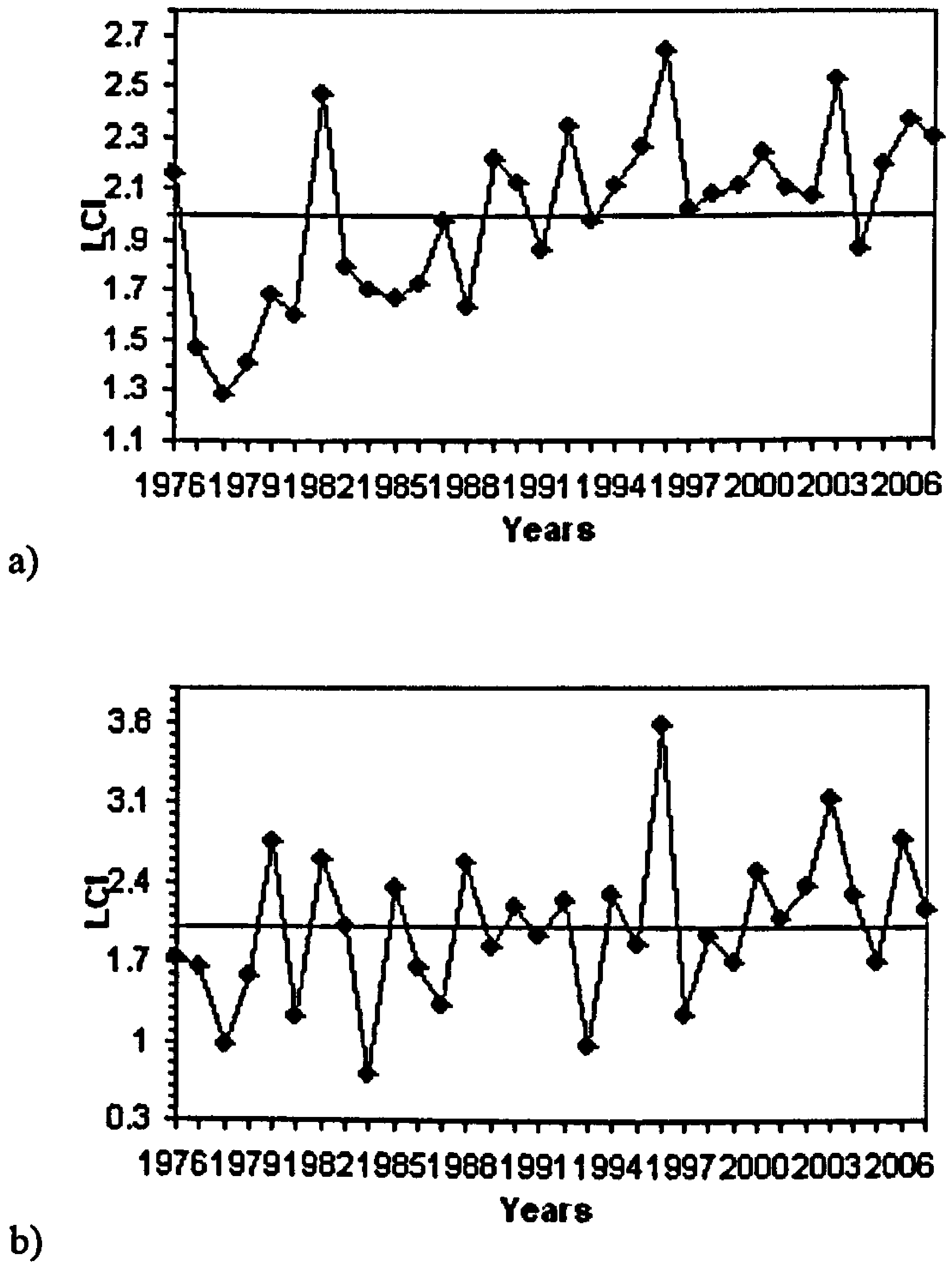


Figure 2.5. Changes in the index of abundance (log collated index, LCI, see below) over time since 1976 for a) *Vanessa atalanta* and b) *Vanessa cardui* (Greatorex-Davies *et al.*, 2007). The LCI is scaled so that the average index over the whole series is equal to 2 (horizontal line) (www.ukbms.org)

### 2.2.3 Hoverflies:

In Britain, there are five hoverfly species that fit the migration criteria specified above (section 2.2.1): *Episyrphus balteatus, Scaeva pyrastri, Sphaerophoria scripta, Syrphus vitripennis* and *Xanthandrus comtus*. These are species whose populations in Britain are boosted substantially each year by influxes from further south in continental Europe. However only four of these species are recorded regularly, with only very few sightings of

*X. comtus* in Britain, such that I was unable to include this species in analyses presented in Chapter 3.

### 2.2.3.1 Taxonomy and Distribution:

All four species of hoverfly are members of the family Syrphidae. *E. balteatus* is widely distributed across much of Asia and Europe, overwintering in low latitude regions before migrating northwards in spring to summer breeding territories (www.aphidweb.com). *S. pyrastri* occurs throughout Europe, Africa, and North America (Eaton & Kaufman, 2007), and occurs in summer throughout much of Britain (Stubbs & Falk, 2002). *S. scripta* occurs throughout Europe and Asia and although it occurs in Britain, populations rely heavily on immigration in the spring (Stubbs & Falk, 2002). *S. vitripennis* is widespread throughout Europe, Asia, and North America (Vockeroth, 1992).

### 2.2.4.1 Life History:

*Episyrphus balteatus*

This species is an important aphidophagous hoverfly, occurring in Britain in spring and summer months in meadows, heath, moorland, gardens and parks (Hart *et al.*, 1997; Ball & Morris, 2000). Adults feed on flowers, predominantly on pollen, while larvae occur more frequently on trees, shrubs, cereal crops and cabbages, preying on a variety of aphid species (Gilbert, 1981; Ball & Morris, 2000). *E. balteatus* can be multi-voltine (Hart *et al.*, 1997), but is univoltine in Britain (Pollard, 1971). Migrants arrive into the UK from southern Europe during late June and July, at a time when aphid numbers are at a maximum. Larvae develop during the summer, and adults emerge in late summer (Ball & Morris, 2000). During the autumn, adults migrate southwards and overwinter in southern Europe as adults (Gilbert, 1986; Gatter & Schmid, 1990; Hart & Bale, 1997; Ball & Morris, 2000). Swarms of adults have been monitored flying southwards in autumn with small numbers of adults detected heading northwards in spring providing evidence of migration (Pollard, 1971; Stubbs & Falk, 2002). *E. balteatus* may occasionally overwinter in Britain, however they have very little ability to tolerate cold conditions and can only survive in sheltered, artificially-heated environments (Hart & Bale, 1997; Sadeghi & Gilbert, 2000; Sutherland *et al.*, 2001; Stubbs & Falk, 2002; Hondelmann & Peohling, 2007). Laboratory experiments on cold tolerance in *E. balteatus* showed 100% adult mortality after exposure to 10 weeks of British winter temperatures, and even after a period of acclimation, larvae died after being subjected to freezing temperatures (Hart & Bale, 1997). Unlike females, males have no ability in increase the size of their fat bodies thus preventing them from entering diapause and overwintering in temperate regions (Hondelmannn & Poehling, 2007). Further evidence for the migratory status of this species comes from mature females not being recorded until late spring/early summer, if the females overwintered they would be expected to be recorded earlier (Pollard, 1971).

### *Scaeva pyrastri*

Adult *S. pyrastri* are commonly recorded feeding on flowers, in particular white umbels, *Cirsium* spp. and *Carduus* spp., while larvae feed on a wide range of ground-layer aphid species, and less frequently on arboreal aphids (Gilbert, 1986; Ball & Morris, 2000). This species is a regular migrant, arriving in Britain from overwintering habitats in southern and central Europe during early summer (Ball & Morris, 2000). *S. pyrastri* develops through one generation per year in Britain, with adults emerging in late summer. Although this species regularly overwinters in central Europe there is little evidence to suggest that individuals are capable of overwintering in western Europe and thus UK population dynamics are dependent upon immigrants arriving each summer (Ball & Morris, 2000). However in recent years the sightings of individuals in early May has started a debate as to whether or not individuals have overwintered, or are early migrants (Stubbs & Falk, 2002).

### *Sphaerophoria scripta*

*S. scripta* is one of the most common open grassland hoverflies in Britain (Stubbs & Falk, 2002) but is restricted to more coastal habitats at the northern edge of its European range (Ball & Morris, 2000). Larvae feed on a range of aphids and other soft-bodied Homoptera within ground layer vegetation, including a number of agricultural pests species (Gilbert, 1986; Ball & Morris, 2000).

*Syrphus vitripennis*

*S. vitripennis* is another common and widespread hoverfly in Britain (Stubbs & Falk, 2002). Adults occur in a range of habitats including woodland, scrub, hedgerows, parks and gardens feeding on nectar from a wide range of flowers (Ball & Morris, 2000). Larvae feed on aphid species on a wide range of trees and shrubs (Gilbert, 1986; Ball & Morris, 2000). This species is highly migratory, undergoing regular migration events during the spring and autumn (Pollard, 1971), There is increasing evidence for a resident population within southern Britain, but numbers are boosted each spring by immigrants arriving from the continent (Ball & Morris, 2000).

### 2.2.4 Dragonflies:

Of the 34 species of dragonfly recorded in Britain, 8 species are migrants according to my initial definition i.e. they do not overwinter in Britain in significant numbers and UK population dynamics are dependent on spring immigrants. However, there are very few distributional records for these species and so they could not be included in analyses presented in Chapter 3. These species include *Hermianax ephippiger* (8 records for the time period analysed, see below), *Aeshna affinis* (1 record), *Crocothemis erythrae* (0 records), *Sympetrum vulgatum* (0 records), *Sympetrum fonscolombii* (17 records), *Sympetrum pedemontanum* (0 records), *and Pantala flavescens* (0 records). However, according to my broader definition of migration, there are two species available for analysis: *Aeshna mixta* (migrant hawker) and *Sympetrum striolatum* (common darter).

#### 2.2.4.1 Taxonomy and distribution:

*Aeshna mixta* is a member of the family Aeshnidae, of which there are 11 other species recorded in Britain, while *Sympetrum striolatum* is one of 16 species occurring in Britain of the family Libellulidae. *A. mixta* and *S. striolatum* are both common and widespread across southern Europe, extending their ranges as far north as northern Germany, Denmark and southern Sweden and Norway (Gibbons, 1986; Brooks, 1997). Mass migrations across Europe (David, 2003), from North Africa (Nelson *et al.*, 2000) have been recorded regularly for *A. mixta* (Brooks, 1997), and large numbers of both male and female *S. striolatum* individuals have been sighted migrating south-west-south through a Pyrenean pass in autumn (Lack & Lack 1951).

#### 2.2.4.2 Life History:

*Aeshna mixta*

*Aeshna mixta* occurs within most habitats although mature adults show a preference for woodland and sheltered areas (Gibbons, 1986; Brooks, 1997; Nelson *et al.*, 2000). Females oviposit on the stems of plants, including *Typha* spp. (bullrush) and *Iris pseudocorus* (yellow flag), around ponds, lakes and gravel pits. Larvae prefer still waters that are acidic to slightly calcareous (Gibbons, 1986; Brooks, 1997). Larvae hatch during early spring, with adults emerging from late July through to September. This species diapauses overwinter as an egg (Brooks, 1997). Prior to the 1940s, this species was a rare migrant in Britain, but recently has been recorded overwintering in southern and eastern Britain forming small resident populations, although it is absent from western and northern Britain (Gibbons, 1986; Askew, 1988; Hammond, 1997). Despite becoming a resident, UK populations are regularly reinforced by migrants from central and southern Europe (Gibbons, 1986; Askew, 1988; Nelson *et al.*, 2000).

*Sympetrum striolatum*

*S. striolatum* larvae are found within a wide range of waterbodies including ponds, ditches and rivers, where slower flowing waters are preferred (Gibbons, 1986; Brooks, 1997). Adults are most often recorded during late summer and early autumn flying in the proximity of water, occasionally moving to woodland rides and clearings and over heathland (Gibbons, 1986). Although a small resident population has recently become established in southern Britain, numbers are heavily boosted by migrants moving northwards from continental Europe in spring (Brooks, 1997; Hammond, 1997).

## 2.3 Datasets included in analyses:

### 2.3.1 Introduction:

To determine the responses of migratory insects to climatic warming I have analysed historical data from a number of datasets for the taxonomic groups discussed above. Within Chapter 3, I analyse distribution data for butterflies, hoverflies and dragonflies. These analyses are limited to distribution changes and do not include comparison of abundance changes because only distribution data are available for hoverflies and dragonflies. In contrast, abundance and distribution data are available for butterflies, which are analysed in Chapters 4 and 5. Chapter 4 compares the responses of migrants versus resident butterflies to recent climatic warming, and Chapter 5 focuses on three migratory butterflies to determine factors affecting observed changes in distribution and abundance. The different data sets analysed are described in further detail below.

### 2.3.2 Butterfly Distribution Data:

Distribution data for butterflies were obtained from the Butterflies for the New Millennium (BMN) data set, a Butterfly Conservation (Wareham, Dorset) recording scheme run in conjunction with the Biological Records Centre (BRC) at CEH Monks Wood, Cambridgeshire (now CEH Wallingford). The BNM began in 1995 as a follow up to a national survey in the 1970s that indicated substantial changes had occurred in butterfly distributions. The BRC was established in 1964 and, working alongside voluntary recording organisations, it holds over 15 million records of more than 12000 species, including 66 butterfly species present in the UK. Records are defined as a particular record card received by the BRC from a recorder for a particular species at a particular location. Data for butterflies have been presented in three national atlases to date (Heath *et al.*, 1984; Asher at al. 2001; Fox et al, 2006).

Together, Butterfly Conservation and the BRC hold distribution data for both resident (N=57) and migrant (N=9) butterfly species, from 1832 to 2004. Pre-1970 data comprise information from two sources, the first from journal articles and museum records, and secondly from observation data from recorders. The data are presented at a variety of spatial resolutions, however data for the other migrant taxa are only available at 10 km Ordnance Survey grid square resolution (Fig 2.6) so in order to compare between the three taxa, I analysed butterfly distribution data at a 10 km grid square resolution.

### 2.3.3 Hoverfly distribution data:

Hoverfly distribution data for migrant species were obtained from the Hoverfly Recording Scheme, by the kind permission of Stuart Ball. The data are available for analysis at a 10 km grid square resolution. The recording scheme was launched in 1976 to collate hoverfly records ready for the publication of a Provisional Atlas in 2000 by the BRC. My analysis of these data was restricted to the time period from 1970 and 1995. Pre-1970 data comprise museum specimens and information from the literature, which are less reliable than observation data in terms of identification and location, and also have poor spatial coverage. Thus pre-1970 data were excluded from my analyses. I also excluded post-1995 data because they are not yet available before the proposed production of a second hoverfly atlas in 2010. (Stuart Ball pers. comm.).

### 2.3.4 Dragonfly distribution data:

Distribution data for migratory dragonfly species were obtained from the NBN Gateway (http://www.searchnbn.net), with distribution records compiled from a variety of recording schemes including the BRC. Data are available at a variety of resolution, but for comparison among taxa were analysed at a 10 km grid square resolution. The data set includes records from 1807 to 1997, although records from before 1960 are scarce.

## 2.4 Selection of Time Periods:

In order to examine distribution changes in these three study taxa over time, distribution data for the three taxa were obtained from the Biological Records Centre (CEH Monks Wood), Butterfly Conservation, NBN Gateway and from Stuart Ball (Hoverfly Recording Scheme). Two time periods were selected for study, spanning a 40 year time period (1960-2000) that coincides with anthropogenic global warming (IPCC, 2001), when the mean annual Central England Temperature (CET) increased by $0.57^{o}$ (data available from the Hadley Centre Climatic Research Unit website: http://www.cru.uea.ac.uk). The time periods were chosen to maximize the quality of the data available for analysis while maintaining a reasonable length of time between the two periods for distribution changes to take place.

### 2.4.1 Butterfly Data Analysis:

For the cross-taxon analysis presented in Chapter 3, distribution data for four migrant butterfly species (as detailed above), were analysed in two time periods, 1970-82 and 1995-99. These two periods were chosen for analysis because they coincide with increased recorded effort prior to the publication of two butterfly atlases in 1984 (Heath *et al*, 1984) and 2001 (Asher *et al*, 2001). Additional data are available up until 2004 but were not included in cross-taxon analysis so that analyses were more comparable to those of hoverflies and dragonflies. This resulted in a 21 year gap between the mid-points of the two time periods during which mean annual UK temperature warmed by $0.51^0$C (calculated from the CET as referenced above). In Chapters 4 and 5, where I focus on analysis of butterflies, I include distribution data up to 2004, thus making use of the most up-to-date data available. This resulted in analysis of distribution changes over a 23-yr period when annual UK temperature warmed by $0.77^0$C.

### 2.4.2 Hoverfly Data Analysis:

Unlike butterflies, distributional data for hoverflies have been collected fairly continuously from 1970 to 1995. Thus, compared to butterflies, there were no clear time periods to select for comparison. When choosing the time periods for study, I made sure there were sufficient data to analyse in each time period whilst keeping a sufficiently large gap between time periods for warming to take place and for species to respond to the warming events. Thus I analysed distribution changes between 1970-1980 and 1990-1995, an 18-yr gap during which mean annual UK temperature warmed by $0.52^0$C (calculated from the CET as referenced above)

### 2.4.3 Dragonflies Data Analysis:

Distributional data for dragonflies have been collected fairly continuously, and I analysed changes in distribution between 1960-1970 and 1985-1995. This resulted in a 25-year gap when UK temperature warmed up by $0.44^0$C (calculated from the CET as referenced above). This selection of time periods for analysis is similar to previous distribution analysis of resident dragonflies by Hickling *et al.* (2005).

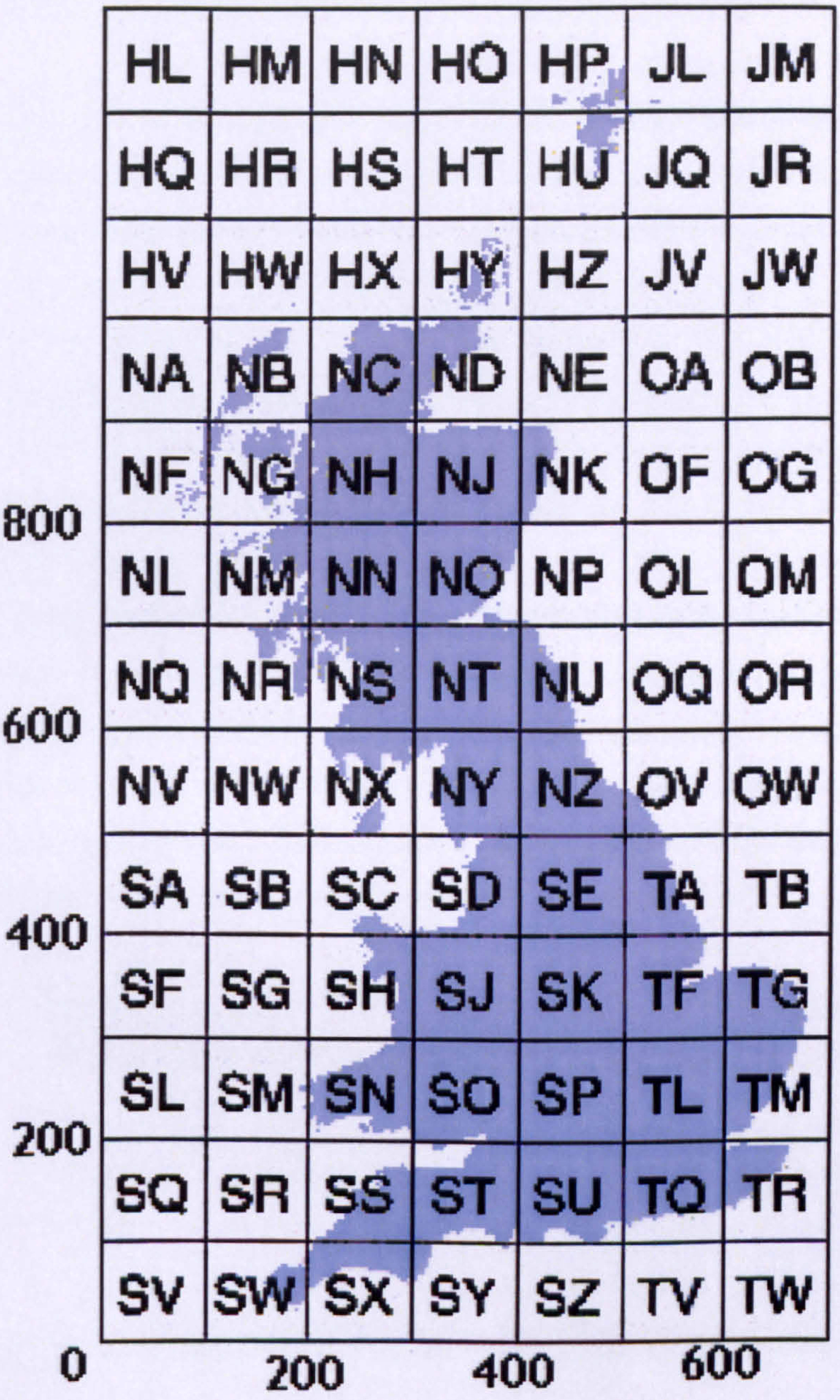


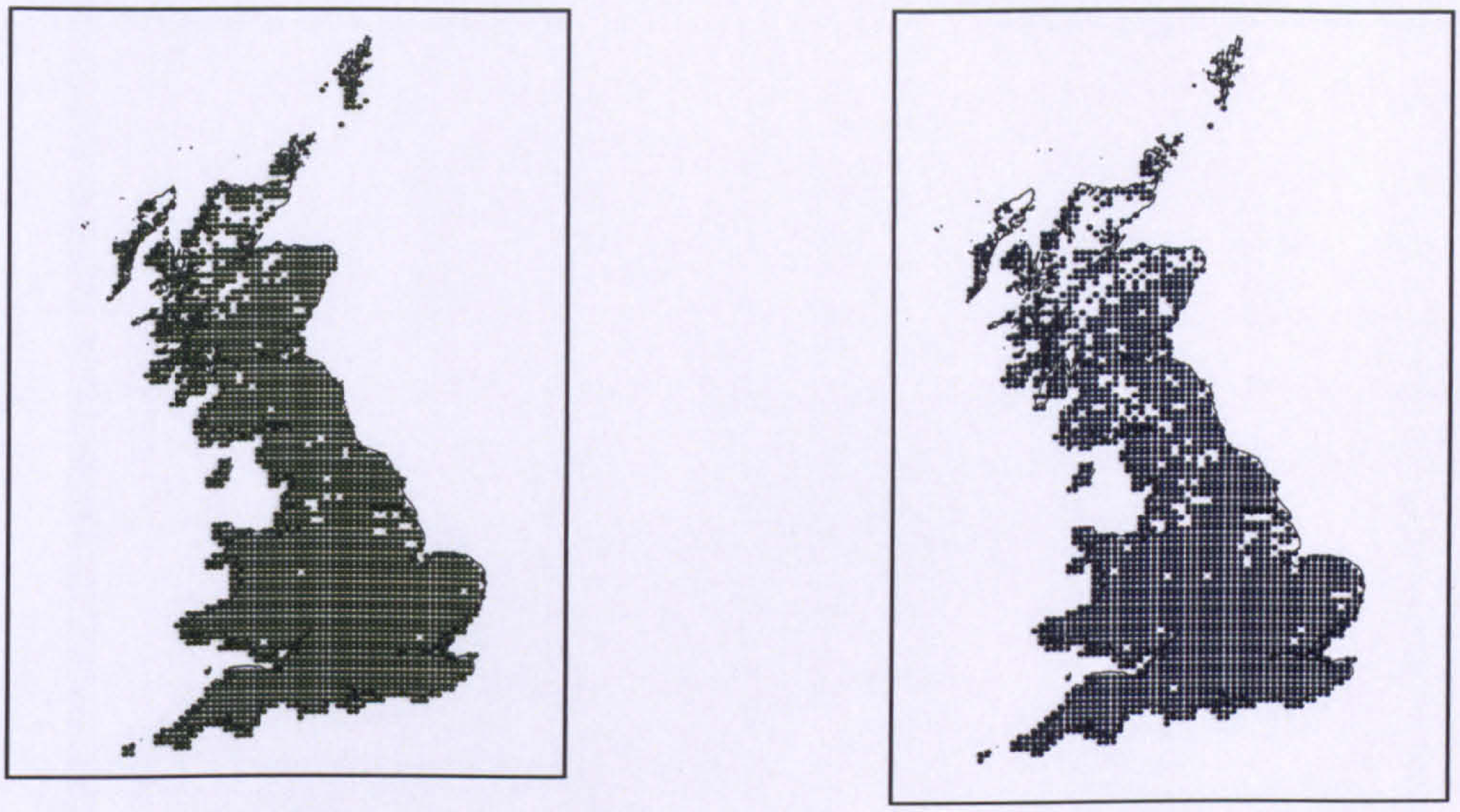
Figure 2.6: The Ordnance Survey 100km grid squares. Each 100km square is identified by a unique pair of letters. Distribution records for butterflies, hoverflies and dragonflies were analysed at 10 km grid resolution (http://storage.plants.ox.ac.uk/eb/images/National%20grid.gif).

## 2.5 Controlling for Recorder Effort in Analyses of Distribution Data:

For distribution data of all taxa, recorder effort has increased greatly over time which may affect the quantification of range shifts (Table 2.1). In Chapter 3, following Hickling *et al.* (2005), four methods were employed to try to account for changes in recorder effort over time. My standard analysis was to include all 10 km squares where any study species from that taxon had been recorded (subsequently termed 'recorded' squares). Thus I excluded grid squares with no records for that taxon. Increasingly strict selection criteria were imposed by repeating the analyses but only including grid squares where at least 5%, 10% and 25% of the total species richness for a particular taxon had been recorded in both time periods (following Hickling *et al.*, 2006). Distribution maps for each study taxon show the localities of the 10 km grid squares analysed under the different levels of recorder effort control (figs 2.7, 2.8 and 2.9). An additional fifth method was employed in butterfly analyses presented in Chapter 4 where sub-sampled data from Fox *et al.* (2006) were also analysed. In this method, Fox *et al.* account for increases in butterfly recorder effort over time by randomly subsampling the number of records in the second time period to equal that in the first time period.

| Taxa | number of records | | number of 10 km squares visited | |
|---|---|---|---|---|
| | 1st time period | 2nd time period | 1st time period | 2nd time period |
| Butterflies | 171,363 | 1,642,432 | 2536 | 2688 |
| Hoverflies | 40,748 | 113,513 | 1339 | 1636 |
| Dragonflies | 3,697 | 16,779 | 658 | 1853 |

Table 2.1: Change in recorder effort, measured as the total number of records (sightings of species received from the general public) and the total number of 10 km squares visited for each taxa.



10 km grid squares recorded in both time periods

At least 5% of species recorded

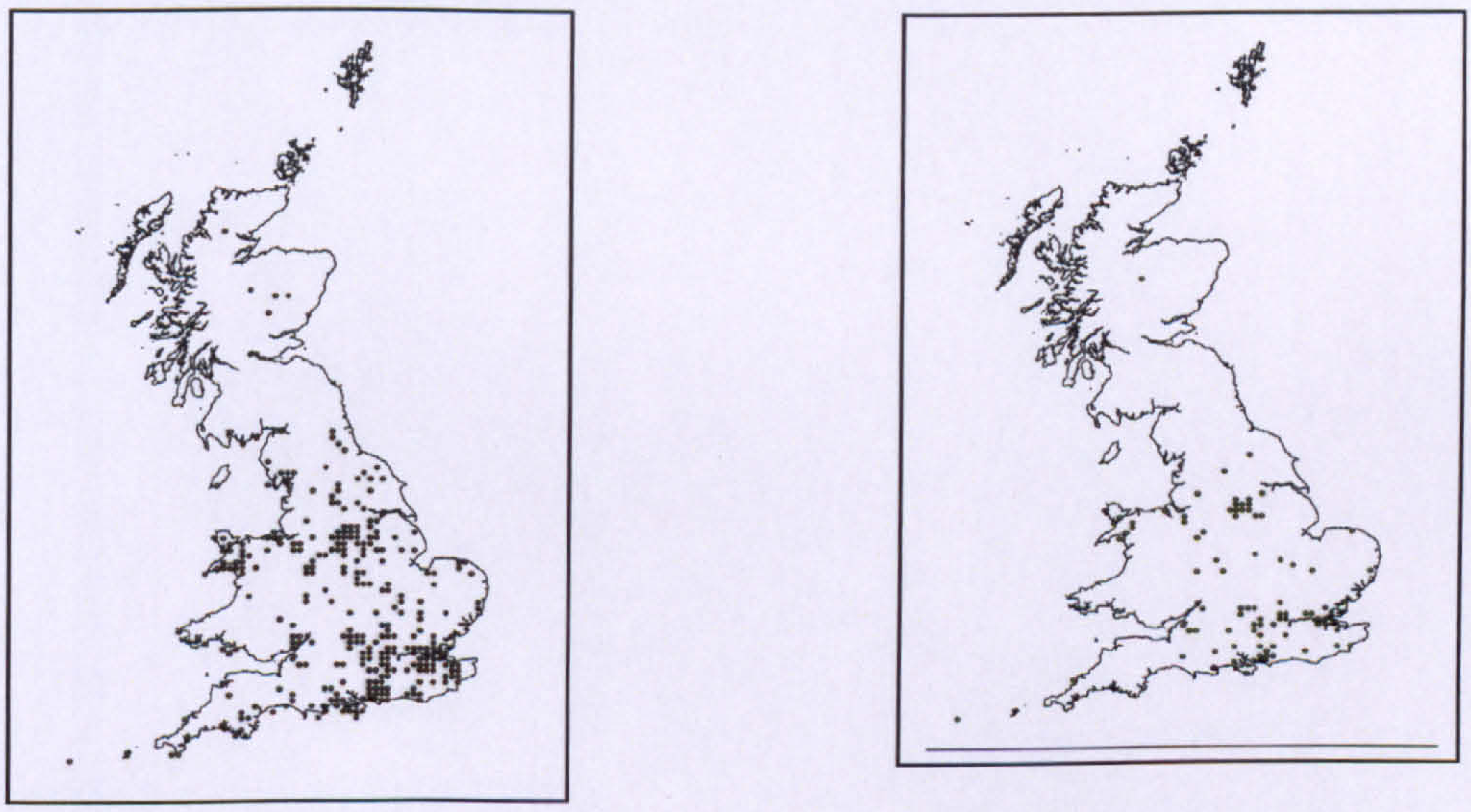
At least 10% of species recorded

At least 25% of species recorded

Figure 2.7: Location of the 10 km grid squares included in butterfly analyses under the different measures of recorder effort control.

Squares recorded in both time periods

At least 5% of species recorded



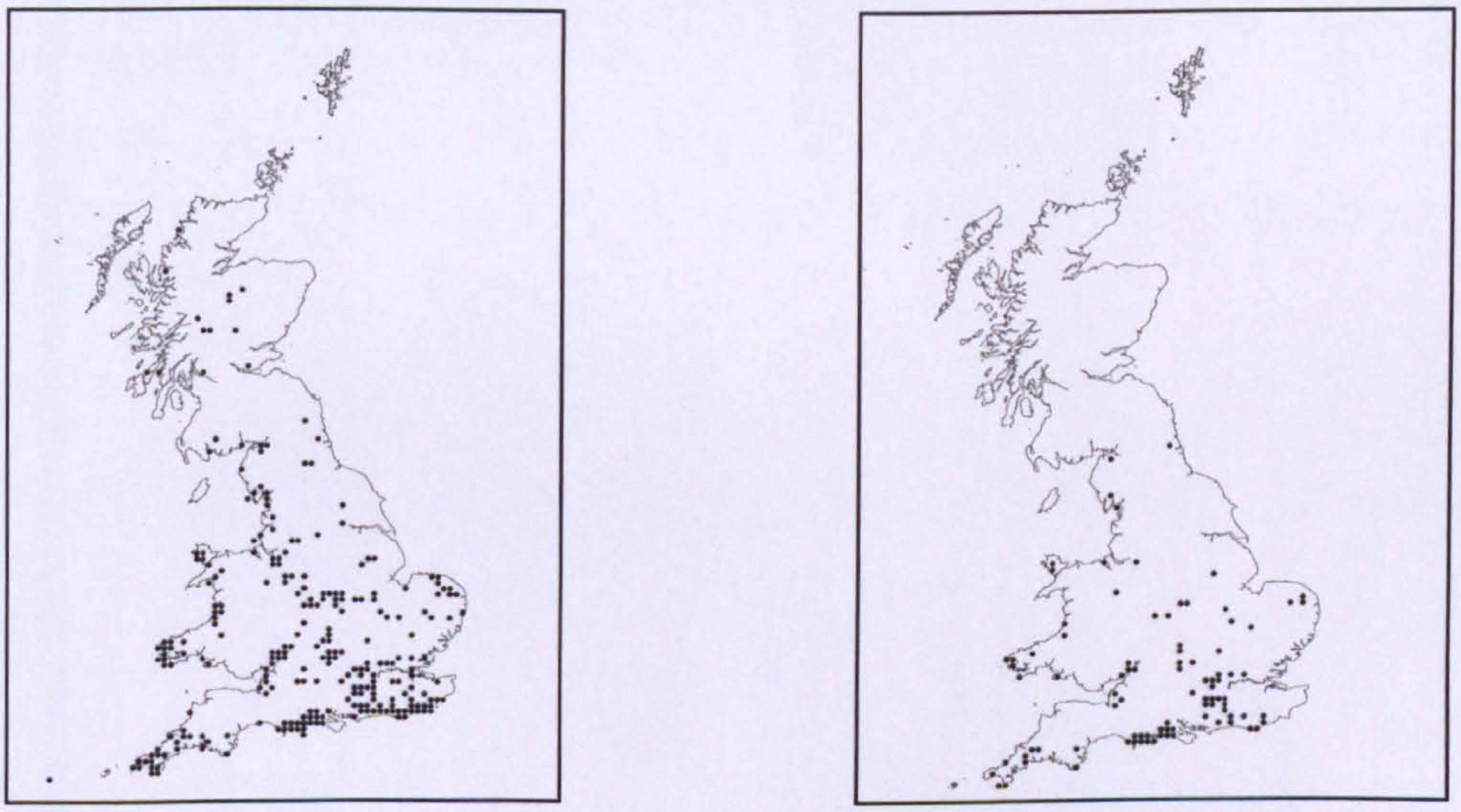
At least 10% of species recorded

At least 25% of species recorded

Figure 2.8: Location of the 10 km grid squares included in hoverfly analyses under the different measures of recorder effort.

Squares recorded in both time periods

At least 5% of species recorded



At least 10% of species recorded

At least 25% of species recorded

Figure 2.9: Location of the 10 km grid squares included in dragonfly analyses under the different measures of control for recorder effort.

## 2.6 The Butterfly Monitoring Scheme:

Chapter 4 includes analysis of changes in butterfly abundance over time based on analysis of UK butterfly monitoring scheme data (UKBMS; Pollard & Yates, 1993; http://www.ukbms.org). The UKBMS was initiated in 1976, with transect counts of butterflies made at 34 sites, mainly in southern Britain. During the late 1970s the number of transect sites increased to about 100, remaining at that number until 1989, since when there has been a steady increase (Fig. 2.10). Transects are walked weekly, under specified weather conditions, from the 1st April to the 29th September making up 26 weeks of recording per year. Transects are walked only if temperatures are greater than $17^0$C, unless there is more than 60% sunshine in which case recording can also take place in temperatures between $13^0$C - $17^0$C. Transects are walked between 10.45 and 15.45 British Summer Time so as not to bias against species that are restricted in the time of day during which they can fly. All butterflies seen within a 5 m distance of the observer are recorded (see Fig 2.12). Although the number of transect sites has increased since 1976 (currently ~700 site, Fig 2.10), the greater proportion of sites still remain in the south of Britain.

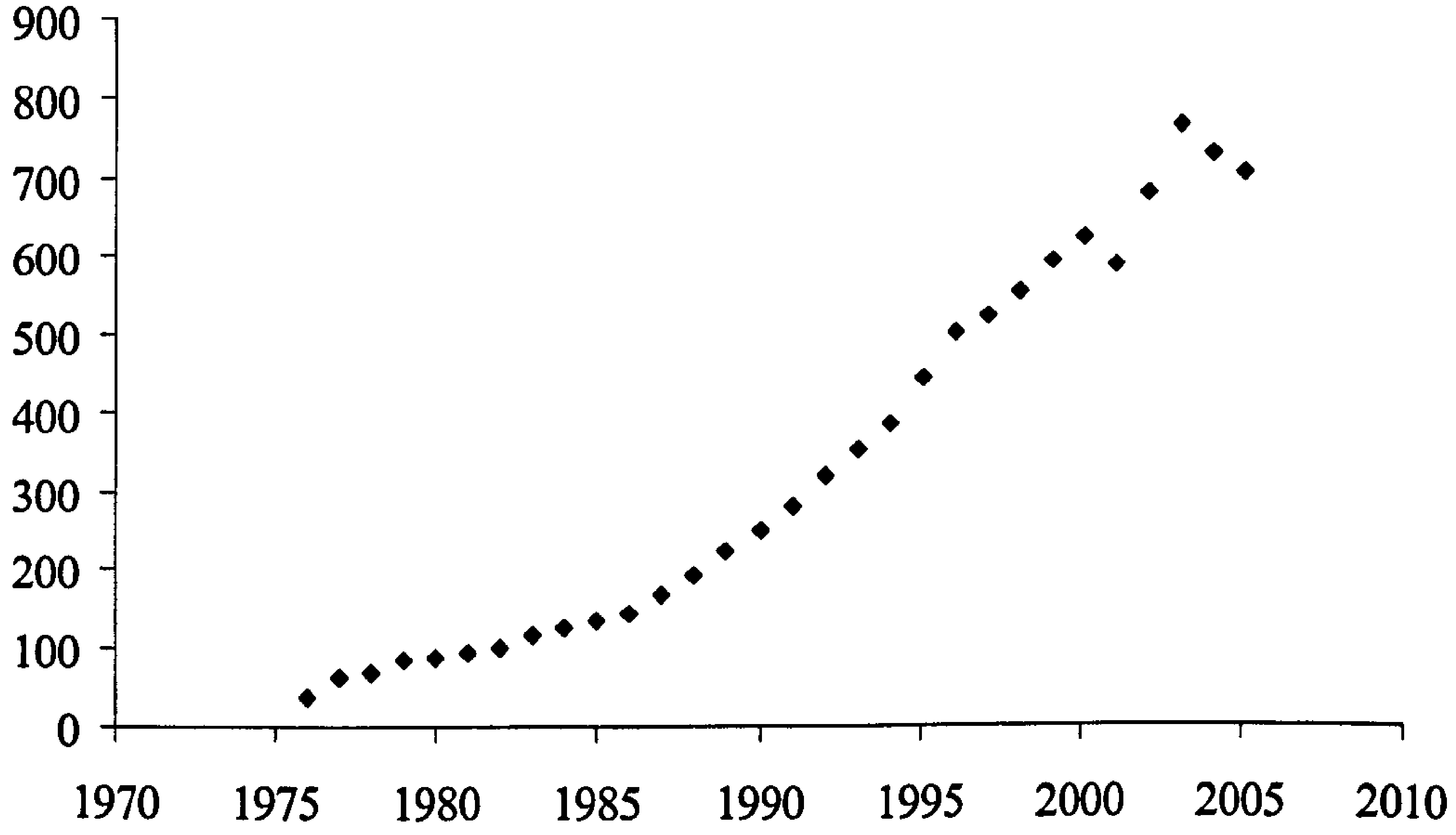


Figure 2.10: The number of sites submitting data to the BMS

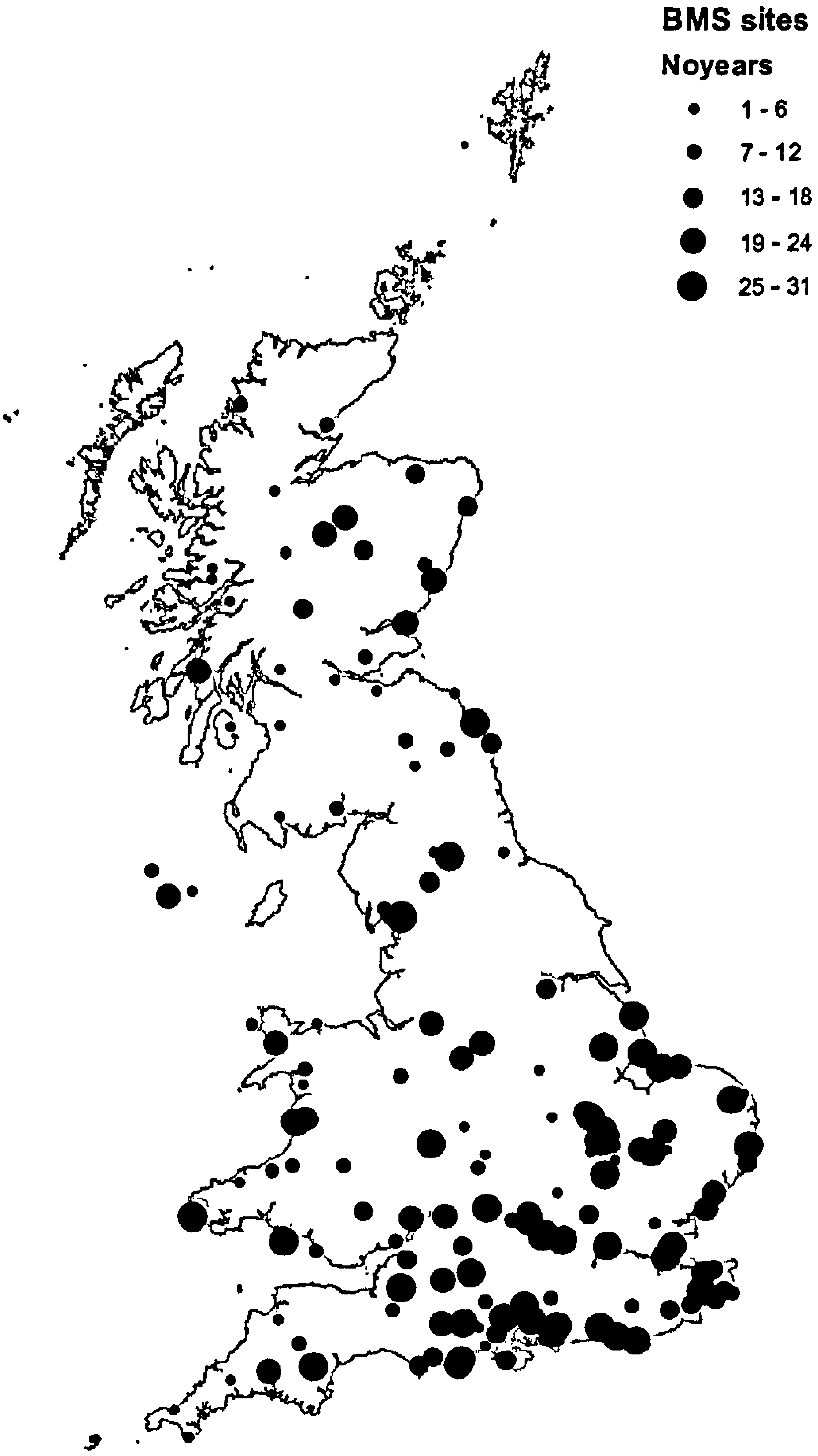


Figure 2.11: Location of the BMS transect sites used in analyses in Chapters 4 and 5, and the number of years that the site was visited by recorders.

The lengths of transects at sites range from 2 - 4 km and incorporate a wide variety of habitat types including chalk and limestone grasslands, different woodland types and coastal habitats. However transect site locations are biased to nature reserves, and agricultural areas are poorly represented.

Transects are walked weekly, but data will inevitability be missing for some weeks, for example due to poor weather conditions, holidays and illness of recorders. For this reason, a collated index is calculated that takes into account these missing weeks (as discussed in Rothery & Roy, 2001, and described at www.ukbms.org). An annual collated index is calculated for each of the 63 species of butterfly that are recorded regularly on transects species using a $\log_e$-linear Poisson regression model and performed using the statistical software package TRIM (Pannekoek & van Strien, 2001). The expected number of butterflies at each site and in each year is assumed to be a product of a site ($i$) and a year effect ($j$), i.e. Expected number of butterflies ($mij$) = site effect * year effect.

Equation 2.1 $$\log (mij) = ai * bj$$

Where $ai$ and $bj$ denoted the effect on a log scale for the $i$th site and the $j$th year.

The model takes into account that some years are more favourable than others in terms of the number of butterfly species ( = the year effect), and that some sites support higher numbers of a particular species than others (= the site effect). Therefore for any particular year where a site has not been recorded, an index value can be computed based how favourable that particular site is and on the general conditions of that year. A collated index across all sites for each year is then calculated for each species as the mean of all recorded site indices in addition to estimated values for missing data. These indices are then presented as log10 values of species abundance.

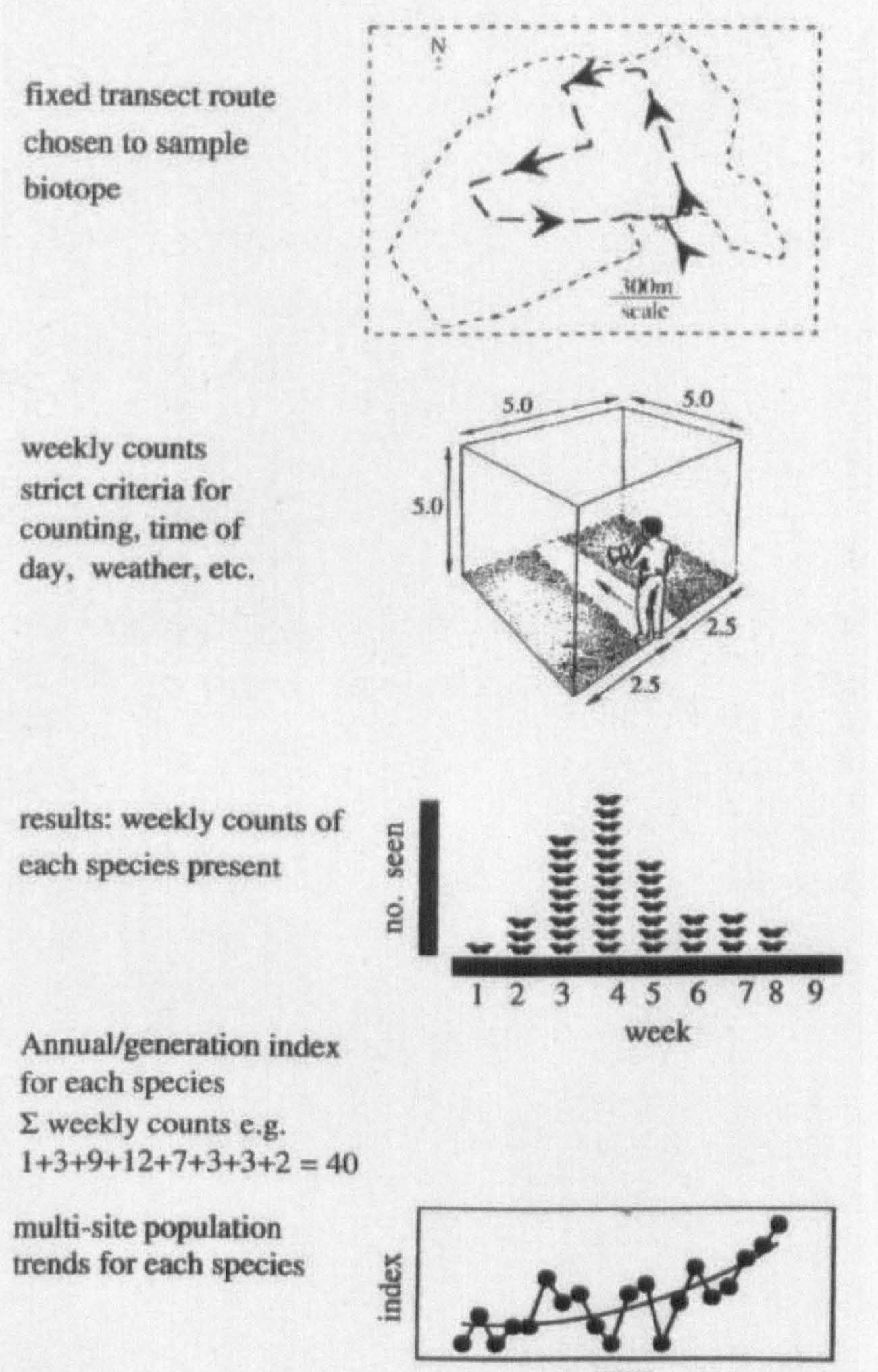


Figure 2.12: Diagram of the five steps used by UKBMS for using transect counts to determine time-series of butterfly population changes (Thomas, 2005).

# Chapter 3

# Comparing Distribution Changes in Migrant Species from Three Insect Taxa

## 3.1 Abstract:

Shifts in species distributions have been documented in many resident species from a variety of taxonomic groups in Britain during a period of climate warming over the past 30 – 40 years. By contrast, less is known about the responses of migratory species. Analysis of distribution data for a range of migratory insects (butterflies: N = 4 species, hoverflies: N = 4 species, dragonflies: N = 2 species) showed that species increased their ranges, and shifted their northern boundaries northward in Britain by 23 - 75 km over a 40-year period during which the climate warmed by 0.44 – 0.52°C. The three study taxa showed similar responses to climate warming in terms of shifts at their northern range boundaries, although butterflies showed significantly greater increases in overall distribution extent compared with the other two taxa. These data suggest that migrant insects from three taxonomic groups are responding to current climate warming, successfully tracking shifting isotherms, and are expected to continue to do so in future.

## 3.2 Introduction:

Many species are changing their distributions in response to climate warming, and shifting their ranges to higher latitudes and /or altitudes (Chapter 1; Parmesan & Yohe, 2003; Root *et al.*, 2003; Parmesan 2006; Wilson *et* al., 2005; Franco *et al.*, 2006; Hickling *et al.*, 2006). Changes in distribution in response to climate changes are not novel responses for species, with glacial/interglacial climatic oscillations during the last 2.4 million years resulting in large changes in insect distributions, demonstrating the ability of some species to track climate (Lawton, 1995). Coope (1995) examined changes in the geographic ranges of Coleoptera in Britain by examining sub-fossil records from the Quaternary period. Coope (1995) showed that many beetles shifted their ranges to track climate, and he found little evidence of extinction or adaptation. However, by comparison with beetles, many British butterflies are failing to track recent climate changes with two thirds of British species declining in abundance and distribution size due to problems of habitat loss and fragmentation (Warren *et al.*, 2001). This suggests that extinction and/or adaptation responses to climate change may be observed in future (Thomas *et al.*, 2004). Many sedentary and specialist butterflies have difficulties colonising new climatically-suitable habitats that are isolated and fragmented, thus preventing range shifts. In contrast, migrants are highly mobile with generalist habitat requirements, and are thus likely to track climate change more successfully, although data are lacking.

Studies over the past decade have documented northward range shifts in a range of taxa, including birds (Thomas & Lennon, 1999), trees and shrubs (Kullman, 2002), marine fish (Perry *et al.*, 2005), butterflies (Parmesan *et al.*, 1999; Hill *et al.*, 1999; Warren *et al.*, 2001), and odonata (Hickling *et al.*, 2005), as well as uphill shifts in regional distributions of butterflies (Parmesan *et al.*, 1999; Hill *et al.*, 2002; Konvicka *et al.*, 2003; Wilson *et al.*, 2005; Wilson *et al.*, 2007). However these studies have focused primarily on resident species and little is known of how climate warming is affecting the distributions of migrant species, especially migrant insects. Previous studies have examined changes in the abundance of migratory Lepidoptera in response to climate warming, with increases in the abundance of migrant species arriving in Britain being positively correlated with rising temperatures (Pollard & Greatorex-Davies, 1998; Sparks *et al.*, 2005; Sparks *et al.*, 2007).

Phenological changes in response to climate warming have been observed in a number of migratory species, particularly in birds (Cotton, 2003) and in the migrant butterfly *V. atalanta* (Roy & Sparks, 2000), with some migrants arriving earlier in spring with increasing temperatures (Roy & Sparks, 2000). Changes in the abundance and phenology of several Lepidoptera species have been reported in response to climate change, although the extent to which their distributions in Britain have been affected by climate warming remains unclear.

Lepidoptera have been the focus of a number of studies examining species' responses to climate change (Sparks *et al.*, 2005; Franco *et al.*, 2006; Wilson *et al.*, 2007; González-Megías *et al.*, 2008; Hellman *et al.*, 2008), being a popular indicator taxon for the following reasons. Butterflies are sensitive to changes in climate because they are poikilothermic, and as such their activities (e.g. development time, fecundity, dispersal) are closely associated with temperature and their geographical ranges often limited by climatic conditions (Roy & Sparks, 2000). Butterflies also have relatively high reproductive rates with one generation or more each year, as well as relatively high dispersal rates (Roy & Sparks, 2000) and as such can show rapid changes in distribution and abundance in response to climate changes over a relatively short period of time (Pollard & Yates, 1993; Parmesan *et al.*, 1996; Roy & Sparks, 2000), while adult butterflies are also conspicuous and have a high public profile with over 10,000 recorders in Britain and Ireland (Roy & Sparks, 2000; Asher *et al.*, 2001). However, the ability of this well-studied taxonomic group to represent changes in other less well-studied taxa is questionable. In studying species responses to climate warming, very few authors have focused on multi-taxon comparisons, with resources and time being a considerable limiting factor. The reliability of using indicator species has been questioned in a number of studies investigating species conservation (Prendergast *et al.*, 1993; Sebastio & Gelle, 2009) and extinction rates (Thomas *et al.*, 2004), where severe underestimations have been recorded. A problem with focusing on a specific taxon, and in particular, limiting investigations to just a few species within that taxon, is that life history characteristics may vary greatly between groups, such that species with different generation times, habitat requirements, thermal requirements and dispersal capabilities are likely to respond differently to changes in environmental variables (Thomas *et al.*, 2001; Warren *et al.*, 2001; Hill *et al.*, 2002; Wallisdevries & Van Swaay, 2006; Fox *et al.*, 2006; Sebastio & Gelle, 2009). As such, comparing species across a range of taxonomic groups will give a much better picture of responses to climate warming. This was shown by Hickling *et al.* (2006), who found that in some cases the responses of resident species of less well-studied taxa exceeded those of better-known groups. Migrants vary greatly within and among taxonomic groups, for example in their natal origin (short-distance vs long-distance migrants), breeding territories (site-specific vs broad-front migrant) and habitat requirements (aquatic/terrestrial vs terrestrial). Therefore, to get a better understanding of the effects of climate warming on migrant species it is important to study responses in a variety of taxonomic groups, an area of research that is under studied.

### 3.2.1 Rationale for Further Work:

Previous work has examined how distributions of resident species have changed over the past 40-50 years, but less is known of how climate warming is affecting the distributions of migratory insects. It is important to study responses of migrants to climate warming because some migrants are pests (Werker *et al.*, 1998; Frost, 2003; Venette *et al.*, 2003) and thus positive responses of migrants to climate warming may have negative economical consequences (Cannon, 1998). By contrast, some migrant insects are economically valuable, with the migrant hoverfly *Episyrphus balteatus* being among one of the most important predators of cereal aphids throughout Europe (Tenhumberg & Poehling, 1995; Almohamad *et al.*, 2007). While there is some understanding of how migrant butterflies are responding to current climate warming, these responses may not be representative of other migratory insects. In this chapter. I analyse changes in the British distributions of migrant insects from three taxonomic groups in order to investigate the degree to which species are tracking recent climate change, and whether these responses are similar among taxa.

### 3.2.2 General Aims and Predictions of this Chapter:

This chapter investigates how the distributions of migrant insects from three taxa (butterflies, hoverflies and dragonflies) have changed over the past 30-40 years, a period of significant anthropogenic climate warming (IPCC, 2001; 2007). I analyse distribution data from the Biological Records Centre (CEH Monks Wood, now Wallingford), Butterfly Conservation and from Stuart Ball (Hoverfly Recording Scheme). I quantify changes in distribution extent and shifts at the northern range boundaries in 10 migrant species over a 40 year period. I examine whether species are responding to climate warming to a similar extent, and the impacts of changes in recorder effort over time on findings. This chapter has the following objectives:

1. Quantify changes in distribution extent and shifts at northern range margins in 10 migrant species over the past 40 years in Britain.

2. Investigate how changes in recorder effort over time affect reported responses of species over the past 40 years.

3. Determine whether the three taxa are responding to climate warming to the same extent, in terms of changes in distributional extent and latitudinal shifts.

4. Determine whether migrants have shown greater rates of distribution change at more northern latitudes.

5. Determine the reliability of the methods used in this Chapter for comparing between different groups of species

## 3.3 Materials and Methods:

### 3.3.1 Study Species Selection:

As described in Chapter 2, distribution data were obtained for Britain for 10 migrant species from three taxa; butterflies (Rhopalocera), dragonflies (Odonata) and hoverflies (Syrphidae). All 10 study species arrive in Britain during spring and summer and develop through at least 1 generation before returning to over-wintering sites. All study species reach their northern range limit in the UK, and with the exception of *Nymphalis antiopa,* all follow approximately the same migratory route, moving north in the spring, and south in the autumn. *Nymphalis antiopa* was included in this study, because although it follows a west-east migration pattern, as shown in Fig 2.4 (Chapter 2), the species has a southern distribution within Britain, thus having the potential to expand northwards. All study species are highly mobile, and have generalist habitat requirements.

### 3.3.2 Data Analysis:

I examined changes in distribution size and shifts at the northern range margin over time. As discussed in Chapter 2, the time periods chosen for analysis were selected to exploit the greatest number of distribution records available whilst maintaining a sufficiently large gap between the two time periods to allow species to respond to climate warming (see Table 3.1). Due to the nature of the different distribution datasets for the three taxa, it was not possible to analyse exactly the same time periods for all species. However, the length of time periods differed by only a maximum of 7 years among species, resulting in temperature increases differing by only a maximum of 0.08°C among species (as shown in Table 3.1).

### 3.3.3 Distribution Size:

For each species, the absolute change in distribution extent was calculated as the difference in the number of 10 km OS grid squares occupied between the first and second time periods. However, the 10 study species varied considerably in terms of their initial distribution size, and so I also computed proportional changes in distribution extent. For each species I calculated the difference in the number of 10 km grid squares occupied between the first and second time period divided by the total distribution extent over the whole time period.

Increases in recorder effort over time may bias estimates of species' responses to climate. Thus for both measures of changes in distribution size, I used four methods described in Chapter 2 to try and account for increased recorder effort over time. For all species, I analyzed all data ("no control"), only those grid squares with >5% species richness, >10% species richness and >25% species richness of the taxonomic group being studied (see Chapter 2 for more details).

### 3.3.4. Range Margin Shifts:

The location of each species' northern boundary in each time period was defined as the mean latitude of the ten most northerly occupied 10-km grid squares (following Hickling *et al.*, 2005). Shifts at the range boundary were calculated as the difference in the mean latitude between the two time periods. As described above, four methods of recorder effort control were applied to these analyses to take into account changes in recording effort over time.

### 3.3.5 Latitudinal Affect:

The study species migrate into the UK from overwintering areas in southern and central Europe and North Africa. I examined whether changes in distributional extent of study species in Britain are related to latitude. The area of Great Britain was divided up into 13 100-km latitudinal bands based on the OS grid system. Both the absolute change and proportional change in distribution extent over time (as described above) was calculated for each latitudinal band for each of the 10 migratory species. As before, this analysis was repeated for each level of recorder effort control (see above). For each species, I used regression analysis to compare slope values from relationships between proportional change in distribution extent and latitude. In addition, for each species I also carried out regressions to determine if latitude has any effect on the absolute change in distribution extent as measured above. For these analyses, I carried out stepwise regressions in which I regressed the number of records in the later time period against latitude and number of records in the first time period. Thus this analysis tested whether there was any effect of latitude on distribution change, after taking account of initial distribution size.

The distribution extent (number of 10 km grid squares with records) in both time periods for each species and latitudinal band is shown in Table 3.2, with the total number of 10-km grid squares present in each band also stated.

As shown in Figs 2.7, 2.8 and 2.9, in Chapter 2, as the control for recorder effort becomes increasingly strict, northern squares begin to be lost. Therefore in the latitudinal band analysis I was unable to apply the fourth recorder effort analyses (>25% species richness) due to the lack of records at higher latitudes, as shown at Table 3.2.

| Taxa | n | Time periods | | Mean temperature in 1st time period (°C) | Mean temperature in 2nd time period (°C) | Overall change in temperature (°C) |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | | | |
| Dragonflies | 2 | 1960-70, | 1985-95 | 9.3 | 9.74 | +0.44 |
| Butterflies | 4 | 1970-82, | 1995-99 | 9.53 | 10.04 | +0.51 |
| Hoverflies | 4 | 1970-80, | 1990-95 | 9.53 | 10.04 | +0.52 |

Table 3.1: Information on study species used in analyses. The table shows the number of species analysed (n) and the degree of climate warming between the mid-points of each time period. (Climate data available from the Hadley Centre Climatic Research Unit website: http://www.cru.uea.ac.uk).

| Latitude | *V.atalanta* | *V.cardui* | *C.colias* | *N.antiopa* | *E.balteatus* | *S.pyrastri* | *S.vitripennis* | *S.scripta* | *A.mixta* | *S.striolatum* | Total number of 10 km squares available |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 141 | 141 | 127 | 20 | 85 | 58 | 56 | 58 | 9 | 46 | 141 |
| 100 | 373 | 372 | 341 | 87 | 204 | 123 | 147 | 169 | 82 | 119 | 376 |
| 200 | 435 | 425 | 293 | 111 | 140 | 68 | 90 | 102 | 37 | 81 | 439 |
| 300 | 356 | 349 | 195 | 89 | 176 | 91 | 121 | 118 | 19 | 59 | 356 |
| 400 | 211 | 200 | 78 | 48 | 80 | 35 | 52 | 34 | 3 | 21 | 227 |
| 500 | 206 | 180 | 46 | 20 | 42 | 25 | 22 | 9 |  | 10 | 191 |
| 600 | 210 | 177 | 34 | 15 | 18 | 9 | 3 | 1 |  | 4 | 270 |
| 700 | 144 | 113 | 9 | 5 | 16 | 4 | 6 | 2 |  | 4 | 263 |
| 800 | 157 | 104 | 6 | 16 | 17 | 3 | 7 | 1 |  | 11 | 284 |
| 900 | 39 | 35 |  | 2 | 1 | 1 |  |  |  | 4 | 142 |
| 1000 | 15 | 14 | 1 |  | 1 | 1 | 1 |  |  |  | 32 |
| 1100 | 22 | 15 | 1 |  | 1 | 1 | 1 |  |  |  | 38 |
| 1200 | 3 | 3 |  |  |  |  |  |  |  |  | 6 |

Table 3.2: Distribution extent (total number of 10-km squares occupied) in both time periods (such duplicates present in both years were not included) in each 100 km latitudinal band for 10 migratory species. There is no control for recorder effort.

## 3.4 Results:

### 3.4.1 Distribution Size:

The number of records vary considerably among the study species, as indicated in Table 2.1 (Chapter 2). Butterflies have the greatest number of records, with an average of 1532 per species for the 1st time period and 4182 per species for the 2nd time period, while dragonflies have the least number of records for the 1st time period (average of 187 records per species), and hoverflies have the least number for the 2nd time period (average of 2562 records per species). Across all species, *V. atalanta* has the greatest number of records (3418 and 8259 records for the 1st and 2nd time period, respectively), while overall *N. antiopa* has the least with 99 and 658 records respectively.

All ten migratory species showed an increase in their distribution extent over the last 40 years, with an average increase across all species of 129-320 10-km grid squares occupied (depending on level of recorder effort control). Comparing among the three taxonomic groups, butterflies increased their distribution extent significantly more than hoverfly species (ANOVA, Tukey, $p<0.05$ for all levels of recorder effort; butterfly, mean increase = +526 10 km grid squares; Fig 3.1a). Butterflies also increased more than dragonflies (ANOVA, Tukey, $p<0.05$ for all but the strictest level of recorder effort when $p=0.081$; Fig 3.1a). There was no significant difference in the extent to which migrant hoverfly and dragonfly species changed their distribution over time (ANOVA, Tukey, $p>0.05$). For both butterflies and dragonflies, applying sub-sampling methods to account for changes in recorder effort had little impact on the results. However for hoverflies, distribution size apparently decreased over time at the strictest levels of sub-sampling (10% and 25% level; Fig 3.1a).

Analysis of proportional data generally supported these analyses and showed that all ten study species increased their distribution extent over the last 40 years by 18-45% depending on the level of recorder effort control, although increases were not always significantly different from zero (Fig 3.1c). However, compared with analyses of raw data, there were few differences observed among the three taxonomic groups although butterflies increased significantly more than hoverflies at the 10% level of recorder effort control (ANOVA, Tukey, $p=0.041$).

The three taxa differed slightly in the temperature increases experienced over time because of differences in the lengths of time periods examined, and this may have affected the comparisons among taxa. Thus the results for the absolute change in distribution size were adjusted relative to the temperature change between the two time periods for each taxonomic group. This showed a similar pattern to that described above, with all species showing an increase in distribution, with an average over all the species of 264 – 661 10-km grid squares per °C (depending on level of recorder effort control). Similarly butterflies increased their distribution extent significantly more than hoverflies (ANOVA, Tukey, $p<0.05$ for all levels of recorder effort control) and dragonflies (ANOVA, Tukey, $p<0.05$), except at the 25% level (ANOVA, Tukey, $p=0.083$).

### 3.4.2 **Range Margins Shifts**:

All ten species showed a northward shift at their northern range boundaries. The average shift over all species was 23-75 km northwards, depending on the level of sub-sampling, although this shift was rarely significantly different from zero (Fig 3.1b). There were no significant differences in northward shifts between the three taxa (ANOVA, $p<0.05$, for all levels of recorder control). For all three taxa, the methods applied to control for changes in recorder effort over time appear to have little qualitative impact on the shifts in the northern range boundary.

As above, the northward shift was also computed as the range shift per °C. The average northward shift over all species was 47-134 km northwards per °C, depending on the level of subsampling, with no significant differences observed between the three taxonomic groups (ANOVA, Tukey, $p>0.05$ for all levels of recorder effort control).

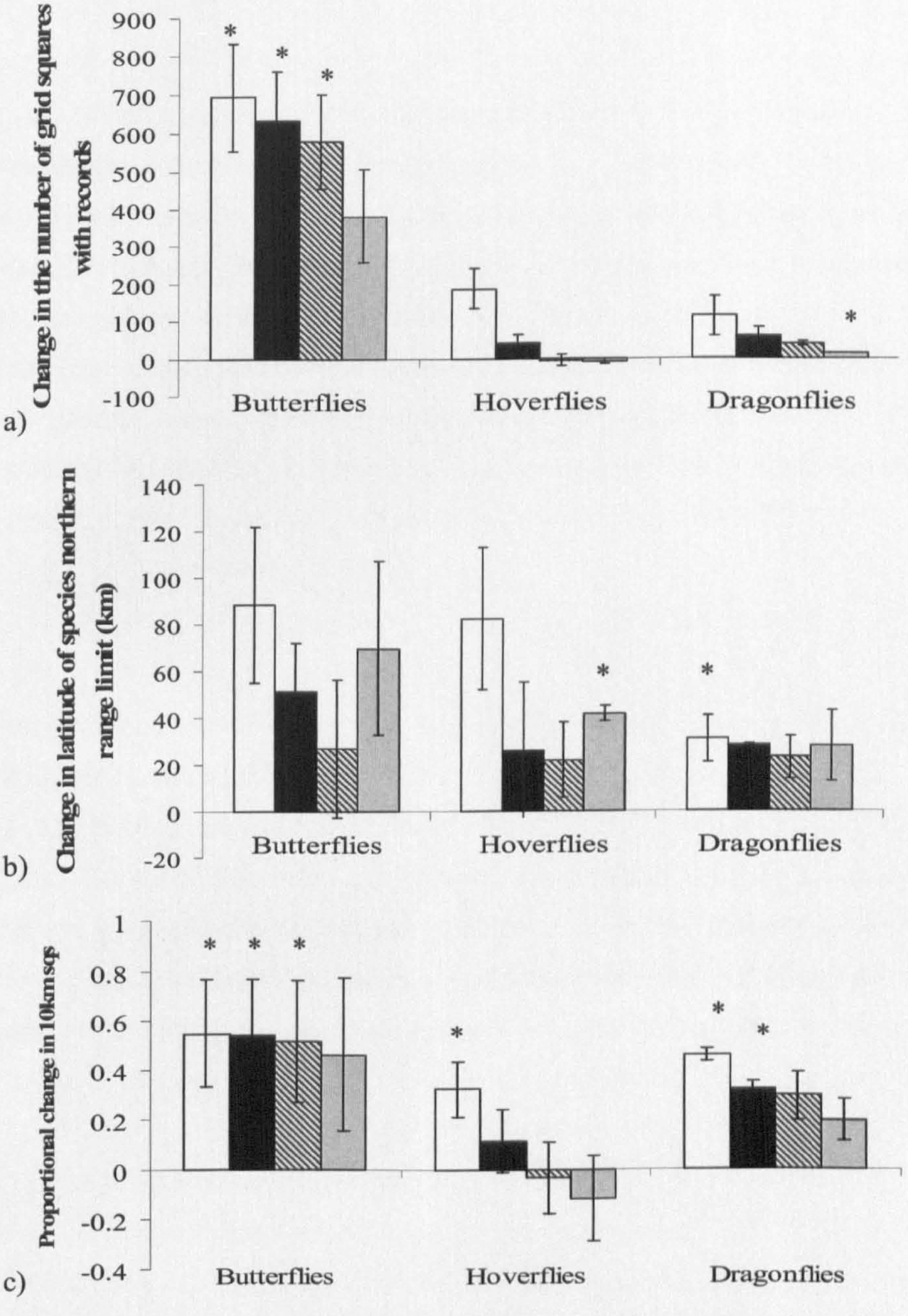


Figure 3.1: Changes in (a) distribution extent (b) northern range boundary and (c) proportion change in distribution extent of migratory butterflies, hoverflies and dragonflies under different levels of recorder effort. White = all recorded squares, black = only including squares with ≥ 5% species richness in both time periods, hatched = ≥ 10% species richness, grey = ≥ 25% species richness. Means and standard errors are shown. Significance is based on one-sample t-test: * $p<0.05$

### 3.4.4 Latitudinal Affect:

The affect of latitude in the proportional change in distribution differed among the 10 species, with the level of recorder effort having a big effect on the outcome. Six out of the 10 species showed a positive relationship between latitude and distribution change, with greater increases in the number of 10-km squares occupied at higher latitudes (Fig. 3.2). However the two butterflies, *C. crocues* (Regression, $p>0.05$, 25% recorder effort control level) and *N. antiopa* (Regression, $p>0.05$. 10%, 25% control level), appear to have increased their distribution to a greater extent at lower latitudes (Fig. 3.2). Latitude appears to be having the greatest effect on dragonflies, with *A. mixta* and *S. scripta* exhibiting the steepest regression slopes, albeit this was only significant for *S. scripta* at the lower levels of recorder effort (Regression, $p<0.05$ for 'no-control' and 5% level of recorder effort control).

The output from the stepwise regression analyses for the effect of latitude on the absolute change in distribution extent showed that for all 10 species, the number of records in a latitudinal band in the later time period was dependent on the number of records in the first time period (as predicted, Regression, $p<0.05$ for all species). However the effect of latitude of distribution change was significant in only two species, *E. balteatus* (Stepwise Regression, latitude effect $p=0.043$) and *S. pyrastri* (Stepwise Regression, latitude $p=0.031$). For both these species, increase in distribution extent over time was negatively related with latitude (*E. balteatus* Stepwise Regression, $b = -0.158$; *S. pyrastri* Stepwise Regression, $b = -0.352$; where b is the regression coefficient), showing that expansion was greater at low latitudes, the opposite of what I predicted. These significant effects of latitude were evident only in the 'no control' treatment, and were no longer evident under more stricter controls for recorder effort.

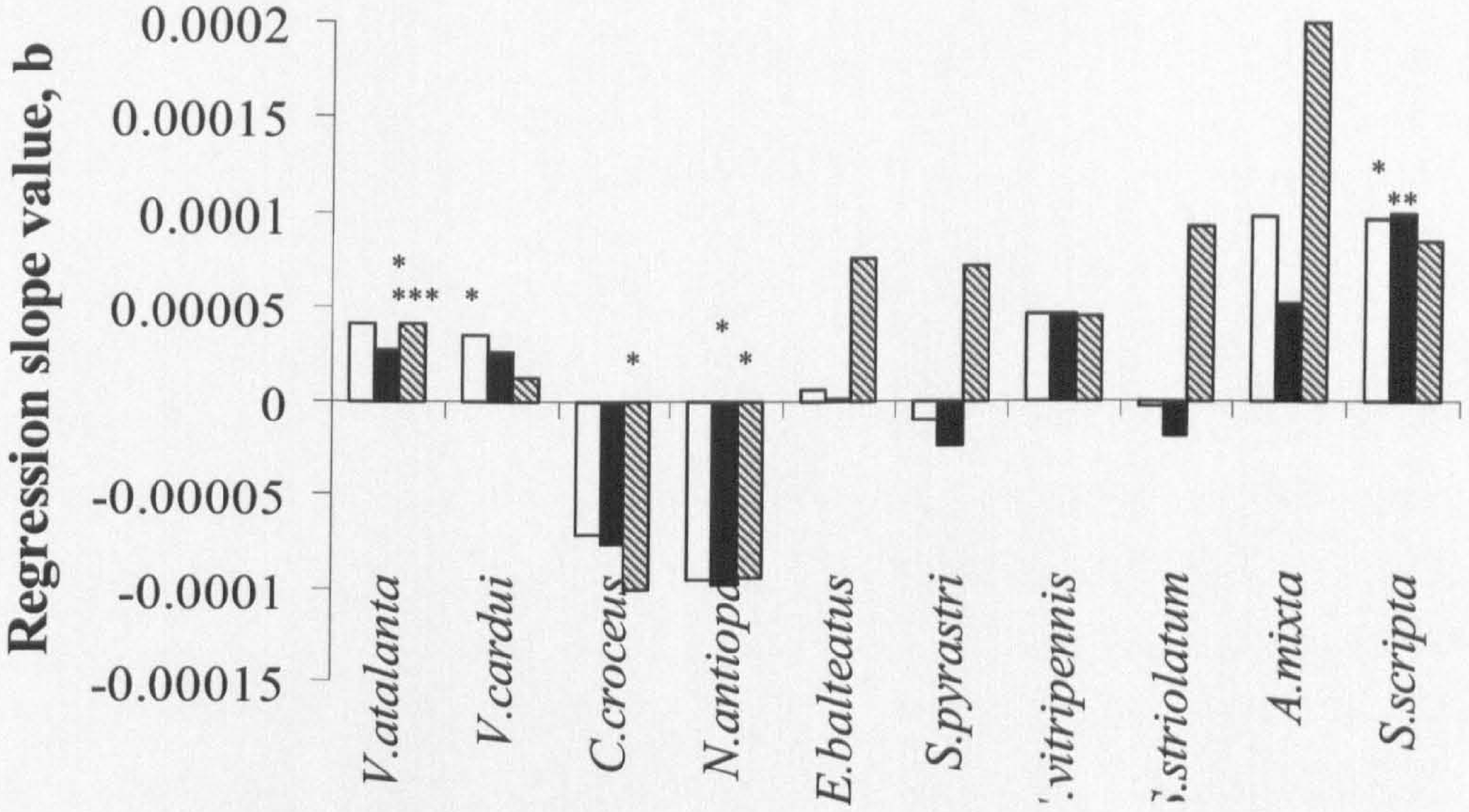


Figure 3.2: The linear trend (b) from regressions of proportional change in distribution extent with latitude for migratory butterflies, hoverflies and dragonflies. White = recorded squares in both time periods, black = only including squares with ≥ 5% species richness in both time periods, hatched = ≥ 10% species. Significance is based on the regression; * p<0.05, ** p<0.01, *** p<0.001 (N = 4 butterfly, 4 hoverfly and 2 dragonfly species).

## 3.5 Discussion:

### 3.5.1 Methodological Issues:

A number of problems arise with comparing data among different taxonomic groups. The greatest problem that arose during this study was in the selection of species, and the low samples sizes that this created such that any findings lack statistical power. As shown in Chapter 2 (Table 2.1) the recording scheme for butterflies has received a much greater number of records than for the other taxonomic groups (10-fold greater than hoverflies, and 100-fold greater than for dragonflies). However, the analyses presented in this Chapter suggest that shifts in ranges of species from less-well recorded taxonomic groups do not differ greatly from those that have received much greater recorder effort. Overall, these data indicate that migrants are expanding northwards in the UK, as has been demonstrated for resident species from a wide range of taxonomic groups (Hickling *et al.*, 2006).

Within the data, the greatest problem for analysis arises from the increase in recorder effort that has occurred over time (as discussed in Chapter 2), and this has the potential to result in overestimation of distribution increases. To account for this I followed the methods used in a cross-taxon comparison analysis by Hickling *et al.* (2006). I only included grid squares that were surveyed in both time periods, and then repeated the analysis with sub-sampled data based on species richness at each 10-km grid square. In many cases there was little qualitative change in findings for different levels of recorder effort, but the strictest recorder effort control often resulted in few data for analysis.

A further problem arose from having to analyse different time periods for the three taxonomic groups. The temperature change between the two time periods differed for the butterflies, dragonflies and hoverflies, by a maximum of 0.08°C. To account for this difference, the relative changes in distribution extent and northward range shifts were calculated per 1°C, thus making comparisons between the species directly comparable. However, this adjustment made little impact on the results, as shown in section 3.4 above, with all species exhibiting increases in their distribution extent and northward range shifts, regardless of whether length of time was accounted for.

### 3.5.2 Cross-taxon comparison:

The results show that all three taxonomic groups have increased their distribution extent over the past 40 years. Initial analyses suggested that butterflies responded more than hoverflies and dragonflies. However, once differences in recorder effort among taxa were controlled for, no significant difference among taxa was observed. Amongst all species, *Colias croceus* exhibited the greatest increases in distribution extent over time, with *Syrphus vitripennis* showing the least change.

Northward shifts in range margins were observed in all three taxonomic groups, with an average shift north of 23-75 km, with no significant differences among taxa. These values were adjusted to take into account the change in temperature between the two time periods, resulting in average northward shifts of 47-134 km per degree Celsius (for 25% level and 'no-control' respectively). Isotherms shift northwards at a rate of 100-133 km per degree Celsius (Hughes, 2000), so it is apparent from the results that the insect migrants analysed in this study are successfully tracking climate change. *Nymphalis antiopa* exhibited the greatest northward shift and the hoverfly *Scaeva pyrastri* showed the smallest northerly shift. These northward shifts (not adjusted values) are comparable to previous studies of resident species by Hickling *et al.* (2006) who found that dragonflies and damselflies showed a mean northward shift of 80 km over the same time period, while butterflies have shifted their northern range margin by a mean of 35 km, again over the same time period.

Although migrants appear to be shifting their northern range boundaries to higher latitudes, increases in distribution extent was not related to latitude, with the majority of species not showing evidence of greater range expansion at northern latitudes (Fig 3.2). Dragonflies appear to be increasing their distribution extent at a greater extent at higher latitudes than the other taxonomic groups, however this was only significant in *S. scripta* when comparing proportional changes with latitude. From this result it is concluded that migrant are 'filling in' the gaps, colonising habitats at lower latitudes before they begin to colonising more northerly habitats.

The methodology used within this chapter have proven to be robust for a multi-taxon comparison, producing results that are easy to interpret across the suite of species while being equally comparable between groups. As such, this methodology can be used further to examine responses between migrants and non-migrants within chapter 4.

## 3.6 Conclusion:

Migrant species from three taxonomic groups are increasing their distribution extent, and shifting their northern range limit to higher latitudes during a period of climate warming. All three groups are apparently responding to the same extent. These results were generally robust to different methods for accounting for recorder effort; butterflies were the best and hoverflies the worst recorded taxa. These results suggest that migrants are successfully tracking climate, and are likely will continue to do so in future as the climate continues to warm.

# Chapter 4

# Migrant Butterflies Respond Rapidly to Climate Warming

## 4.1 Abstract:

Many species have expanded their distributions polewards during recent climate warming, but most studies have focussed on resident species and information on migrants is relatively limited. Butterfly data were analysed to compare how the distribution and abundance of migrants and residents (generalist and specialist species), have changed in Britain over the last 30 years. Migrants have become more abundant and widespread, and these responses to climate warming were generally greater than those of generalist and specialist resident species. It is concluded that relatively short-distance, broad-front migrants are successfully tracking changes in current climate. The concerns that have been raised about the ability of trans-continental, site-specific migrants to track climate change do not seem to apply to these insects.

## 4.2 Introduction:

### 4.2.1 Previous Studies of Distribution Change:

Studies over the past decade have documented range shifts towards higher latitudes – in the northern hemisphere - in a wide range of southerly-distributed taxa (Parmesan *et al.*, 1999; Warren *et al.*, 2001; Kullman, 2002; Perry *et al.*, 2005; Hickling *et al.*, 2006), as well as shifts to higher elevation (Parmesan, 1996; Hill *et al.*, 2002; Konvicka *et al.*, 2003; Wilson *et al.*, 2005; Franco *et al.*, 2006). Many resident insect species have expanded their distributions northwards in Britain during recent climate warming (Hickling *et al.,* 2006). However, range expansions do not necessarily occur in all species, and they are most frequent in generalist species which are mobile and whose breeding habitat is widely available across the landscape. By contrast, specialist species have failed to track recent climate warming due to difficulties in colonising newly-available, climatically suitable habitats in highly fragmented landscapes (Warren *et al.*, 2001). However, these studies have focused on resident species and less is known of how climate warming is affecting the distributions of migrant species.

### 4.2.2 Possible Responses of Migrants to Climate Change:

Perspectives on how climate warming may affect migrant species seem to differ among researchers studying different taxa. For example, there are concerns that climate warming will affect migratory bird species not only in their summer breeding territories but also along migration routes and in their over-wintering habitats (Ahola *et al.*, 2004; Huntley *et al.*, 2006). Long-distance migrant birds may be particularly vulnerable to climate warming, and mismatches between breeding and food availability of these species have been shown to result in declines in the abundance of migrants (Both *et al.*, 2006). The alternative perspective is that migrants with their high mobility and often generalist habitat requirements should be able to track climate change. For example, migratory Lepidoptera have increased in abundance during recent climate warming (Pollard & Greatorex-Davis, 1998; Sparks *et al.*, 2007) and are likely to continue to do so in the future (Sparks *et al.*, 2005). Given that many migrant insects are pests, there are concerns that increases in their abundance and distribution under future climate warming may have negative economic consequences (Cannon, 1998).

### 4.2.3 Rationale for Further Work:

Distribution changes in terms of both distribution extent and range shifts have been documented in resident butterfly species, showing that range expansions are generally confined to generalist species (Warren *et al.*, 2001). Migrants share similar traits to generalist resident species, in having broad habitat requirements and high mobility, and therefore might be expected to be tracking climate changes to a similar extent, although data are lacking. In Chapter 3, I compared distribution changes in migrants from three different taxa, and in this chapter I will focus on one taxon, butterflies, and examine responses of three different ecological groups: migrants, and resident generalists and specialists.

### 4.2.4 General Aims and Predictions of this Chapter:

This chapter investigates distribution and abundance changes in three groups of butterflies in Britain, generalist and specialist resident species, and migrant species arriving in Britain each spring. Data from the UKBMS and BMN (see Chapter 2 for details of the datasets) are analysed to examine how migrants are responding to recent climate change. I compare the responses of migrants with those of generalist and specialist resident butterflies in Britain to determine if changes exhibited by migrant species are similar to those of non-migratory species. This chapter has the following objectives:

1. Investigate the degree to which migrants are tracking climate change by examining change in abundance, distribution size and shifts at the northern range margin over the past 30 years.

2. Compare the responses of migrants and resident species, and test the hypothesis that migrants are tracking climate change to a similar extent to resident generalist species.

## 4.3. Method and Materials:

### 4.3.1 Selection of Species:

In total, I considered data from 48 southerly-distributed resident butterfly species (24 generalists, 24 specialists) and 10 migrant species, although sample sizes varied according to analysis. Twenty of the 24 specialists were common across all analyses. The specialists *Papilio machaon* and *Strymondia pruni* are not recorded regularly on transects in the UKBMS but were included in distribution analyses, whereas *Melitaea athlaia* and *Thymelicus action* are very restricted specialist species that are recorded in the UKBMS but are not sufficiently widespread to be included in the distribution analyses (see below). Two generalist species (*Thymelicus lineola* and *T. sylvestris*) are difficult to distinguish and so data for these species are combined in UKBMS data sets as '*T. sylvestris*' (Pollard and Yates, 1993), and so sample sizes for generalists were one fewer in the abundance analysis compared with the distribution analyses. Three of the ten migrant species were included in the main analysis: *Vanessa atalanta*, *Vanessa cardui* (both Nymphalidae) and *Colias croceus* (Pieridae). Seven additional migrant species (*Pontia daplidice*, *Aporia crataegi*, *Issoria lathonia*, *Lampides boeticus*, *Danaus plexippus*, *Colias hyale* and *Nymphalis antiopa*) could not be included in the main analyses because they are not recorded by the UKBMS, and distribution records have not been collated systematically since 1999. However, data for these species were included in a separate analysis of range changes.

Definitions of generalist (wider-countryside species with broad habitat requirements) and specialist (restricted to localized, patchy habitats) resident species follow Asher *et* *al.* (2001), while the definition of a migrant is discussed in Chapters 1 and 2. Four species classified as generalist species by Asher *et al.* (2001) had previously been classified as habitat specialists by Pollard & Yates (1993). All analyses were repeated with these species treated as specialists rather than generalists, but none of the overall conclusions were altered by the way in which these four species were classified, and so I only present analyses treating these species as generalists.

### 4.3.2 **Changes in Abundance:**

Abundance data for migrants, and for southerly-distributed specialists and generalist butterflies, were obtained from the UKBMS (see Chapter 2 for details). The dataset runs from 1976 through to 2004 giving a 28-year period to measure abundance changes. The indices for all site (maximum number of sites = 1500) were combined to produce a collated index for each species for each year using a log-linear Poisson regression model, as performed by the statistical software TRIM (Pannekoek & van Strien 2001). Collated indices were calculated for three migrants, 22 specialist and 23 generalist species. The regression slope of the $\log_{10}$ collated index on year was used to measure the trends in abundance of species between 1976 and 2004, and the average slope estimated separately for migrants, specialists and generalists.

### 4.3.3 **Changes in Distribution Extent:**

Distribution data were obtained from the Butterflies for the New Millennium (BMN) data set (see Chapter 2 for details). Species that had fewer than 35 distribution records in total during the study period were excluded because poorly-recorded and/or scarce species are likely to have high sampling errors, such that three migrants, 22 specialist and 24 generalist specie were included in the analyses. Two time periods, 1970-1982 and 1995-2004, were selected for studying changes in distributional extent and shift in the northern range margin, as they coincide with periods of extensive recording for distribution atlases (Heath *et al.*, 1984; Asher *et al.*, 2001; Fox *et al.*, 2006), and also coincide with a period of rapid anthropogenic global warming (IPCC, 2001). For each species, the change in distribution extent was calculated as the difference in the number of 10-km Ordnance Survey grid squares occupied between the first and second time period.

As discussed in Chapter 2, recorder effort for all species has increased greatly over time which may affect quantification of range changes. Thus, five methods were used to try and account for changes in recorder effort over time. Neither method is necessarily better than another, but in the absence of documentation of precise recorder effort in the original data collection, over which I had no control, examination of the five sets of results provides an indication of whether the results are likely to be robust to this potential source of error. Four of these methods, following Hickling *et al.* (2006), are described in Chapter 2. The fifth method uses data presented in Fox *et al.* (2006), in which authors have limited the effects of recorder effort by randomly sub-sampling the number of records in the second time period to equal that in the first time period.

To ensure that no bias was introduced by analysing only the three most common migrants, a further analysis was undertaken that included an additional seven rare UK migrants: *Pontia daplidice*, *Aporia crataegi*, *Issoria lathonia*, *Lampides boeticus*, *Danaus plexippus*, *Colias hyale* and *Nymphalis antiopa*. A binomial test examined whether or not distributional extent had increased or decreased in the 10 migrant species over the two time periods.

### 4.3.4 **Shifts at the Northern Range Margin:**

Distribution data were analysed for three migrant, 22 specialist and 24 generalist species to determine the degree to which species' ranges had shifted northwards between 1970-1982 and 1995-2004. I could not use abundance data in this analysis because transect sites are not randomly distributed throughout Britain, with a greater proportion occurring in the south and only a few sites in the north. The northern limit of each species' range was defined as the mean latitude of the ten most northerly occupied 10-km grid squares (following Hickling *et al.*, 2006). Shifts in the range limit were calculated as the difference in the mean latitude of grid squares between the two time periods. This shift was calculated for each of the four levels of control for recorder effort, but not computed for sub-sampled data (Fox *et al.*, 2006; fifth method). These data are based on a sub-sampling process that randomly selects the same number of records in the second time period as were present in the first time period (see above). The data presented in Fox *et al.* (2006) are averaged across 30 replicates of this sub-sampling process, and it is not possible to calculate the location of the range margin from these data in a way that would be comparable with the other methods.

### 4.3.5 Statistical Analysis:

All analyses were carried out using parametric tests with species as independent data points. In order to control for phylogeny, analyses were also repeated using independent phylogenetic contrasts (CAIC program, Purvis & Rambaut, 1995). However due to the low number of migrants, only two independent contrasts were calculated which could not be tested statistically. Thus only analyses from parametric tests are presented.

## 4.4 Results:

### 4.4.1 Changes in Abundance:

Migrants were the only group to show a significant increase in abundance on transects over the past 30 years (one sample *t*-test, $t = 5.214$, df = 2, $P = 0.035$; Fig 4.1), and thus migrants increased in abundance significantly more than either specialists or generalists (ANOVA, F = 7.988, df = 2,43, $P = 0.001$).

### 4.4.2 Changes in Distribution Extent:

Migrants increased their distribution extent over time regardless of the method of control for changes in recorder effort (Fig 4.2). Comparing the three group of species, migrants increased their distribution extent significantly more than either generalist or specialist species for all levels of recorder effort (ANOVA, Tukey, $P < 0.05$ for all methods of control for recorder effort). As shown previously (Warren *et al.*, 2001), generalists increased distributional extent more that specialists (ANOVA, Tukey, $P < 0.05$) for all but the strictest level of recorder effort control (ANOVA, Tukey, $P = 0.258$). For all types of control for recording effort, estimated range size increases were always largest for migrants and smallest for specialists (Fig 4.2). For analyses with no recorder effort control, there was an average increase in mean distribution extent over time of 1076 grid squares for migrants, 503 grid squares for generalists, and 123 grid squares for specialists. These results were supported by analyses of all 10 migrant species. Over the past 30 years, 9 out of 10 migrants have shown increases in their distributional extent (Binomial test, N = 10, $P = 0.021$).

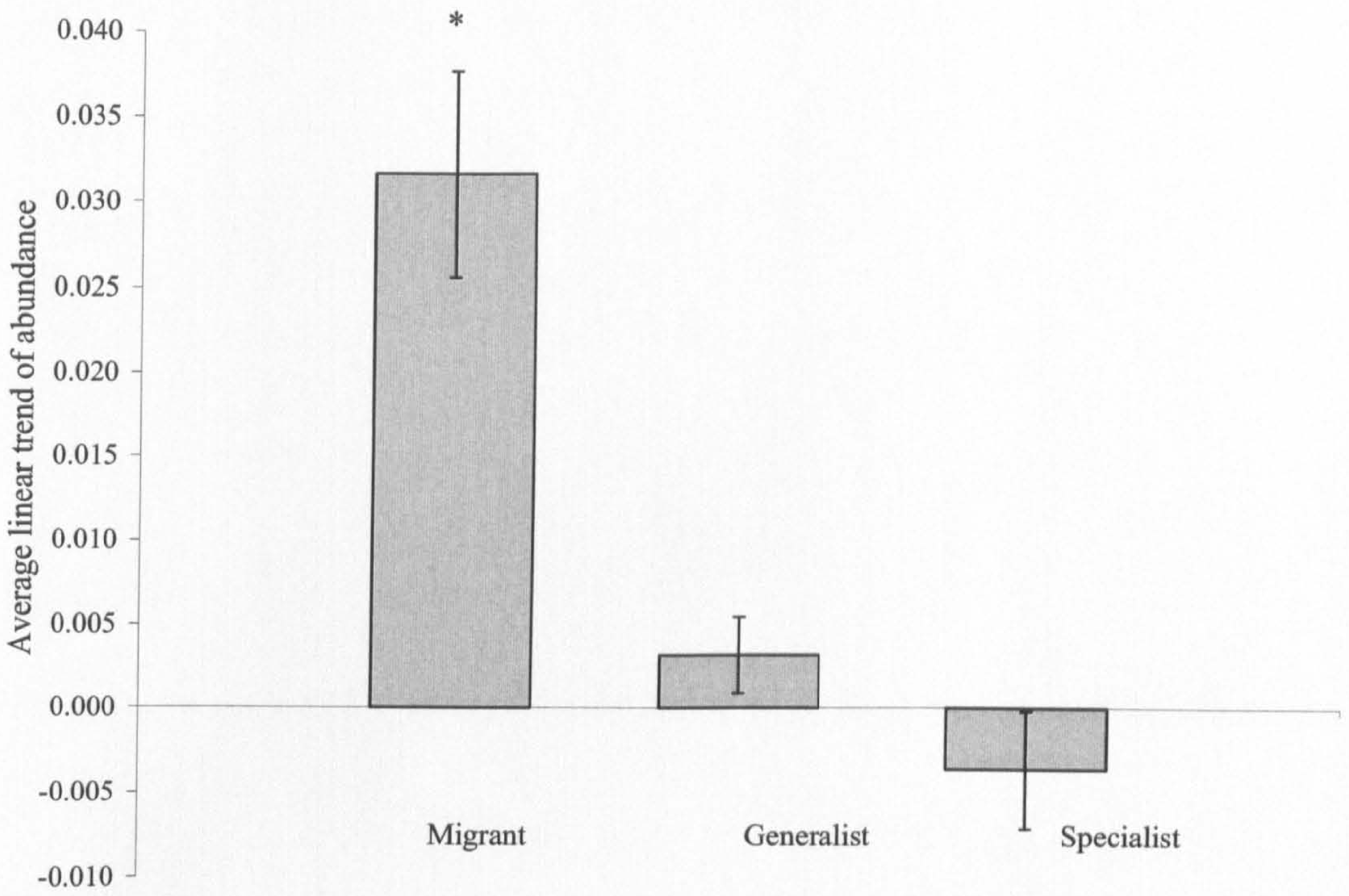


Figure 4.1: Changes in the abundance of migrants, generalist and specialist butterfly species. Data are from UKBMS transect data and show the linear trend in abundance for each species from regressions of $\log_{10}$ collated species index against year (1976 to 2004). Means and standard errors are shown. Significance is based on one-sample t-tests; * $p<0.05$ (N = 3 migrant, 23 generalist and 22 specialist species).

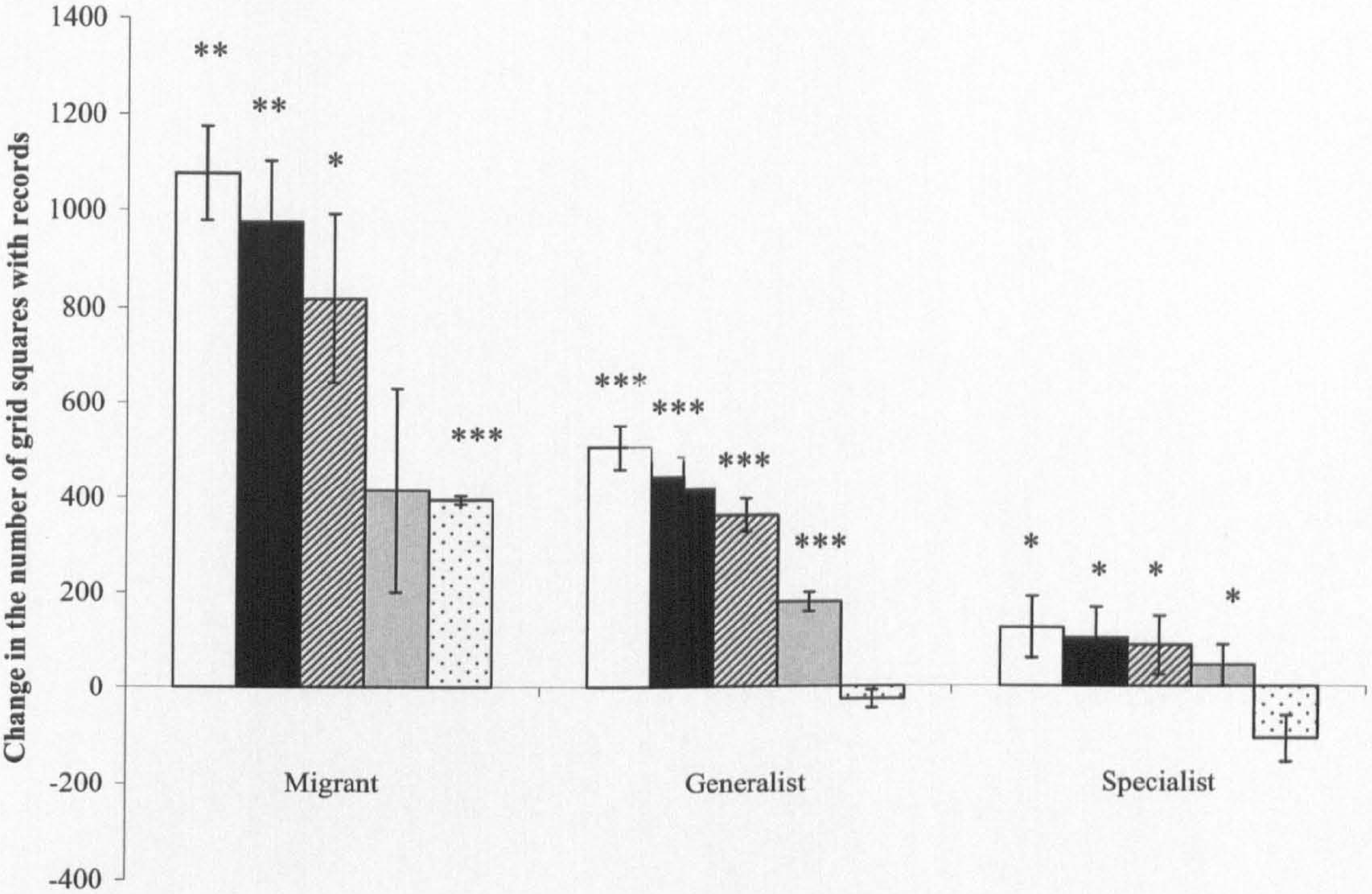


Figure 4.2: Changes in the distribution extent (number of 10 km grid squares occupied) from 1970-1982 to 1995-2004 for migrant, southerly-distributed specialist and generalist butterflies under different levels of control for recorder effort (see text for details); white = recorded squares in both time periods, black = only including squares with ≥5% species richness in both time periods, hatched = ≥10% species richness, grey = ≥25% species richness, dotted = sub-sampled data from Fox *et al.*, 2006. Means and standard errors are shown. Significance is based on one-sample t-test showing a significant difference from zero: *** p<0.001, ** p<0.01, * p<0.05 (N = 3 migrant, 24 generalist and 22 specialist species).

### 4.4.2. **Shifts at the Northern Range Margin**:

Migrants, specialists and generalists all showed northward shifts at their range limits regardless of control for recorder effort (Figure. 4.3), with average northward shifts of 49 to 63 km for migrants, 28 to 43 km for specialists and 31 to 58 km for generalists (depending on the level of recorder effort control). There was no difference between migrant, specialist and generalist species in the degree to which they shifted north (ANOVA, *P* >0.73 across all level of recorder effort control). Significant northward shifts in specialists and generalists were evident at all levels of recorder effort control (one-sample *t* tests, p<0.05), however average range shifts for migrant species were not significantly different from zero (Figure 4.3).

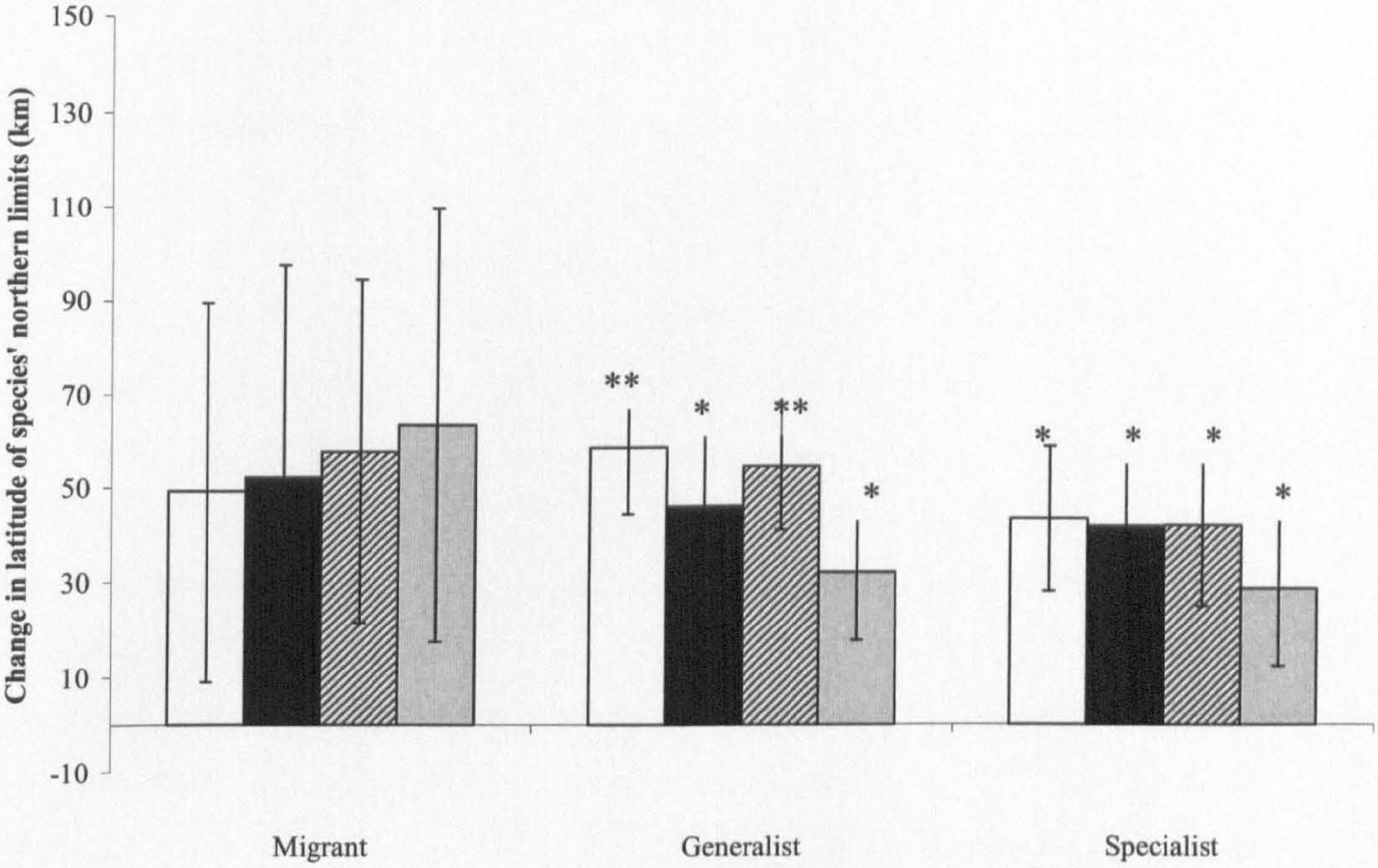


Figure 4.3: Changes in the northern range limit of migrant, specialist and generalist butterflies from 1970-1982 to 1995-2004 under different levels of recorder effort control; white = recorded squares, black = only including squares with >5% species richness, hatched = >10% species richness, grey = >25% species richness. Means and standard errors are shown. Significance is based on one-sample t-tests: ** p<0.01, * p<0.05 (N = 3 migrant, 24 generalist and 22 specialist species).

## 4.5. Discussion

Over the past 30 years, nine out of ten migrant butterfly species have been recorded more frequently in Britain. The more detailed analyses for three of these species showed that migrant butterflies in Britain have increased in abundance and distribution size more than either generalists or specialists. There are only three species of migrant occurring in Britain in sufficient numbers to be included in these comparisons, and so these findings may lack power in this respect. However, these findings support those of other studies showing responsiveness of migrant to climate warming (Sparks *et al.*, 2007), and thus I conclude that migrants are showing greater responses to climate warming than are resident butterflies in the UK.

It is widely argued that increases in recorder effort over time may bias estimates of distributional shifts (Rich, 1998; Dennis *et al.*, 1999). The significant increase in abundance of migrants at monitored transect sites does not suffer from this potential criticism because recorder effort is the same at monitored sites over time. However, analyses of changes in the distribution extent and the range margin could be affected by changes in recorder effort. Nonetheless, at different levels of recorder effort, changes in the distribution extents were apparently robust among the three butterfly groups such that for any all levels of recorder effort control, migrants increased more than generalists, which increased more than specialists (Fig 4.2). For migrants, range expansion remained significantly greater than zero for all but one level of recorder effort control (25%; Fig 4.2). However, even for this level of control, range expansion remained higher than for generalists and specialists. Given that some parts of northern and upland Britain contain less than 25% of total British species richness, this high level of recorder effort control will tend to disproportionately remove northern and upland grid squares from analyses, and so this fourth method is liable to underestimate the true amount of range change. The fifth method of accounting for recorder effort by sub-sampling supported these findings and confirmed that migrants increased more than generalists, and that specialists significantly declined over time.

By contrast with the results for the changes in abundance and distribution extent, shifts at northern range margins did not significantly differ among the three groups. Although all three groups showed northward shifts for all level of recorder effort, these shifts were significant only for generalists and specialists (for all levels of recorder effort). The lack of statistical significance for migrants may be due to the small number of species analysed and high variation among these species (Fig 4.3). In addition, individuals of all three migrants, *V. atalanta*, *V. cardui* and *C. croceus* had already been recorded at relatively northern latitudes in the first time period, and so the potential of their range margins to shift further northwards between the two time periods may also have been somewhat constrained.

It is clear from this study that migratory butterflies appear to be responding rapidly to climate change, as measured by changes in abundance and distribution size in Britain.

Most migrant butterflies are generalists in their habitat requirements, and so habitat is unlikely to limit their northwards expansion, and their high mobility has enabled them rapidly to exploit new, climatically suitable areas. Increased abundance and distribution of migrants in the UK in summer may be due to increased influx of spring migrants form over-wintering sites, increased summer breeding success in the UK (e.g. by increasing the number of generation ) due to warmer climates (Bryant *et al.*, 1997), and/or increased incidence of over-wintering in Britain. Migrant butterflies are arriving into the UK earlier each spring (Roy & Sparks, 2000), and a previous study of *V. atalanta* indicated that the increased abundance in the UK was attributable to increased spring migration, with little evidence of either increased summer reproductive success or over-wintering (Pollard & Greatorex-Davis, 1998). Nonetheless there is some anecdotal evidence for recent over-wintering in migrant butterflies in Britain (Skelton 1999; Fox *et al.*, 2006); although the degree to which this could influence the population dynamics of migrant in summer compared with potentially large influxes of migrants in spring needs more study.

This study shows how broad-front migratory butterflies arriving into Britain each spring appear to be responding to climate warming as well as, or better than, generalists, with marked increases in abundance and distribution extent being apparent near their northern range margins. However, data on changes in their status at southern over-wintering range margins are lacking. Such information will be important if UK population dynamics are driven primarily by spring influxes. Given that studies on the other taxa have shown negative effects of climate warming on migrants (Both & Visser, 2001; Rainio *et al.*, 2006; Post & Forchhammer, 2008), knowledge of the behaviour and ecology of migrants is crucial for determining how they may respond to future climate warming.

Broad-front migrants may have an advantage over site-specific migrants when responding to climate warming. For example, some migrating birds follow remembered routes and refuel at specific stop-over sites, some of which may become unsuitable as the climate changes. Migrants included in this study do not over-winter in specific sites in the Mediterranean and so are unlikely to be affected by habitat loss in over-wintering sites. This contrasts with site-specific migrants species such as the monarch butterfly *Danaus plexippus* (Brower *et al.*, 2002; Wassenaar & Hobson 1998) which may be adversely affected if specific over-wintering sites in Southern USA and Mexico become climatically unsuitable in future (Oberhauser & Peterson, 2003). For migrant vertebrates, there is evidence that arrival times of migrants are now asynchronous with their food resources, resulting in population declines in some birds (Both *et al*, 2006) and mammals (Post & Forchammer, 2008). Relatively short-distance migrants, such as the European butterflies considered in this study, may have the potential to respond rapidly to climate changes if weather conditions in the over-wintering grounds are correlated with those in summer breeding areas. Species initiating their migrations in southern Europe or North Africa, as the study species do, may be able to track earlier spring events, whereas bird species that overwinter in sub-Saharan Africa may not be able to 'predict' when spring conditions in northern Europe are unusually early (Both *et al.*, 2006). It has also been suggested that some migrants may eventually stop migrating (Butler, 2003), as is already apparent in some butterflies in Japan where ten previously migratory species have become permanently established (Kiritani, 2006). There is no evidence for this behaviour occurring yet in northern Europe, but this deserves more study.

As global climates continue to warm, we can expect these short-distance migrants to continue shifting their distributions further northwards coupled with increased abundance (Sparks *et al.*, 2007). Previous studies have shown that resident generalists are tracking climate warming better than specialists (Warren *et al.*, 2001). Findings within this study that distributions of migrants are expanding more than generalists indicate that their greater mobility may be important in this context. Non-migratory generalist butterflies make use of some of the same larval host plants and habitats as migrants, and have similarly flexible patterns of voltinism. This suggests that it is likely to be the difference in mobility, rather than in habitat associations and voltinism patterns that is important. Factors such as increased fecundity or reduced parasitism in summer ranges (Altizer *et al.*, 2000) and recently-colonised sites (Menendez *et al.*, 2008) may also contribute to species' responses to climate warming and require further study. Nonetheless, the fact that both migrants and generalists are tracking climate (albeit with variation among species) whereas specialist are not (Warren *et al.*, 2001) highlights the importance of habitat availability for range expansion. The failure of specialist species to keep track of climate warming because of the loss of breeding habitat, combined with the greater ability of migrants and generalists to expand their ranges, will result in local species assemblages becoming increasingly dominated by generalists (Menendez *et al.*, 2006).

## 4.6 Conclusion:

Migrant butterflies in Britain have increased their abundance and distribution extent to a greater extent than either resident generalist or specialist butterfly species over the past 30 years of climate warming. This suggests that relatively short-distance, broad-front migrant species respond to climate change more rapidly than non-migratory species. Concerns raised as to how migratory species will respond to climate change do not seem to apply to these insects.

## Chapter 5

# Factors Affecting Changes in the Distribution and Abundance of Migratory Butterflies: Analysis of Spring and Summer Populations.

## 5.1 Abstract:

Increases in the abundance of migratory butterflies have been recorded in recent decades, but little is known about what factors may be driving these increases. Changes in the abundance of resident species have been associated with fluctuations in temperatures, but it is not known whether temperature affects the arrival and abundance of migrant species in Britain. Analysis of distribution and abundance data of three migratory butterflies (*Vanessa atalanta, Vanessa cardui, Colias croceus*) showed that over time the number of immigrants arriving each spring has increased, resulting in increased summer populations over the past 30 years. Observations of the arrival patterns of migrants suggest that migratory butterflies are exploiting fast flowing airstreams above their flight boundary layer to aid their migration, although further work using radar is necessary to confirm this hypothesis. It is apparent that the three study species are following different migratory routes, with *V. atalanta* arriving into Britain from the south east, while *V. cardui* and *C. croceus* arrive from due south. I conclude that migrant butterflies are responding positively to climate change both in their overwintering sites, producing an increased immigration into Britain, and at their summer breeding sites in Britain, although it is apparent that temperature is not driving these responses.

## 5.2 Introduction:

Over the past few decades, correlations between the abundance of a wide range of plant and animal species and climate variables have been observed (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Root *et al.*, 2003; Leech & Crick, 2007). A meta-analysis by Parmesan & Yohe (2003) showed that 80% of 282 species showed changes in abundance that were in agreement with climate change predictions. Climate appears to have both positive and negative effects on species abundance, with climate change having been identified as the underlying cause of population declines in amphibian species (Pounds 2001), whereas most butterfly species are predicted to increase in abundance as climate warms (Roy *et al.*, 2001).

In Chapter 4, I analysed changes in butterfly distribution data over time. However, as highlighted in the Chapter, there are problems with analyzing distribution data because of the potential confounding effects of increases in recorder effort over time. For example, there was an increase from 171,363 distribution records for 1970-1982 to 1,642,432 records for 1995-1999. In this Chapter, I analyse abundance data from transects (UKBMS; see Chapter 2 for details) that have constant recorder effort over time, and so do not suffer from the same recorder effort issues as the distribution data sets. The lack of abundance data for other migrant taxa means that it was not possible to examine changes in abundance across taxa. However butterflies are ideal indicator species because temperature has an important influence on most aspects of their ecology (Dennis, 1993; Hill *et al.*, 1999; Roy & Sparks, 2000). The UK Butterfly Monitoring Scheme (UKBMS) consists of transects walked by observers under standardised conditions at a variety of sites across Britain since 1976.

### 5.2.1 **Changes in Abundance**:

Studies into resident butterfly species in Britain have found strong associations between weather variables and population fluctuations and trends, especially temperature and monthly rainfall (Roy *et al.*, 2001). Common and widespread butterflies in Britain have increased in abundance over the past 30 years, with steeper increases observed in the east of the country compared with the west (Pollard *et al.*, 1995; Roy *et al.*, 2001). However, rarer specialist species have had the tendency to decline in numbers over this period (Pollard *et* al., 1995). Declines have also been observed in British moths, with 54% of species showing marked declines in abundance compared to 22% showing increases (Conrad *et al.*, 2004). By contrast with butterfly species, the areas of greatest decline in moth abundance are occurring in the south east of Britain (Conrad *et al.*, 2004).

Increases in abundance have been observed in migrant species in their summer breeding sites (Figure 5.1). A study of historical records suggested a higher abundance of migrant individuals in Britain when 'en route' temperatures in mainland Europe were higher (Sparks *et al.*, 2005), resulting in increased numbers of migrant species arriving into Britain in spring (Sparks *et al.*, 2007). *Vanessa atalanta* has significantly increased in abundance in Britain over the past 30 years, reflecting increases in spring arrival of individuals from southern Europe (Pollard & Greatorex-Davis, 1998).

### 5.2.2 **Migratory Behaviour:**

Migratory flight can be under the control of the individual, in which case individuals fly just a few meters above the ground within their flight boundary layer. Alternatively, individuals can climb to higher altitudes where their speed and direction is controlled predominantly by the wind (Gatehouse & Zhang, 1995; Pedgley *et al.*, 1995; Gatehouse, 1997; Coulson *et al.*, 2002; Wood *et al.*, 2006; Stefanescu *et al.*, 2007, Chapman *et al.*, 2008a,b). It has been generally accepted that while smaller migrant species, for example carabids and moths, traverse great distances with the aid of high altitude airstreams (Drake & Gatehouse, 1995; Gatehouse & Zhang, 1995; Feng *et al.*, 2007; Chapman *et al*, 2008a,b), larger day-flying insects, for example butterflies and dragonflies, remain primarily within their flight boundary layer, thus exhibiting more control over their directionality (Gatehouse & Zhang, 1995; Pedgley *et al.*, 1995; Syrgley *et al.*, 1996; Gatehouse, 1997; Stefanescu, 1997; Syrgley, 2001; Srygley & Oliveira, 2001; Cardé, 2008; Srygley & Dudley, 2008). If this is the case, it would be predicted that these larger flying insects (such as butterflies) would initially arrive in Britain at coastal sites before colonising the rest of Britain during late spring and summer. However, recent evidence suggests that migratory behaviour in larger day-flying insects may not be so different from other insect taxa that embark on wind-bourne migration (Gatehouse, 1997; Mikkola, 2003; Feng *et al.*, 2006; Stefanescu *et al.*, 2007). For example, dragonflies have been observed to fly at night at speeds ranging between 5 – 11ms$^{-1}$ in high altitude airstreams, with individuals concentrated at altitudes of between 200-500m, thus allowing them to migrate up to 400km per night (Feng *et al.*, 2006). Similar flight behaviour has been suggested for migratory butterflies, with migrating *V. atalanta* and *V. cardui* observed flying 100m above the ground and potentially reaching altitudes of up to 3000m (Mikkola, 2003). In addition, Stefanescu *et al* (2007) have found strong correlations between the arrival of *V. cardui* in northeastern Spain in spring and North African wind currents, providing evidence for the exploitation of high altitude winds by migratory butterflies.

### 5.2.3 Rationale for Further Work:

Previous work on migrant butterflies has focused on how their abundance in the UK has changed over time, but little is known about the contribution of migrant individuals to abundance of subsequent summer populations, or the pattern of arrival and subsequent colonization of Britain. The abundance of migrant species in Britain has increased over the past 30 years, associated with climate warming, but it is not known whether this increase is due to increased influx of spring migrants, or due to increased summer breeding success of migrants once they have arrived in Britain.

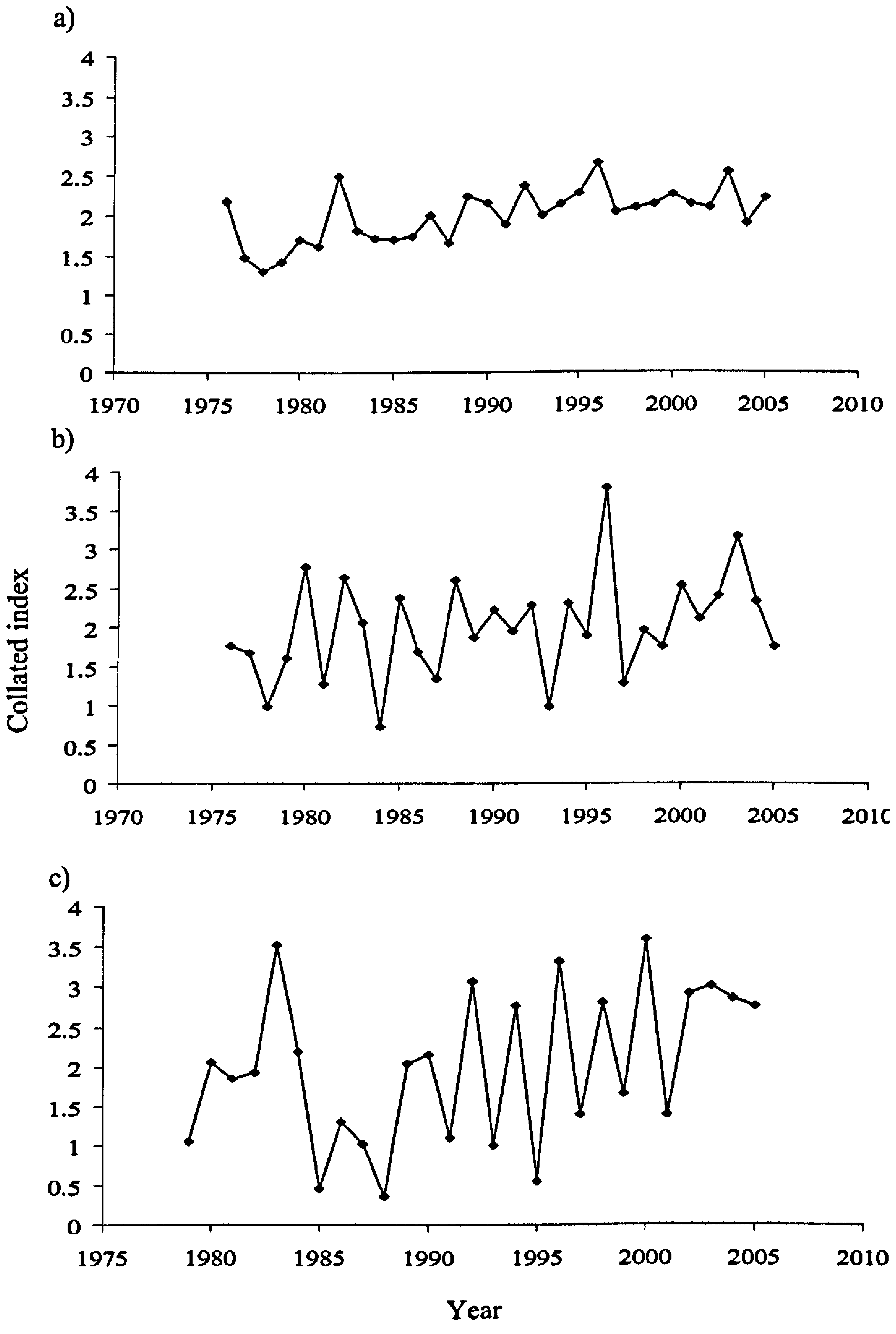


Figure 5.1: Changes in abundance over time as calculated as a collated index from UKBMS data (as described in Chapter 2) for a) *V. atalanta,* b) *V. cardui,* and c) *C. croceus.*

### 5.2.4 General Aims and Predictions of this Chapter:

This Chapter investigates changes in abundance of spring and summer populations of three migrant butterflies that arrive in Britain each spring: *Vanessa atalanta*, *Vanessa cardui* and *Colias croceus*. I analyse historical data from the Butterflies for the New Millennium (BMN) and UKBMS (see Chapter 2) between 1970 and 2005 to examine how spring (subsequently termed 'migrant') and summer (subsequently termed 'resident') populations have responded to recent climate warming, and whether the three species are exhibiting similar responses. Further, I examine factors affecting observed changes by examining whether increases in abundance are correlated with temperature data from Spain and Britain. I also examine migration routes of the study species by examining their spring arrival patterns. This chapter has the following objectives:

1. In light of findings from Chapters 3 and 4, this chapter will determine if increases in migrants recorded in Britain are due to an increase in the numbers migrating into Britain in spring or due to increased breeding success of migrant species once they arrive, resulting in greater numbers of second generation individuals.

2. To determine if changes in yearly abundance of migrant butterflies in Britain are driven primarily by temperatures experienced during larval development in over-wintering sites in Spain, or by temperatures experienced during 2nd generation larval stage in the UK .

3. To determine if butterflies migrate within the flight boundary layer, and hence arrive in the UK at coastal locations before subsequently colonising locations further inland. In addition, to determine if migrants butterflies arriving in the UK each spring initially populate southern sites, before populating sites at higher latitudes later in the year.

4. To compare changes in the abundance of the three study species to determine if they have responded similarly to recent climate warming and whether they exhibit similar migratory behaviour.

## 5.3 Materials and Methods:

Abundance data for migratory butterflies are available from the UKBMS (as described in Chapter 2). Data were available for only three migrant species: *V. atalanta*, *V. cardui* and *C. croceus.*

### 5.3.1 Determining Cut-off Point Between Spring Arrivals and Summer Residents:

In order to determine whether the overall increase in migrant butterflies in Britain is due to an increase in spring arrivals or to increased reproductive success once they arrive, it was necessary to split the data into two time periods. Mean weekly plots of butterfly abundance from 1976 to 2005 for *C. croceus* and *V. cardui* show two clear peaks in abundance, the first representing spring arrivals in to the UK, and the second representing the progeny of spring arrivals, as well as any further migrants from the continent (Figure 5.2). The trough of the peaks lay mid way through July, so I used the 14th July as the cut off point, thus any counts before the 14th are regarded as 'migrant' spring individuals and counts from 14th July onwards as 'resident' summer individuals. Plots of weekly abundance of *V. atalanta* did not show two distinct peaks, but a gradual increase in abundance over time, and so I also used the same cut-off day for this species. I examined the robustness of this cut-off date by plotting weekly transect data for each year. For *C. croceus* and *V. cardui,* the trough between the two abundance peaks lay in mid July in 93% (27/29 years for *C. croceus* and 28/30 years for *V. cardui*) of cases. Example of weekly plots for 1976, 1990 and 2005 are shown in Figure 5.3. To test the sensitivity of my findings to the exact cut-off day, I repeated all the analyses with the split occurring 2 weeks before and 2 weeks after July 14th.

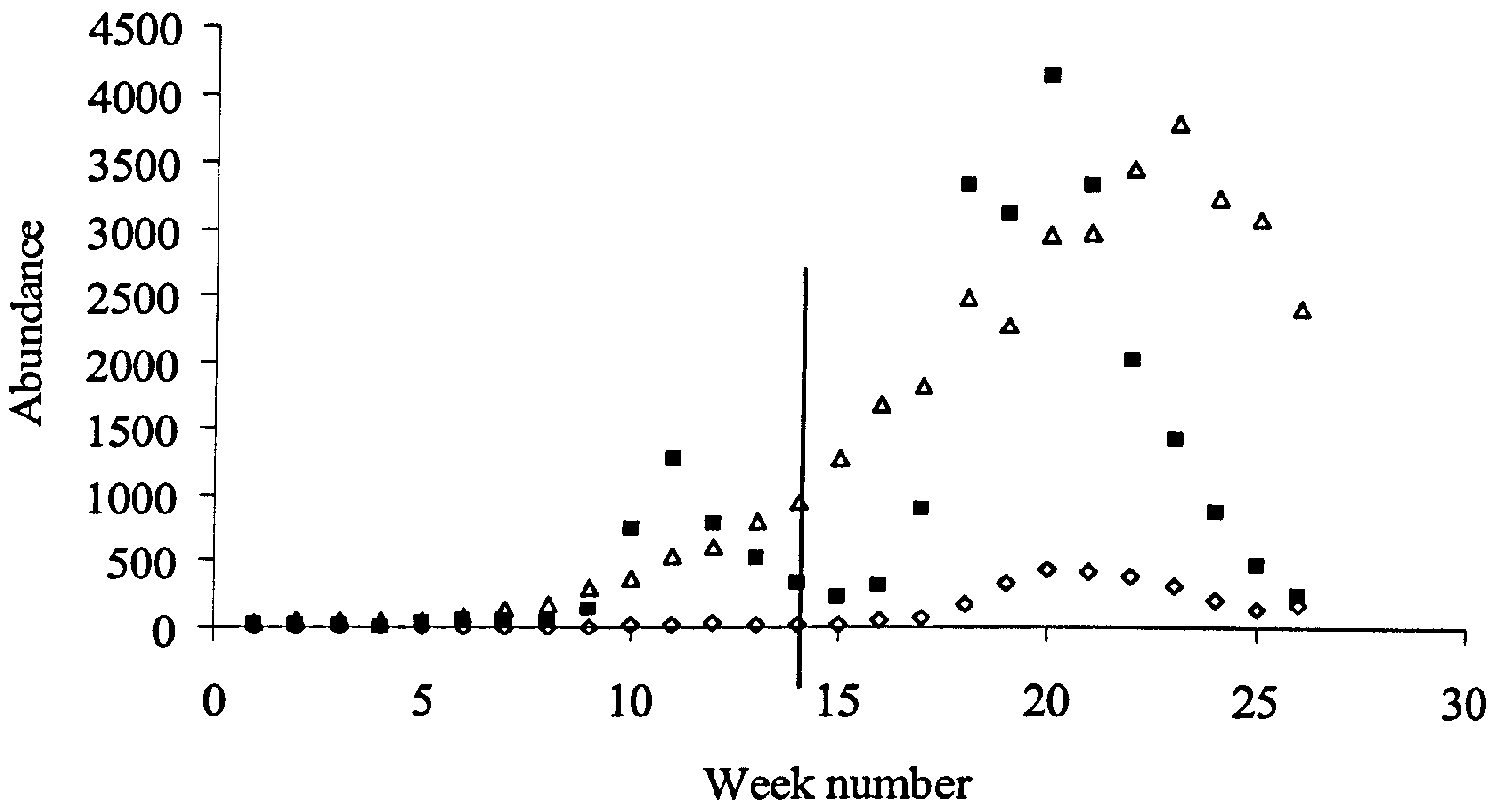


Figure 5.2: Plot showing how mean abundance (all data from 1976-2005) of *V. atalanta* (triangles), *V. cardui* (black squares) and *C. croceus* (diamonds) varies between April (week1; UKBMS) and September (week 26). The vertical line shows the cut off point (14th July) separating spring (migrant) and summer (resident) individuals.

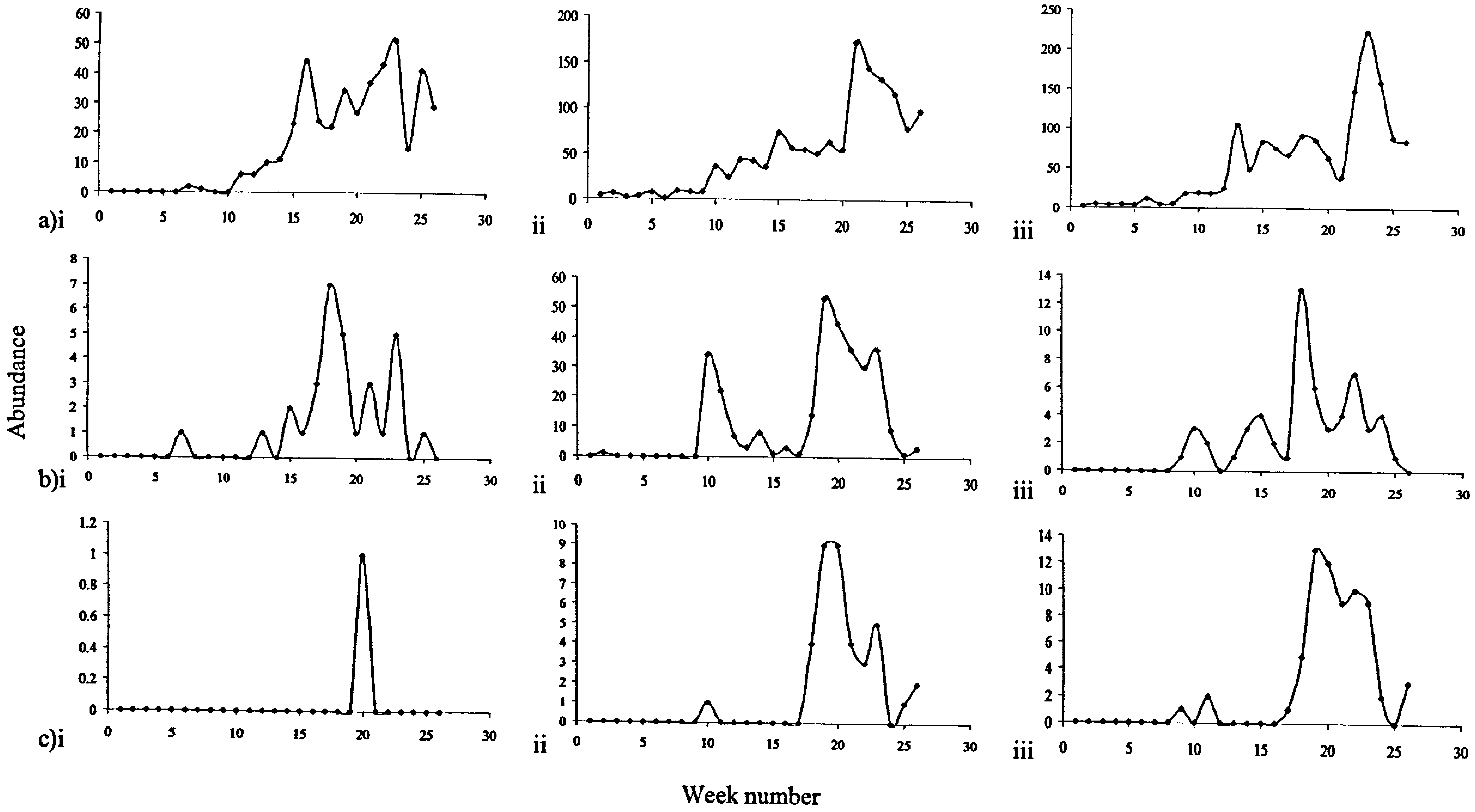


Figure 5.3: Weekly abundance plots for a) *V. atalanta,* b) *V. cardui* and c) *C. croceus* in (i) 1976,(ii) 1990 and (iii) 2005. Note that the y axis scaling is different on different plots.

### 5.3.2 Abundance Data:

Raw abundance data were transformed so that abundances could be compared between years and species. Transects at different sites vary greatly in length (from 0.761 km to 6.289 km), and so I calculated abundance per km (density) for each species at each site for each year. These data were log (x+1) transformed prior to analysis to minimize the influence placed on extremely large counts. Over the past 30 years of recording, the number of sites walked each year fluctuates considerably (ranging from 38 to 771 sites per year, see Fig 2.10 in Chapter 2). To examine whether or not this affected my conclusions, I carried out analyses on the full data set from all sites, as well as restricting the analyses to only those sites that ran for >25 years (N= 25 transects).

### 5.3.3 Distribution Data:

In order to compare abundance data from the BMS with distribution data (used in Chapter 3), I used the number of records for the three migrant species as a measure of their abundance. As with the UKBMS data, I split the distribution data into two time periods. Not all the distribution record cards were dated (approx 20% of all records), and were not used in the subsequent analyses. Distribution records from November to March were excluded because it is difficult to determine whether these records represent UK overwintering individuals or are very early migrants, while using the same time period as that of the UKBMS abundance data allowed a more robust comparison between the two datasets. In any case, there were very few records for these months (0.7% of *V. atalanta* records, 3.6% of *V. cardui* records, 0.4% of *C. croceus* records). Distribution data for October were retained for analysis, although this period is beyond that covered by transect data. The number of records were log10 (x+1) transformed to minimise the influence placed on extremely large counts (Sparks *et al.*, 2005).

### 5.3.4 Analyses:

The density of individuals per km of transect was calculated for both spring and summer time periods for all three species. For each species, mean density was calculated across all transect sites for each year. In order to determine whether migrant or resident populations are contributing most to the overall change in migrant butterflies in Britain, I used these density values to computed a 'summer index' for each year as the number of summer individuals recorded as a proportion of the yearly total number of individuals (equation 1).

This index was also calculated where the cut-off point occurred two weeks earlier and two weeks later than the 14th July.

Equation 5.1 $$\text{Summer index} = \frac{resident\#}{migrant\#+resident\#}$$

To examine patterns of migrant arrival and subsequent colonisation of the UK, butterfly density per week was calculated for coastal and inland sites. I focused on the years 1982, 1996 and 2003 as these were the most abundant years for migrants. The summer index and the date of the first recorded arrival was calculated for coastal and inland sites for 1982, 1996 and 2003.

To determine if butterfly migrants arrive first into the south of Britain, and then subsequently colonise more northerly latitudes, Britain was divided into three regions (north, south east and south west) following Conrad *et al.* (2004; Figure 5.5) Analyses described above, comparing migrant and resident populations, summer index and arrival dates were then repeated for each of the three regions.

The collated index from the BMS data was plotted against number of 10km squares with records to see if there was any relationship between distribution and abundance in the two data sets.

In order to investigate factors affecting changes in abundance of migrants in Britain, changes in abundance and distribution were correlated with temperature variables. Monthly mean temperature data from the Central England Temperature (CET) series were analysed (Parker *et al.*, 1992; http://www.cru.uea.ac.uk). Mean monthly temperatures were also obtained for Spain (Mitchell *et al.*, 2004).

For Spain, average monthly temperatures from November to March were computed, covering the period of over-wintering larval development. Average monthly temperatures from November to March were also calculated for UK, to investigate evidence for larval over-wintering in the UK. For the UK, average monthly temperature between May and July were also computed, covering the period of larval development of 2nd generation individuals. For each study species, initially two regressions were run for each species for migrant abundance against year and resident abundance against year, saving the residuals for each individual regression. The residuals were then correlated with each of the three temperature variables using a bivariate correlation to examine factors affecting spring and summer abundance changes, after taking account of under-lying trends in abundance.

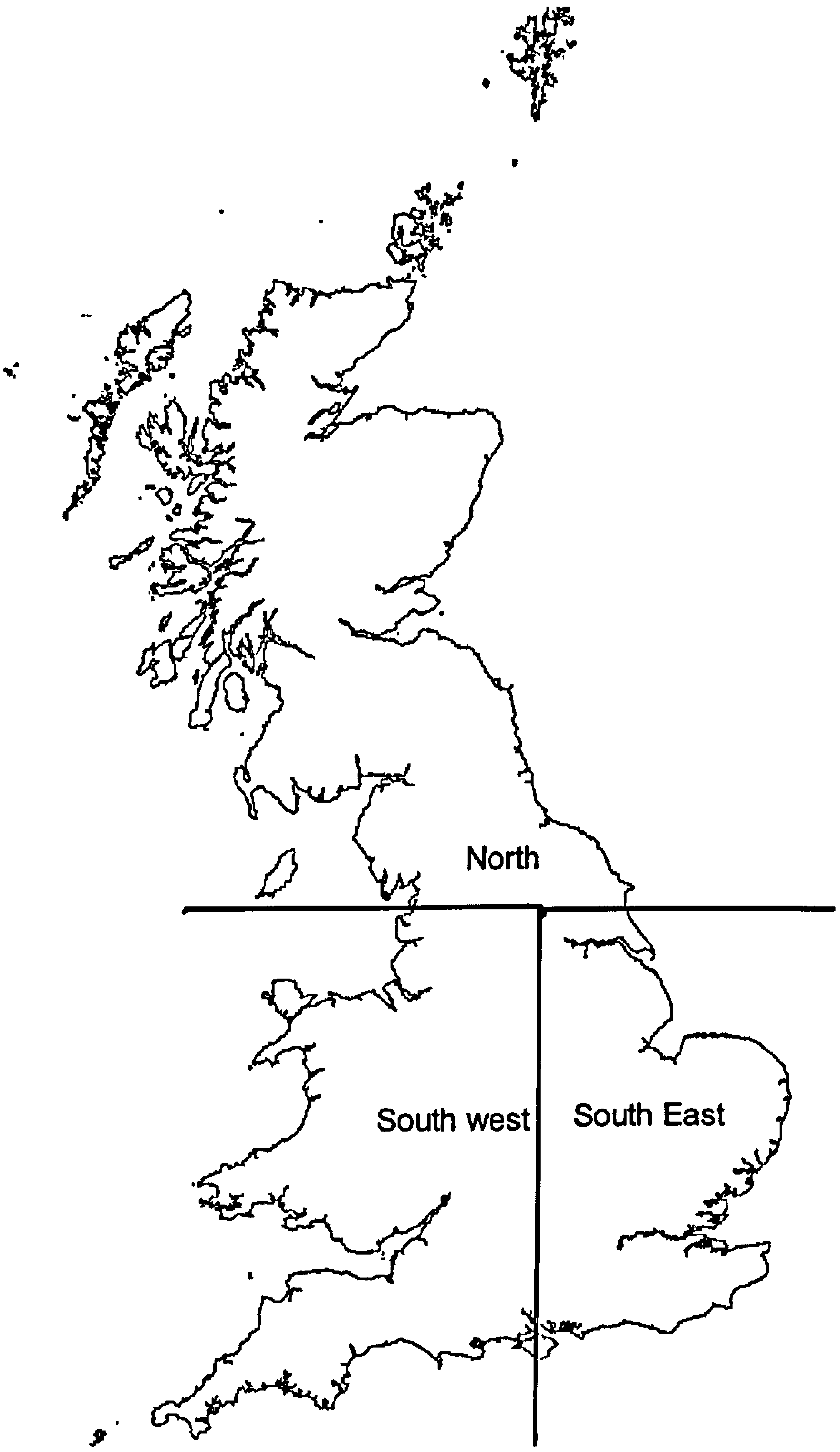


Figure 5.4: A map of the UK showing the division into three regions (north, south east and south west) along OS grid lines 450 km E and 450 km N.

## 5.4 Results:

### 5.4.1 Migrant and Resident Abundance Trends:

With the exception of *C. croceus* abundance in spring, all three species have increased significantly in abundance and distribution over the past 30 yrs in both their spring and summer populations (Table 5.1). The choice of cut-off date for splitting data into spring and summer individuals made little difference to these conclusions (Table 5.1). Similar results were also obtained when only those sites that had been running for >25 years (LR) were included in the analysis compared with when all sites were included (Table 5.1).

With the exception of the distribution data, there was a highly significant interaction between the migrant and resident populations for all three species (Table 5.1). When analysing the abundance data, all three species showed a greater rate of increase in migrant abundance compared to the resident abundance, whereas for the distribution data they increased at a similar rate.

### 5.4.2 Summer Index:

The trend in summer index, whereby this measures the proportion of residents contributing to the overall abundance, for each of the three species is shown at Table 5.2. The overall analysis gave few significant results, with those restricted to *V. atalanta* suggesting that over time the proportion of migrants has increased at a greater rate than the residents, echoing the results discussed above.

| Species | Analysis | Migrant trend | P value | Resident trend | P value | Interaction |
|---|---|---|---|---|---|---|
| *V.atalanta* | Distribution 15/16 | Increase | <0.001 | Increase | <0.001 | ns |
| *V.atalanta* | Distribution 13/14 | Increase | <0.001 | Increase | <0.001 | ns |
| *V.atalanta* | Distribution 17/18 | Increase | <0.001 | Increase | <0.001 | ns |
| *V.atalanta* | Abundance 15/16 | Increase | <0.001 | Increase | <0.001 | <0.001 |
| *V.atalanta* | Abundance 13/14 | Increase | <0.001 | Increase | <0.001 | <0.001 |
| *V.atalanta* | Abundance 17/18 | Increase | <0.001 | Increase | <0.001 | <0.001 |
| *V.atalanta* | Abundance LR | Increase | <0.001 | Increase | 0.002 | <0.001 |
| *V.cardui* | Distribution 15/16 | Increase | <0.001 | Increase | <0.001 | ns |
| *V.cardui* | Distribution 13/14 | Increase | <0.001 | Increase | <0.001 | ns |
| *V.cardui* | Distribution 17/18 | Increase | <0.001 | Increase | <0.001 | ns |
| *V.cardui* | Abundance 15/16 | Increase | 0.014 | Increase | 0.016 | <0.001 |
| *V.cardui* | Abundance 13/14 | Increase | 0.024 | Increase | 0.015 | <0.001 |
| *V.cardui* | Abundance 17/18 | Increase | 0.013 | Increase | 0.016 | <0.001 |
| *V.cardui* | Abundance LR | ns | 0.107 | ns | 0.073 | <0.001 |
| *C.croceus* | Distribution 15/16 | Increase | <0.001 | Increase | <0.001 | ns |
| *C.croceus* | Distribution 13/14 | Increase | <0.001 | Increase | <0.001 | ns |
| *C.croceus* | Distribution 17/18 | Increase | <0.001 | Increase | <0.001 | ns |
| *C.croceus* | Abundance 15/16 | ns | 0.239 | Increase | 0.013 | <0.001 |
| *C.croceus* | Abundance 13/14 | ns | 0.227 | Increase | 0.013 | <0.001 |
| *C.croceus* | Abundance 17/18 | ns | 0.231 | Increase | 0.012 | <0.001 |
| *C.croceus* | Abundance LR | ns | 0.314 | Increase | 0.018 | <0.001 |

Table 5.1: Regression analyses of abundance (density per km of transect for BMS data) and distribution (number of records) against year for migrant and resident populations of *V. atalanta*, *V. cardui* and *C. croceus*. Analyses were repeated by altering the cut-off point between spring and summer from 14th July (15/16), as well as two weeks before (13/14) and after (17/18) this date, and restricting analyses to only those BMS sites that ran for >25 years (LR). The significance of the interaction between migrant and resident populations is also included.

| Species | Analysis | Trend | P value |
|---|---|---|---|
| *V.atalanta* | Distribution 15/16 | ns | 0.093 |
| *V.atalanta* | Distribution 13/14 | ns | 0.064 |
| *V.atalanta* | Distribution 17/18 | decrease | 0.017 |
| *V.atalanta* | Abundance 15/16 | decrease | 0.007 |
| *V.atalanta* | Abundance 13/14 | decrease | 0.030 |
| *V.atalanta* | Abundance 17/18 | ns | 0.056 |
| *V.atalanta* | Abundance LR | decrease | 0.001 |
| *V.atalanta* | Distribution 15/16 | ns | 0.659 |
| *V.cardui* | Distribution 13/14 | ns | 0.769 |
| *V.cardui* | Distribution 17/18 | ns | 0.793 |
| *V.cardui* | Abundance 15/16 | ns | 0.069 |
| *V.cardui* | Abundance 13/14 | ns | 0.088 |
| *V.cardui* | Abundance 17/18 | ns | 0.226 |
| *V.cardui* | Abundance LR | ns | 0.258 |
| *C.croceus* | Distribution 15/16 | decrease | 0.005 |
| *C.croceus* | Distribution 13/14 | decrease | 0.002 |
| *C.croceus* | Distribution 17/18 | ns | 0.705 |
| *C.croceus* | Abundance 15/16 | ns | 0.303 |
| *C.croceus* | Abundance 13/14 | ns | 0.279 |
| *C.croceus* | Abundance 17/18 | ns | 0.407 |
| *C.croceus* | Abundance LR | ns | 0.327 |

Table 5.2: Regression of summer index against time for each study species. Decreasing trends indicate a decreased proportion of summer individuals in yearly totals over time. Analyses were repeated by altering the cut-off point between spring and summer from 14th July (15.16) as well as two weeks before (13/14) and after (17/18) this date, and restricting the analyses to only those BMS sites that ran for >25 years (LR).

### 5.4.3 Coastal Versus Inland Sites:

As expected, summer index values were high for all species, showing rapid population increases in summer from offspring of spring migrants. Figures 5.5-5.7 show the summer index values at coastal and inland sites for the years 1982, 1996 and 2003 for *V. atalanta* and *V. cardui*, and for 1996 and 2003 for *C. colias*. Unlike the other two butterflies, very few *C. croceus* were recorded during 1983. Overall, *V. atalanta* and *V. cardui* are well distributed across Britain, whereas the *C. croceus* occurs predominantly in the south. Overall, there was no general pattern of arrival in relation to site location for *V. atalanta* and *C. croceus*, although the summer index of coastal was lower than inland sites in *V. cardui* in 1982 (Wilcoxon, $p=0.035$, $Z=-2.104$) and 1996 (Wilcoxon, $p=0.004$. $Z=-2.857$).

There was a significant trend towards earlier arrival dates over time for *V. atalanta* and *V. cardui* (Figure 5.8; *V. atalanta*, regression of arrival date against time; coastal sites, $p<0.001$, $r^2=0.565$, $F=36.39$, $b=-0.284$, $df=29$; inland sites $p=0.034$, $R^2=0.150$, $F=4.96$, $b=-0.284$, $df=29$; *V. cardui*, coastal sites $p=0.025$, $R^2=0.168$, $F=4.69$, $b=-0.26$, $df=29$). However, there was no significant difference in arrival dates between the two types of sites (*V. atalanta* Wilcoxon, $p=0.536$, $N=30$, $Z=-0.619$; *V. cardui*, Wilcoxon, $p=0.349$, $N=30$, $Z=-0.937$) and so no evidence that individuals initially arrive at coastal sites and subsequently colonise Britain. By contrast, *C. croceus* showed no change in arrival date over time, although arrival dates were earlier at coastal sites compared with inland sites (Wilcoxon, $p=0.017$, $N=16$ $Z=-2.396$).

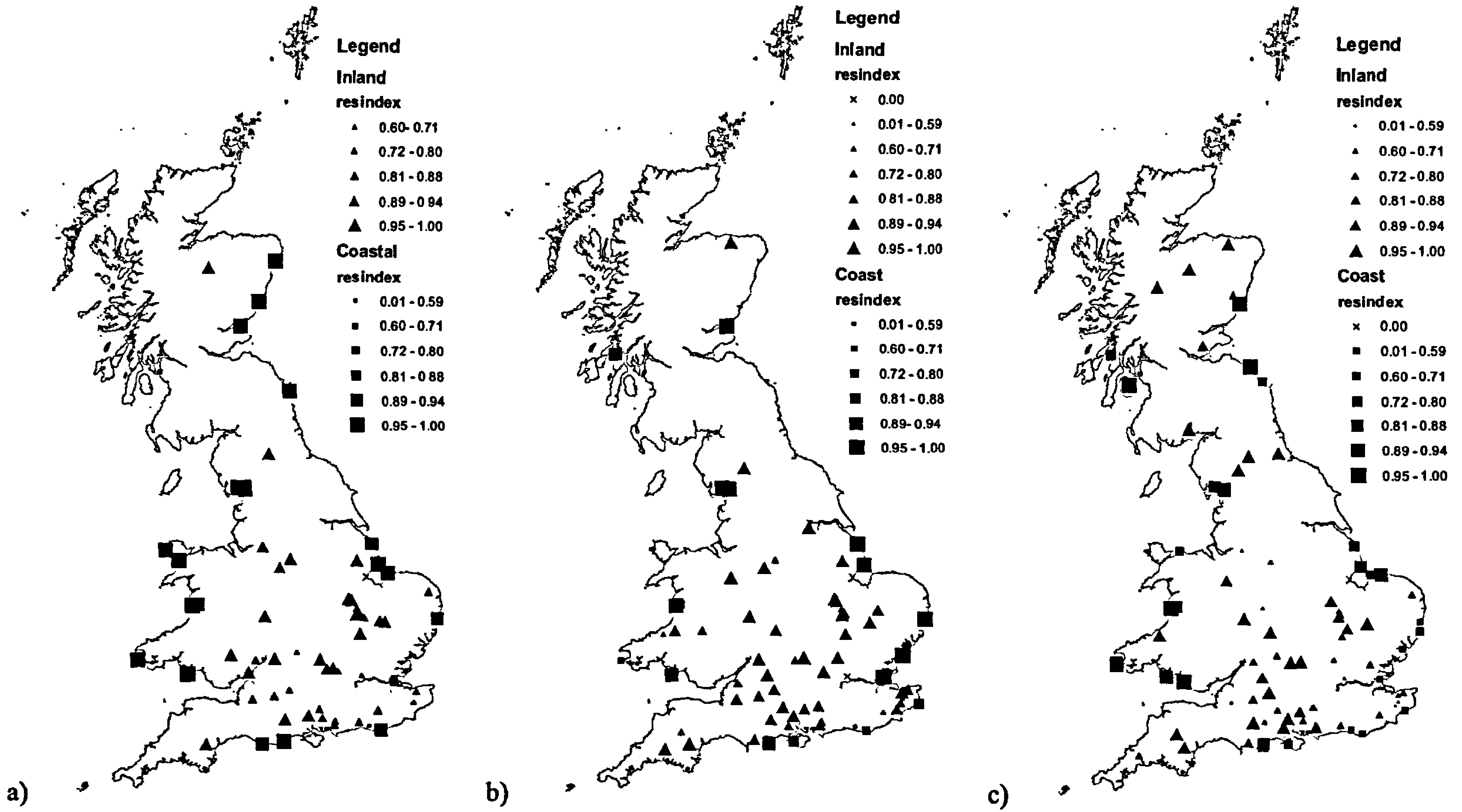


Figure 5.5: Summer index for *V. atalanta* in a) 1982, b) 1996 and c) 2003, for coastal (square) and inland (triangle) sites.

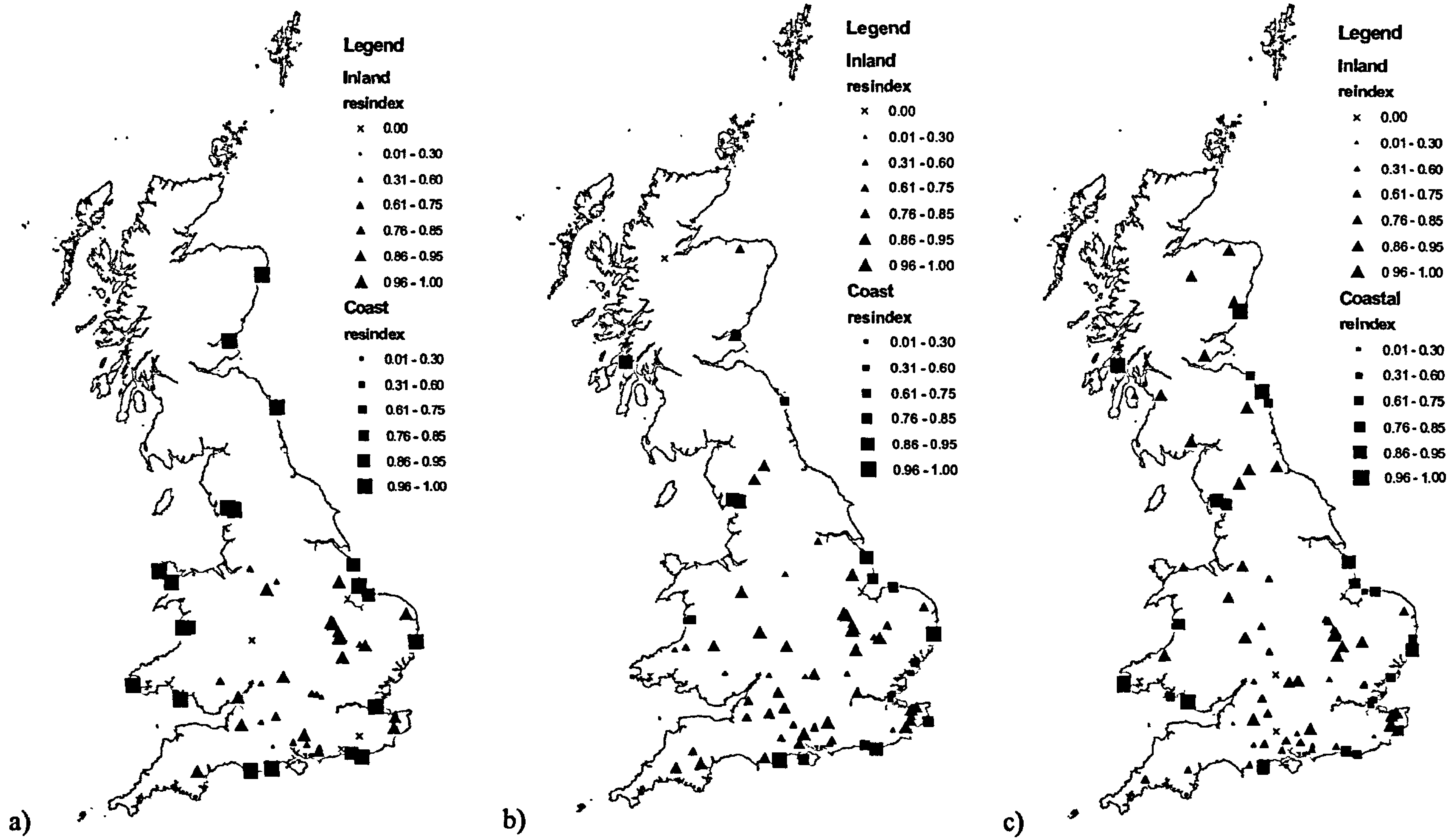


Figure 5.6: Summer index for *V. cardui* in a) 1982, b) 1996 and c) 2003, for coastal (square) and inland (triangle) sites.

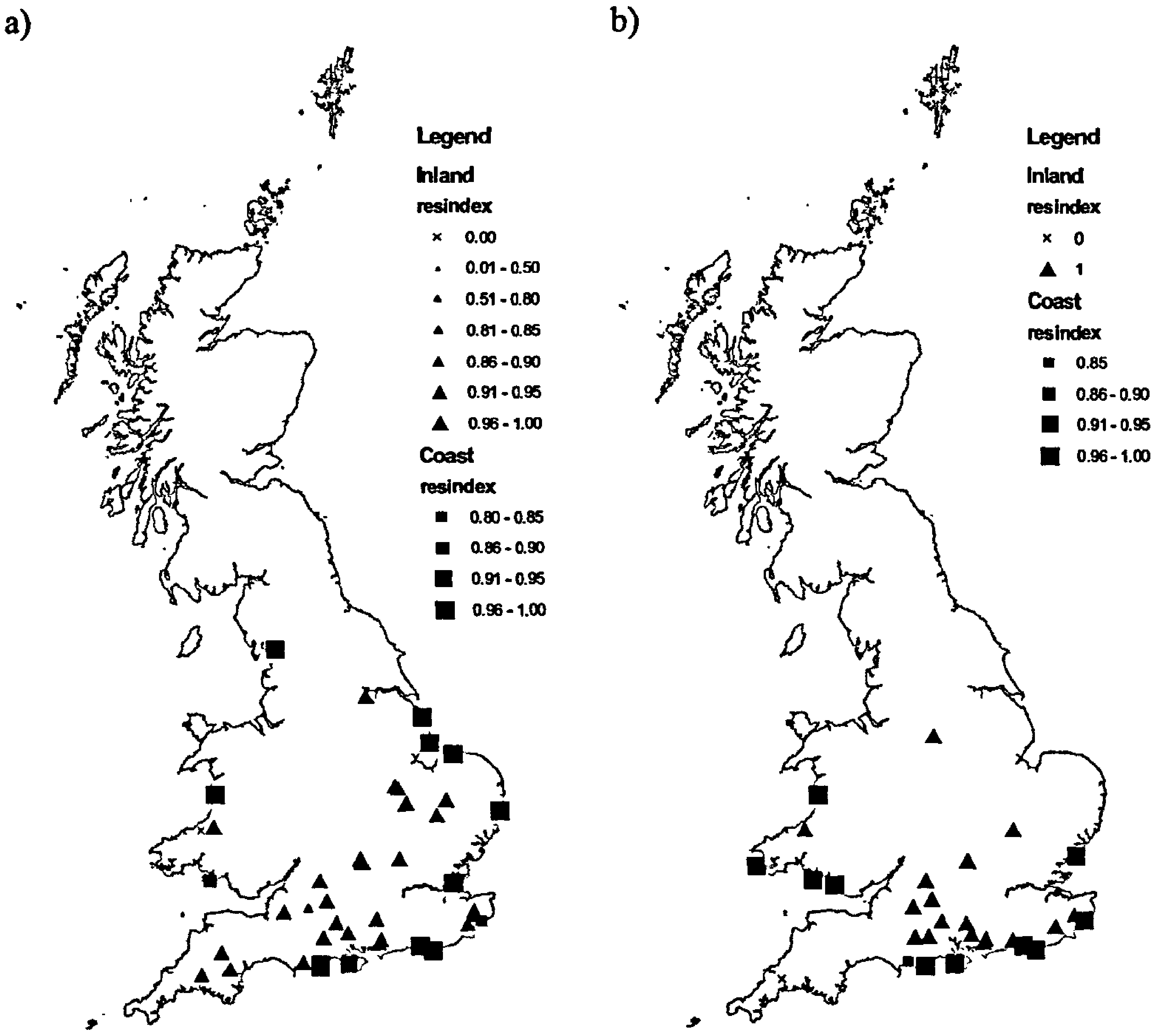


Figure 5.7: Summer index for *C. croceus* in a) 1996 and b) 2003, for coastal (square) and inland (triangle) sites.

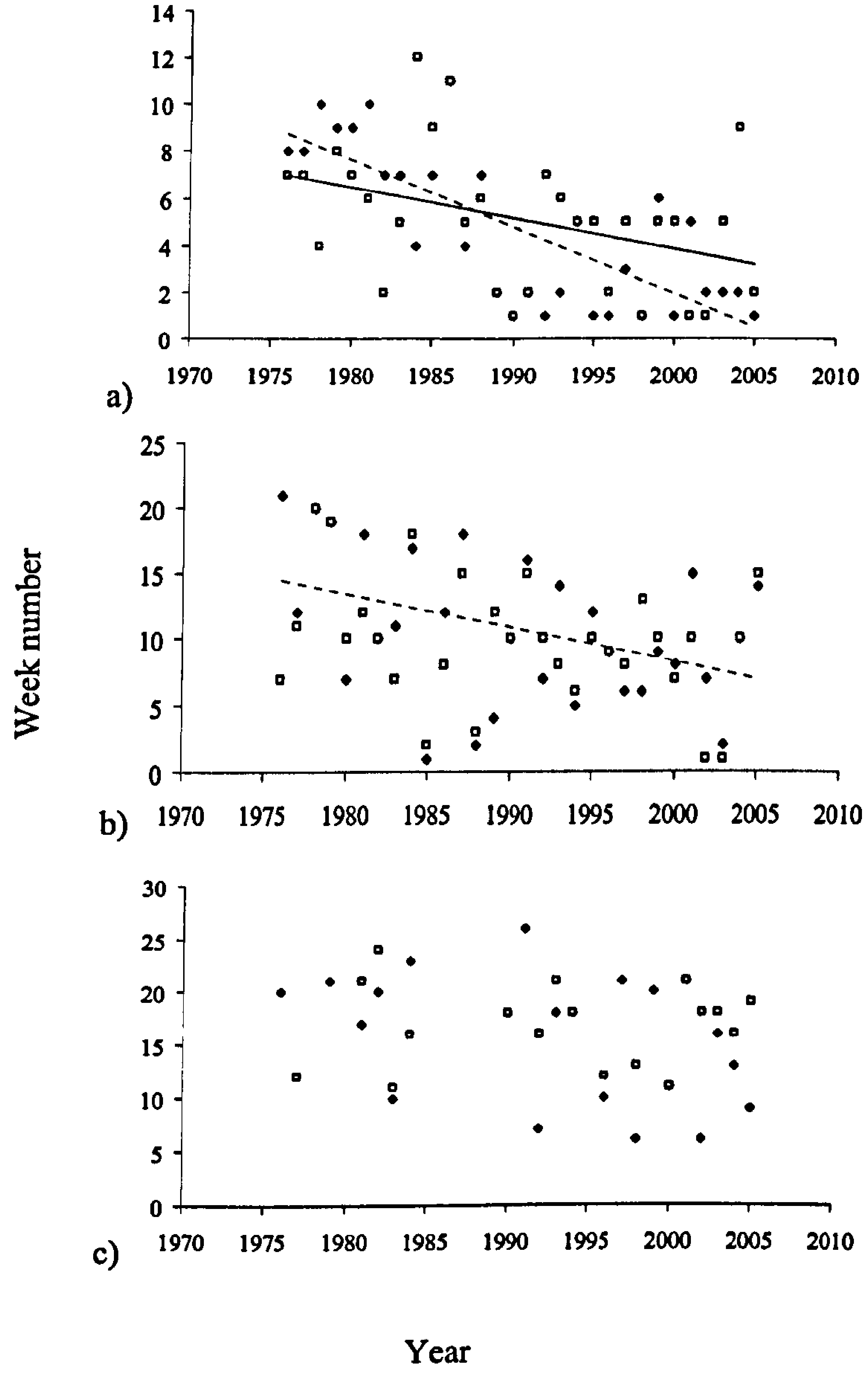


Figure 5.8: The change in arrival date over time of a) *V. atalanta*, b) *V. cardui*, and c) *C. croceus* for coastal (diamond and dashed line) and inland (square and solid line) sites. Only significant regressions are plotted.

### 5.4.4 Regional Abundance:

Figure 5.9 shows the change in summer index over the past 30 years for the three migrant species for three regions in Britian. While *V. atalanta* showed significantly higher summer index values in the southwest and north compared to the southeast (ANOVA, p=0.001, F=7.862, df=87) with the greatest variation occurring in the north, *V. cardui* showed significantly higher summer index values in the north compared to the south (ANOVA, p=0.009, F=5.014, df=81), with the greatest variation in the southwest. Due to the lack of records within the north of Britain, this analysis for *C. colias* lacked statistical power and revealed no regional patterns.

Figure 5.10 shows the change in arrival date (measured in weeks) for the three migrant species. *V. atalanta* has shown a highly significant shift in arrival phenology in both the north (Regression, p=0.003, $R^2$=0.279, F=10.439, df=28) and the southeast (Regression, p=<0.001, $R^2$=0.371, F=16.514, df=29), arriving earlier over time. There was a highly significant difference in the arrival date between the three regions for *V. atalanta* (ANOVA, p<0.001, F=14.320) and *V. cardui* (ANOVA, p=0.001, F=8.068, df=81), with both species arriving earlier in the spring.

### 5.4.5 Climate Correlations:

For all study species, there was no correlation between migrant abundance and temperature during larval development in either Spain or the UK (Table 5.3). Spring distribution of *V. atalanta* was significantly positively correlated with UK spring temperatures in the UK, but this was not the case for the other study species. It is therefore apparent that 'good' butterfly years, for example 1983, 1996 and 2003 do not correlate with high temperatures at either the southern or northern most limits of their range.

### 5.4.6 Comparing Distribution and Abundance Data:

The three migrant species show a significant relationship between their distribution (number of 10km squares with records) and their abundance (measured as a collated index) (Regression, p<0.0025 for each species; Fig 5.11). *C. croceus* showed the strongest relationship ($R^2 = 0.581$).

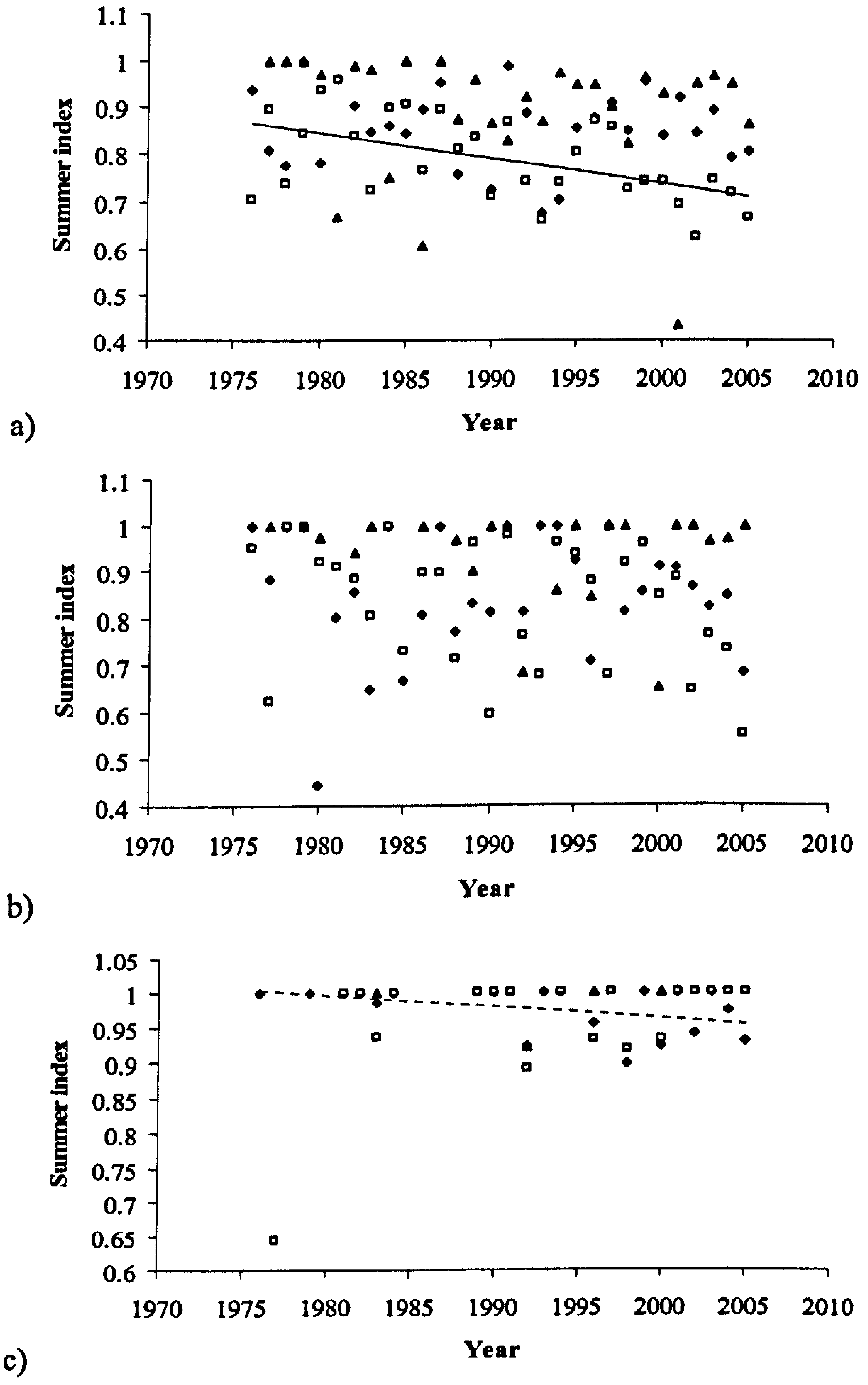


Figure 5.9: Summer index for a) *V. atalanta*, b) *V. cardui*, c) *C. croceus*. North = triangle, Southeast = square, Southwest = diamond, Southwest trend line = dotted, Southeast trend line = black line.

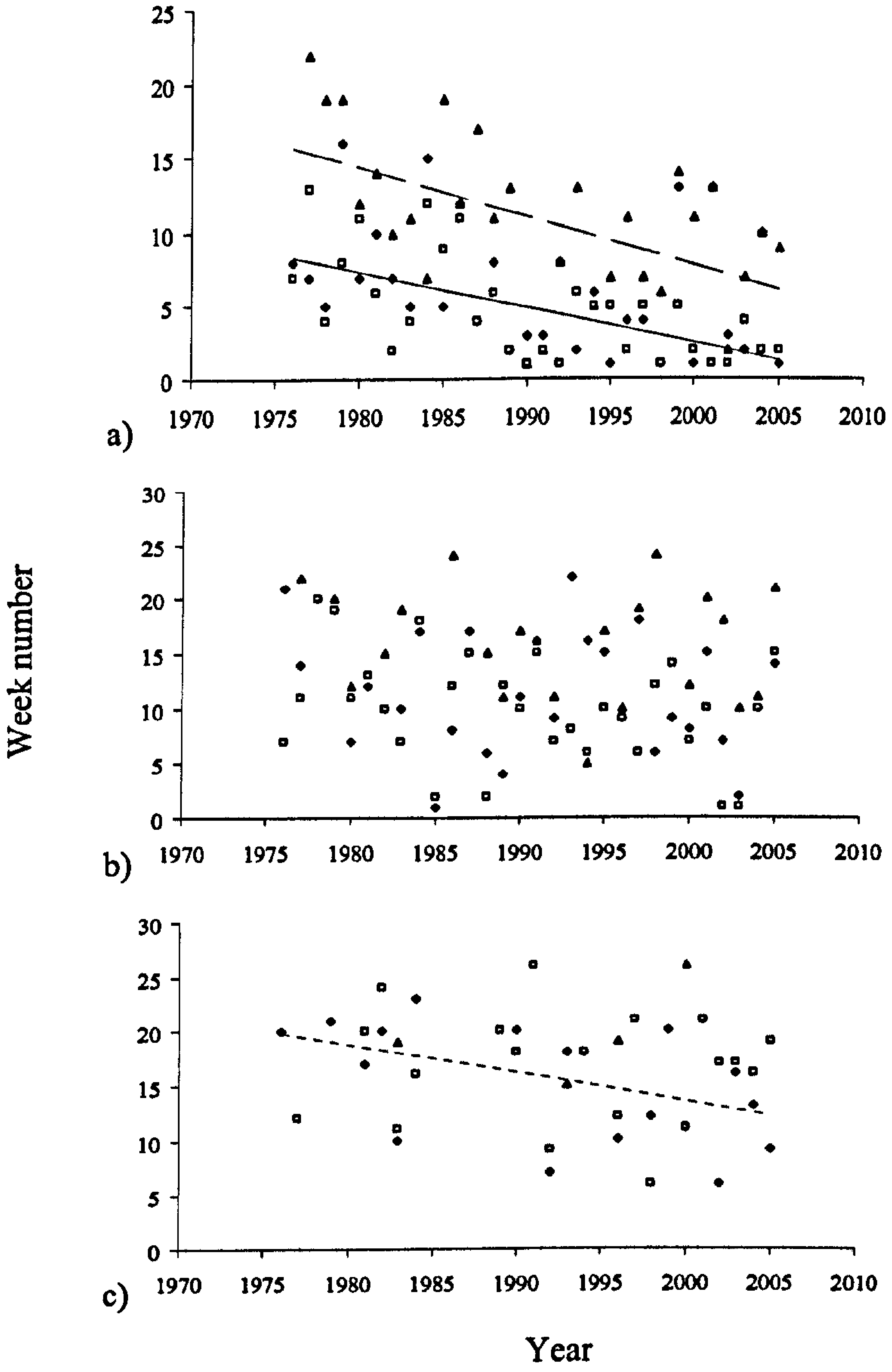


Figure 5.10: First arrival date, in weeks, for a) *V. atalanta*, b) *V. cardui*, c) *C. croceus*. North = triangle, Southeast = square, Southwest = diamond, Southwest trend line = dotted, Southeast trend line = black line, north trend line = dashed line.

| Species | Analysis | Migrant with Spain winter temp | Migrant with UK winter temp | Migrant with UK spring temp |
|---|---|---|---|---|
| *V.atalanta* | Distribution 15/16 | ns | Ns | P<0.001 |
| *V.atalanta* | Distribution 13/14 | ns | Ns | P<0.001 |
| *V.atalanta* | Distribution 17/18 | ns | Ns | P<0.001 |
| *V.atalanta* | Abundance 15/16 | ns | Ns | ns |
| *V.atalanta* | Abundance 13/14 | ns | Ns | P=0.033 |
| *V.atalanta* | Abundance 17/18 | ns | Ns | ns |
| *V.atalanta* | Abundance LR | ns | Ns | P=0.003 |
| *V.atalanta* | Distribution 15/16 | ns | Ns | ns |
| *V.cardui* | Distribution 13/14 | ns | Ns | ns |
| *V.cardui* | Distribution 17/18 | ns | Ns | ns |
| *V.cardui* | Abundance 15/16 | ns | Ns | ns |
| *V.cardui* | Abundance 13/14 | ns | Ns | ns |
| *V.cardui* | Abundance 17/18 | ns | Ns | ns |
| *V.cardui* | Abundance LR | ns | Ns | ns |
| *C.croceus* | Distribution 15/16 | ns | Ns | ns |
| *C.croceus* | Distribution 13/14 | ns | Ns | ns |
| *C.croceus* | Distribution 17/18 | ns | Ns | ns |
| *C.croceus* | Abundance 15/16 | ns | Ns | ns |
| *C.croceus* | Abundance 13/14 | ns | Ns | ns |
| *C.croceus* | Abundance 17/18 | ns | Ns | ns |
| *C.croceus* | Abundance LR | ns | Ns | ns |

Table 5.3: Correlation results between spring (migrant) and summer (resident) populations of three migrant species with Spanish winter temperature (mean of the months November-March), UK winter temperature (mean of the months November-March) and UK spring temperature (mean of the months May-July), NS = non significant.

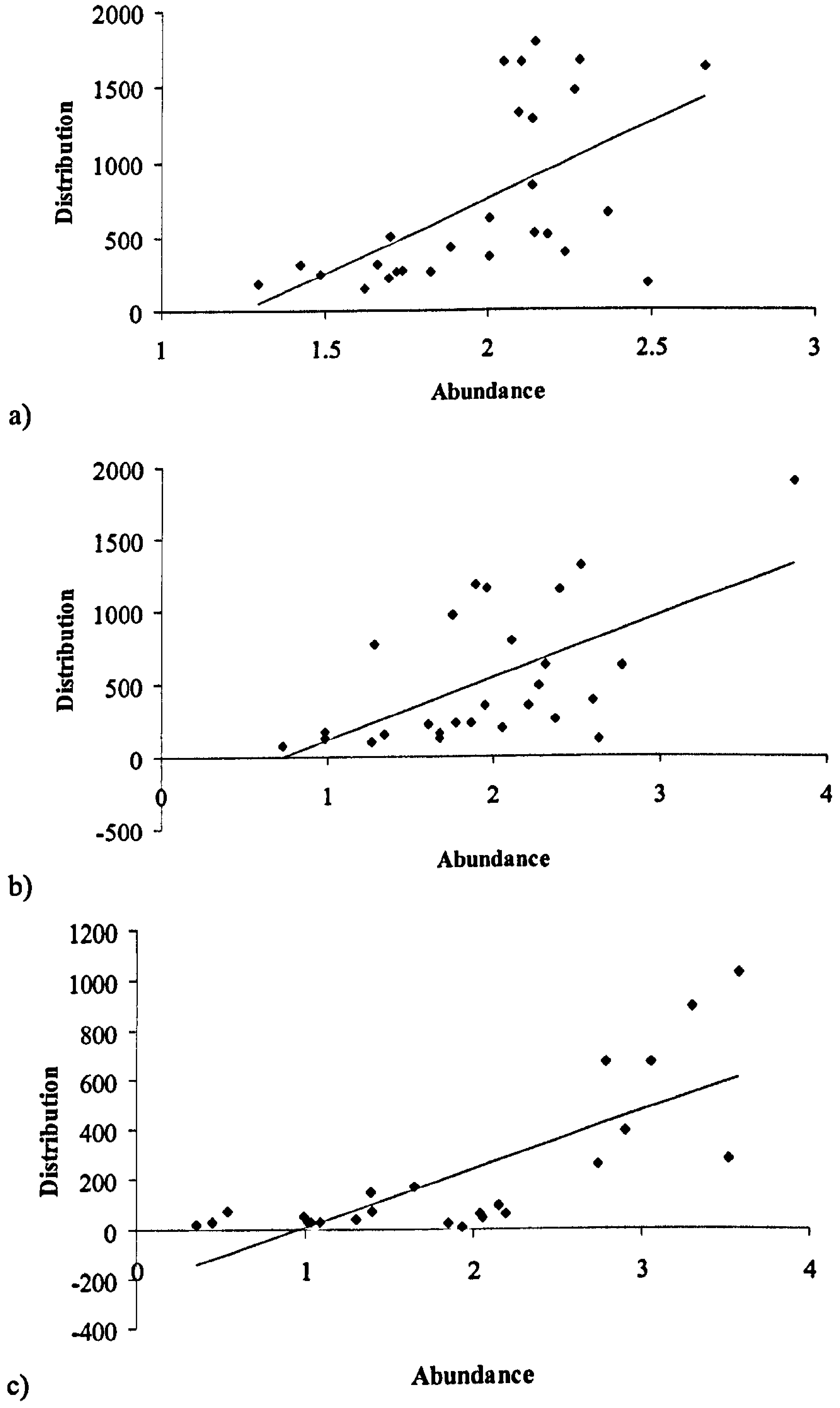


Figure 5.11: Relationship between yearly distribution (number of 10 km squares with records) and abundance (collated index of abundance from BMS) of a) *V. atalanta*, b) *V. cardui*, c) *C. croceus*. Significant regression lines are fitted.

## 5.5 Discussion:

### 5.5.1 Changes in Abundance:

The two migratory butterflies, *V. atalanta* and *V. cardui*, have shown significant increases in the abundance of both their migrant and summer populations in Britain over the past 30years. This suggests that not only is the number of individuals arriving each spring increasing, but that their reproductive success in Britain has increased over time, resulting in a greater second generation during the summer. However, the picture is not so clear for *C. croceus*. Significant increases were observed in analyses of distribution data, but only summer (resident) populations showed significant increases based on analysis of abundance data. This suggests that this species is responding positively to climate warming once it arrives in Britain, in terms of increased reproductive success, but that increased immigration has not been observed, unlike the other two migratory species. These finding largely confirm those of others who have observed increases in the abundance of spring immigrants in *V. atalanta* (Pollard & Greatorex-Davies, 1998; Sparks *et al.*, 2005) and in *V. cardui* (Pollard *et al.*, 1998).

To examine further what is driving the overall observed increases in these three migrants, as detailed in Fig 5.1, the proportion of summer individuals each year was calculated. Both *V. cardui* and *C. croceus* showed no trends in summer index over time, albeit a negative trend was observed in two of the analyses, indicating that migrant and summer populations of *C. croceus* have increased their abundance over time at approximately the same rate. Although studies on the migration of *C. croceus* are lacking, Pollard *et al.* (1998) showed that the abundance of resident European populations of *V. cardui* are determined largely by the size of the spring migration, with subsequent breeding success having little impact on the overall population size. From the findings of this study, it appears that both migrant and resident populations play an equally important role in affecting the overall abundance of *C. croceus*. In *V. atalanta*, the majority of the analyses showed a significant decrease in the proportion of resident individuals over time. This suggests that over time, as the abundance of this species has increased, the number of spring immigrants has increased at a greater rate than the number of resident individuals. As such, increases in British populations over time are apparently being driven by increased arrivals of individuals from the continent, a finding supported by Pollard & Greatorex-Davies (1998), albeit the reliability of my result is somewhat lacking because three out of the seven methods gave a non-significant trend.

### 5.5.2 Arrival Patterns:

The three migrants show different distributions in Britain, with *V. atalanta* and *V. cardui* individuals colonising habitats throughout Britain, while *C. croceus* is limited to more southerly latitudes, reaching only as far north as Lancashire. All three species had high values for the proportion of summer individuals throughout their distribution, with the vast majority of sites having greater numbers of summer resident individuals than migrants. When comparing between inland and coastal sites, there was no significant difference in the summer index between the two locations for *V. atalanta* or *C. croceus* for any of the years investigated. It is therefore concluded that these species colonise both inland and coastal sites on arrival, and are not restricted to coastal sites for re-fueling before continuing their migration inland. It is also apparent that there is no specific pattern in the location of first and second generation individuals, with individuals in the resident population as likely to occupy a coastal site as an inland site. If, as suggested, butterflies migrate within their flight boundary layer, then the minimum distance that they would have to fly over open water in order to reach Britain is 34km (the narrowest section of the English Channel). Butterflies have been recorded to migrate at speeds of 4-7ms$^{-1}$ thus taking approximately 1.3 – 2.3 hours to cross the narrowest section of water between continental Europe and Britain. Migration within the flight boundary layer is energetically expensive causing migrants to make regular stops in order to refuel (Gatehouse & Zhang, 1995). It is therefore expected that once individuals have crossed the large expanse of water they would take the first opportunity to re-fuel at coastal sites. However this idea is not supported by the findings from this study for *V. atalanta* or *C. croceus*, which show no preference of migrant arrivals to coastal locations. The results for *V. cardui* are not consistent, with significant differences occurring between the summer index of inland and coastal sites for 1982 and 1996, but not in 2003. However the differences between coastal and inland sites were in the opposite direction to that expected, with coastal sites having a higher proportion of resident individuals than inland sites.

Further evidence suggesting that migrant butterflies, arriving in Britain each spring, fly above their flight boundary layer comes from examining the arrival dates at coastal and inland sites.

Supporting findings by other authors (Roy & Sparks, 2000), *V. atalanta* and *V. cardui* showed significant trends towards earlier arrival dates (see Fig 5.8), although for *V. cardui* this was only the case for coastal sites. No significant differences were observed between the arrival dates for coastal and inlands sites suggesting that these species colonise the whole of the country at the same time, rather than arriving at coastal sites, re-fueling and then moving inland. This supports evidence that these species fly above their flight boundary layer taking advantage of high altitude airstreams (Stefanescu *et al.*, 2007). However, *C. croceus* has not shown any shifts towards earlier arrival dates, but there are significant differences in their arrival dates at coastal *versus* inland sites.

The findings from this study give somewhat contradictory evidence into the migratory behaviour of three butterfly species. While comparisons between summer index and arrival dates for coastal and inland sites for *V. atalanta* indicate that individuals colonise the country at approximately the same time, the results for the other two species differ. To gain a better understanding of colonization patterns in these three species, I examined differences in summer index and arrival dates between the north, southeast and southwest of Britain, to ascertain 1) whether there was an association between climate warming and the colonization of higher latitudes and 2) whether second generation individuals continue their migration northwards.

For *V. atalanta,* the highest proportion of resident individuals occurred in the southwest and north of the country, whereas the southeast had a higher proportion of migrant individuals, a trend that has increased over time. These results suggest that on their migratory pathway, *V. atalanta* individuals arrive in Britain in the southeast, flying across the Channel from the continent, with the second generation expanding west and northwards in Britain. The summer index in the north of Britain shows the greatest variation over time, with the proportional contribution of summer resident individuals ranging from 0.4 to 1. The high variability in the numbers of migrant individuals in the north could be attributed to the variability in wind currents that high-flying migratory insects are subjected to, such that in some years favourable winds allow individuals to travel greater distances, colonising sites further north. This conclusion is supported by examining trends in the arrival dates in the three regions. Arrival times are significantly earlier in both the southeast and north of Britain, a trend that has already been examined by Roy & Sparks (2005), and confirms that populations in southwest Britain comprise mainly second generation individuals. However, when comparing between the three regions, significant differences were observed between the two southern regions and the north, suggesting that although individuals have advanced their arrival dates over time in the north, they still occur later than in the south, such that northern populations are also mainly comprised of second generation individuals.

In contrast, *V. cardui* appears to follow a slightly different migration trajectory, with the southeast showing the highest proportion of migrant individuals, as does *V. atalanta*, but with the greatest variation in summer index occurring in the southwest, and with the north having very few numbers of migrant individuals. This indicates that *V. cardui* may take a northerly migratory pathway, arriving in Britain from due south, whereas *V. atalanta* takes a north westerly route and also colonising the south of Britain first. The colonisation of the southwest, however, is more variable than the south east, with the subsequent summer generation continuing the northward migration through Britain. In terms of arrival dates, *V. cardui* shows much greater variation with no significant trends over time in any of the three regions, although significant differences between the arrival dates at southern and northern regions substantiates the hypothesis that this species colonises the south of the country first before moving further northwards.

*Colias croceus* individuals appear to colonise Britain much more sporadically than the other two migrant species, with the number of resident individuals outweighing the migrant population, such that the summer index is very high in all regions. However, a significant decrease in the summer index was observed for the southwest, suggesting that the number of migrants colonising the southwest of Britain has increased over time. There are very few numbers of this species recorded in northern Britain, suggesting that this species has a northerly migratory direction, colonising the whole of the south of Britain, similar to *V. cardui.* The increase in migrants in the southwest is confirmed by examining arrival dates, with individuals arriving significantly earlier over time in southwest Britain. This conclusion is supported by anecdotal evidence of *C. croceus* seen flying northwards on the south coast, while individuals have been washed up on beaches along the south coast.

### 5.5.3 Driving Forces:

In order to determine what is driving these observed changes in the abundance of migrant butterflies in the UK, I examined temperature variables for Spain and Britain. I found that there were no correlations between the number of migrant *V. cardui* and *C. colias* and temperature variables in Spain or Britain. Individuals from these species predominately overwinter in North Africa (Tolman, 1997; Stefanescu *et al.*, 2007), and as such it is not surprising that overwinter temperatures in Spain have no effect on population size. However, it is surprising that the number of resident individuals of these two species is not driven by development temperatures in Britain during the spring, and therefore suggests that the reproductive success of these butterflies is governed by some other climatic variable, for example precipitation in North Africa. In contrast, significant correlations were found between British spring temperatures and the resident population of *V. atalanta*, a trend between temperature and development that has been demonstrated by Bryant *et al.* (1997). Therefore, as temperatures rise in Britain, the resident population of *V. atalanta* is expected to continue to increase, although the picture for the other two migrants is not so clear. It would be interesting to extend these analyses to examine additional climatic variables such as precipitation, as well as temperatures encountered along migration routes.

### 5.5.4 Comparing Datasets:

In Chapters 3 and 4, I used distribution data to investigate the responses of migrants to climate warming, however changes in recorder effort over time may have caused complications in the data analysis. In this Chapter, I have repeated some of the analyses with both distribution and abundance data, to determine if the type of data set analysed had any effect on the result. There was a significant relationship between the two data sets for all three species, indicating that distribution data are a good representation of abundance for these species, giving further validity to the results presented in Chapters 3 and 4. Both data sets gave similar results for analyses correlating abundance/distribution with temperature, however differences between data sets were observed when investigating changes in the size of migrant and resident populations. For *C. croceus,* differences between data sets may reflect that there are very few records available for this species in the UKBMS data set compared with the distribution data. From my results, I conclude that distribution data can be used as a surrogate for abundance data for analyses of responses to climate warming, although analyses of transect data where there is equal effort across sites may give more robust results.

## 5.6 Conclusion:

Three migrant butterflies regularly recorded in Britain have increased both their migrant and resident populations over a period of time associated with climate warming, while trends towards earlier arrival dates have also been observed. Evidence from arrival patterns showing that these species are not restricted initially to coastal sites suggests that these species are exploiting fast flowing airstreams above their flight boundary layer to aid migration. Differences in migratory routes taken by these species were inferred, with *V. atalanta* arriving predominantly from the south east, while the other two species arrive into Britain on a more northerly trajectory. Comparisons between distribution and abundance data sets suggest that distribution data can be used as a surrogate for abundance data, giving validity to the results in Chapters 3 and 4.

# Chapter 6

# The Effect of Photoperiod on Flight Directionality in Red Admiral Butterflies (*Vanessa atalanta*)

## 6.1 Abstract:

The abundance of migrants is predicted to increase in future (Chapter 5), but data are lacking on the environmental cues that affect migration behaviour. I investigated the role of photoperiod on migratory flight direction in *Vanessa atalanta*. *Vanessa atalanta* larvae and pupae were reared under three different regimes in the lab, representing Spanish photoperiods in spring (increasing from LD 9:15 to 18:6) and UK photoperiods in summer (constant 18L: 6D photoperiod) and autumn (decreasing LD 16:8 to LD 7:17), and flight direction was determined in a flight chamber. There was no difference in the directionality of adults among the three photoperiod treatment groups, although spring individuals had a preferred NNW flight direction consistent with northward migration from southern Europe into the UK in spring, while summer individuals showed random flight directions, consistent with termination of migration. Examination of ovarian development showed that females from all three treatments had mature oocytes and that the spring and autumn treatments had failed to produce diapausing individuals. I discuss the role of temperature in combination with photoperiod in initiating migration. Directionality of individuals did not change with age, and there were also no differences between males and females.

## 6.2 Introduction:

Migratory activity has been documented in a wide range of insect taxa and occurs regularly in locusts, butterflies, moths, dragonflies, hoverflies and beetles, with migrants travelling distances from a few hundred up to several thousands of kilometres (Williams, 1957). For example, *Locusta migratoria* individuals have been recorded in Britain, migrating up to 1500 miles from their overwintering sites in the Black Sea (Williams, 1957), whereas *Sympetrum striolatum* migrates approximately 500 miles from the Spanish Pennisula into southern Ireland (Longfield, 1948). In most insects, migration in temperate regions involves movements away from the equator during the spring and movements toward equator in autumn (Williams, 1957), involving a switch in the orientation of flight by 180° (Brower, 1996). This phenomenon has been observed in a number of Lepidoptera species worldwide, including *Vanessa atalanta, V. cardui* and *Autographa gamma* in the UK (Baker, 1978a; Chapman *et al.*, 2008a,b), *Danaus plexippus* in North America (Brower, 1996), *Vanessa itea, D. plexippus, D. chrysippus* and *Badamia exclamationis* in Australia (Dingle *et al.*, 1999). Migration can be controlled by genetic and/or environmental cues experienced during development (Rankin & Burchsted, 1992). This chapter will focus on environmental factors affecting migration.

### 6.2.1. **Environmental Factors Affecting Migration:**

To escape unsuitable conditions, which are predominately cold winters and an absence of food in temperate regions, insects have developed a diapause syndrome, in which they undergo a period of arrested development (Southwood, 1977; Leather *et al.*, 1993; Goehling & Oberhauser, 2002). In adults, this is typically characterized by the total or partial suppression of reproductive development (Hodek, 1983 within Campos, 2008), which is trigged by abiotic (photoperiod, temperature and moisture) and biotic (crowding and nutrition) cues (Leather *et al.*, 1993). Of these abiotic cues, photoperiod has been well-studied in insects, with changes in daylength following a regular seasonal pattern, and therefore providing a reliable cue for the forthcoming deterioration of habitat (Leather *et al.*, 1993; Campos, 2008). While diapause is an adaptation to avoid unsuitable conditions in time, species have evolved a spatial response through migration (Southwood, 1977). However a connection arises between diapause and migration, as discussed in Chapter 1, with many insect species entering into diapause before they migrate, embarking on a long-distance migration event in a pre-reproductive state (Johnson, 1969; Dingle, 1972; Wilson & Gatehouse, 1992; Campos, 2008).

Investigations into the effect of photoperiod regimes on arthropods have revealed that the propensity to enter diapause can be controlled by the number of hours of light an individual is subjected to (Spieth, 1995; Spieth *et al.*, 1998; Goehling & Oberhauser, 2002). When subjected to decreasing day length (at a rate of 3min $day^{-1}$), it was found that the incidence of diapause in *Danaus plexippus* increases significantly (Goehling & Oberhauser, 2002). While further experiments found that *Pieris brassicae* individuals reared under short days, interrupted by long days during larval stages were more likely to be non-diapause individuals (Spieth, 1995). The reverse also occurred such that a sequence of short days interrupted by long days was more likely to result in an increase in the number of diapausing individuals. Using these rearing conditions, Spieth *et al.* (1998) reared offspring from *Pieris brassicae*, a species that migrates from southern France to breeding territories in North Germany each spring, that were subsequently flown under semi-natural conditions. Spieth *et al.* (1998) found that the short-day regime produced individuals that flew north and that individuals reared under long days flew south.

### 6.2.2 Directionality Observed in Field Experiments:

Several field observations have been undertaken examining the migration patterns of insects in Europe, USA, Australia, Asia and the tropics including butterflies (Scott, 1992; Benvenuti *et al.*, 1996; Goehling & Oberhauser, 2002; Mikkola, 2003), moths and dragonflies (Feng *et al.*, 2006), and carabids (Feng *et al.*, 2007), with the consensus that spring migrations are directed away from the equator, while the reverse is observed in autumn. As early at the 1930s and 1950s, observations of seasonal changes in flight direction of migrant Lepidoptera were recorded in the UK (Fisher, 1938; Williams, 1951). *Autographa gamma*, *Vanessa atalanta* and *Colias croceus* were observed to show a predominantly northerly direction during May - July with a southerly direction predominating in autumn (Fisher, 1938; Williams, 1951). These findings are supported by observations of *V. cardui* migrating through Colorado (USA), which showed 80% migration efficiency (unidirectionality) in an east-northeast/northeast direction during the peak migration period in spring (Scott, 1992). Migratory *V. atalanta* arriving in over-wintering sites in Catalonia in autumn have a southerly preference (Stefanescu, 2001), with similar patterns also evident in Italy (Benvenuti *et al.*, 1994). These findings are strengthened further by studies undertaken in Australia, where five butterfly species, including *V. cardui* and *D. plexippus*, were observed flying in a predominately southern (polewards) direction during spring-summer, and towards the equator in a northerly direction during autumn-winter (Dingle *et al.*, 1999). Similar seasonal switches in the direction of migratory flights have also been observed in a number of moth species migrating over the Panama canal (Srygley & Dudley, 2008), and in the UK (Chapman *et al.* 2008a,b). However, there is little understanding of the physiological mechanisms of orientation utilized by these species to enable migration in the appropriate direction.

### 6.2.3 Navigation in Insect Migrants:

A number of hypotheses have been put forward as to how insects navigate during migration events, including the use of a sun-compass and the use of the Earth's magnetic field. Coupled with these mechanisms is the ability of some species to compensate for crosswind drift. For species that fly within their boundary layer it is hypothesised that species can fix a bearing using one or more landmarks, and such avoid being 'blown off course' (Srygley & Dudley, 2008). However, this is not possible for species that migrate within high-altitude airstream, and as such they must be fully reliant on a compass system (Chapman *et al.*, 2008a), while species flying within the flight boundary layer become solely dependent upon some other orientation mechanism when crossing open expanses such as water where landmarks are limited.

The use of a sun compass is reliant on a time-compensation mechanism, whereby an individual can compensate for the apparent movement of the sun across the sky and so maintain a constant bearing throughout the day (Oliveria *et al.*, 1998; Åkesson & Hendenström, 2007). The use of a time-compensated sun compass during migratory flights has been demonstrated in a number of species in clock-shift experiments, in which predictable shifts in orientation were caused by advancing or retarding a species circadian clock (Oliveira *et al.*, 1998), including the migrant butterflies *Aphrissa statira, Phoebis argante* (Oliveira *et al.*, 1998), *D. plexippus* (Mouritsen & Frost, 2002; Stalleicken *et al.*, 2005), and *V. cardui* (Scott, 1992).

Although a number of species appear to rely on a sun-compass mechanism to navigate, it begs the question of how nocturnal migrants orientate, and indeed diurnal migrants during overcast periods. Another possible mechanism of navigation is that of a geomagnetic compass in which the Earth's magnetic field provides a source of directional information (Lohmann *et al.*, 1995; Åkesson & Hendenström, 2007). A study into the migratory pathways of the migrant moth *Autographa gamma* (silver Y), demonstrated that individuals were able to compensate for wind drift during high-altitude flight, and as such must use a navigational mechanism (Chapman *et al.*, 2008a, b). Use of a sun compass is ruled out as this species is a nocturnal migrant, while the use of the moon or stars as an orientation cue is improbable as this species continues to migrate during clouded conditions and the resolution of the compound eye is such that the use of a stellar compass is not possible, leaving a geomagnetic compass as the most likely mechanism (Cardé, 2008; Chapman *et al.*, 2008a). Further evidence for the use of a geomagnetic compass as a navigational tool comes from migrating butterflies and dragonflies, both of which were observed to continue at a preferred compass direction under overcast conditions (Srygley & Dudley, 2008). Further manipulative experiments on *Aphrissa statira* provide evidence for the use of a geomagnetic compass, with individuals subjected to a magnetic field of reversed polarity flying in an opposite direction to that of their natural orientation (Srygley *et al.*, 2006).

### 6.2.4 Experimental Studies on Flight Direction

As indicated above, a number of experimental studies have been undertaken on the preferred flight direction of migratory butterflies (Scott, 1992; Benvenuti *et al.*, 1994; Benvenuti *et al.*, 1996; Oliveira *et al.*, 1998; Spieth *et al.*, 1998; Mouritsen & Frost, 2002; Syrgley *et al.*, 2006; Srygley & Dudley, 2008). Across these studies, a number of experimental approaches have been undertaken, the majority of which have relied on field observations of wild individuals or of released individuals that have been experimentally manipulated. Migrants have been tracked, either by researchers on foot (Scott, 1992; Benvenuti *et al.*, 1994; Benvenuti *et al.*, 1996) or by boat (Oliveira *et al.*, 1998; Syrgley *et al.*, 2006; Srygley & Dudley, 2008) or by the use of radar (Chapman *et al.*, 2008). Investigations into the orientation mechanism of insects have also been investigated by placing tethered individuals into a flight simulator (e.g. Mouritsen & Frost, 2002) or by observing free-flying individuals in a choice chamber (Coombe, 1982; Speith *et al.*, 1998).

### 6.2.5 Rationale for Further Work:

A number of studies have examined how migrant insects orientate, with the consensus that a sun and/or geomagnetic compass plays an important role in directionality. However, less work has focused on examining differences in flight direction between migratory and non-migratory populations of the same species. In this chapter I will focus on the butterfly *V. atalanta,* comparing the directionality of migrant and non-migrant individuals.

### 6.2.6 General Aims and Predictions of this Chapter:

This chapter investigates directionality of individuals reared under three different photoperiods characteristic of spring in Catalonia (increasing photoperiod), summer in Britain (constant photoperiod) and autumn in Britain (decreasing photoperiod). It also examines whether flight direction varies with age and sex of individuals. Given that there is a limited period for migration before reproduction, younger adults reared under spring and autumn conditions would be expected to show stronger flight preferences compared with older individuals, as well as compared with summer individuals. This chapter has the following objectives:

1. Determine if 'spring' and 'autumn' individuals exhibit a preferred northerly and southerly flight direction respectively, while summer, non-migratory individuals show no preferred flight direction.

2. Test the hypothesis that younger migrant butterflies exhibit stronger flight direction preferences than older individuals.

3. Determine if *Vanessa atalanta* is using a time-compensated sun compass as a navigational tool.

## 6.3 Materials and Methods:

### 6.3.1 Insect Material:

Material used in this study were reared from four females, three collected from York (two in Haxby (OS grid reference SE610579) on 27th June 2005 and 9th July 2005, and one in Bishop wood (OS grid reference SE544348) on 23rd June 2005)), and one from Witherslack (Lake District (OS grid reference SD4382) on the 28th June 2005). The females were kept in net enclosures in a glasshouse and provided with nettle stems to encourage egg-laying, and fed with honey water. First instar larvae were removed on hatching and reared under three different light regimes. Photoperiod treatments were chosen to represent spring conditions in Catalonia (9L:15D increasing to 19L:5D), summer conditions in the UK (constant 18L:6D) and autumn conditions in the UK (16L:8D declining to 6L:18D; Fig 6.1). These treatments will subsequently be referred to as 'spring', 'summer' and 'autumn' in the rest of the Chapter. Temperature was maintained at 20°C in all treatments. Offspring from each female were split equally among the three treatments, resulting in 270 1st instar larvae per treatment. Offspring from three females caught in June were placed in incubators on 7th July 2005, at a density of ten larvae per nettle stem, with subsequent hatching larvae being added to the incubator a week later, on 15th July 2005. The fourth female caught in July did not produce 1st instar larvae until August, and the majority of larvae were placed in incubators on 21st July 2005, with subsequent individuals added to incubators on 8th August 2005. Despite the discrepancy in the date that larvae were placed in the incubators, individuals spent the same amount of time exposed to the different light regimes, with individuals developing under the spring and autumn conditions experiencing the same net change in daylight. After emergence adults were kept individually under the conditions in which they had developed and fed honey water *ad libitum*. Food was then removed two hours before testing for flight direction in order to encourage flight.

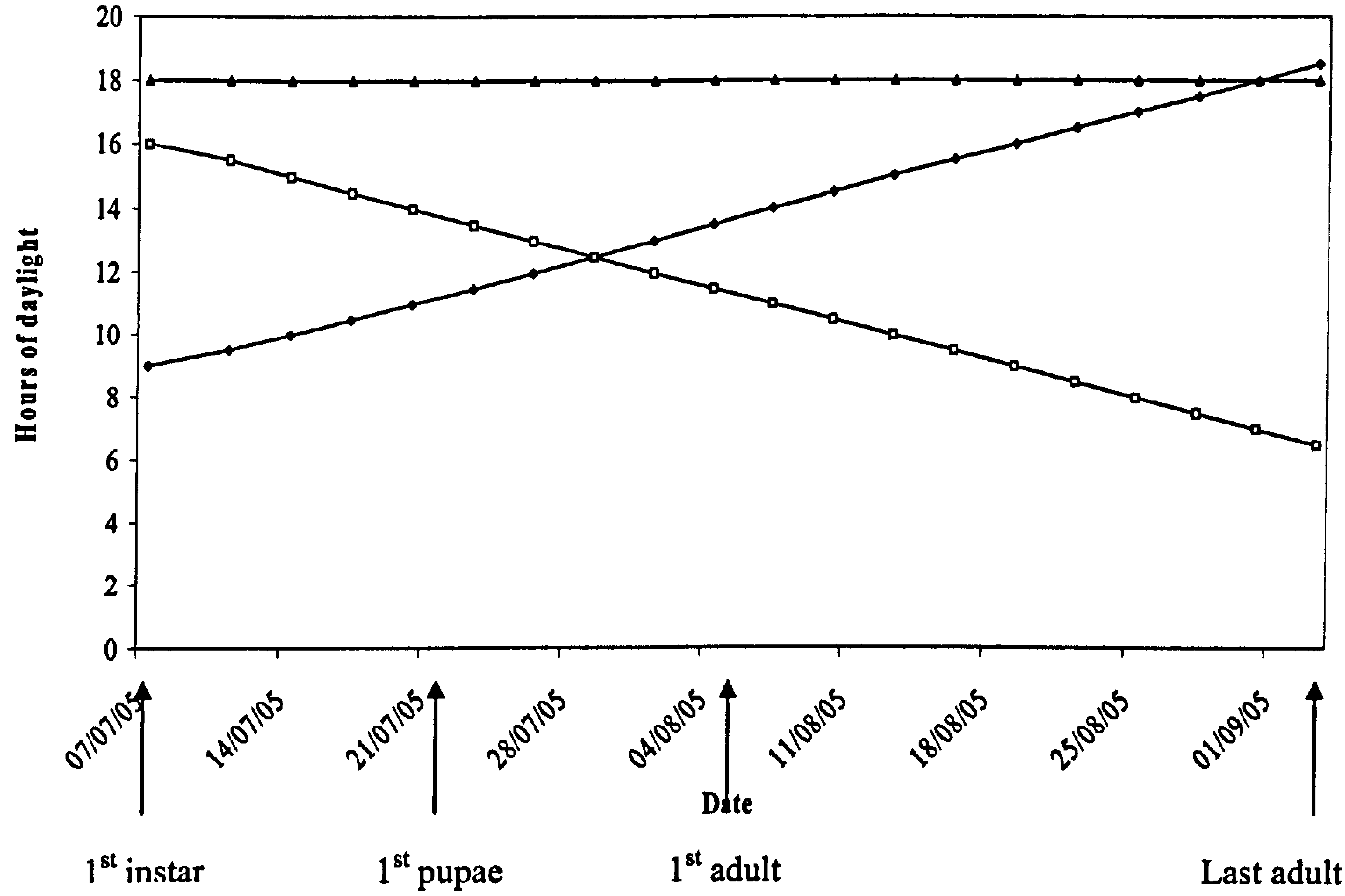


Figure 6.1: The light conditions (number of hours of light per day) in each of the three treatments. The initial number of hours in each treatment correspond with the latitudinal light levels in Spain (spring) and UK (summer and autumn). Diamond = spring, triangle = summer and square = autumn.

### 6.3.2 Experimental Design:

A flight chamber was constructed out of transparent Perspex and consisted of a 1 m diameter cylinder with 25 cm high walls (Fig 6.2). Within the cylinder there were 16 transparent Perspex walls each 25 cm long that divided the chamber up into 16 sections with compass directions, N, NNE, ENE, E, etc. Thus each adult butterfly had a choice of 16 directions in which to move. The flight chamber had a removable lid, and there was a 10 cm diameter hole cut in the lid through which the butterflies could be introduced to the chamber, and later removed. The chamber was placed on a turntable so that it could be rotated without disturbing the butterflies. After a direction was chosen by an individual, the chamber was rotated, leaving the individual to fly around the chamber and choose another direction. This was done to ensure that no bias was introduced into the experiment by the individuals showing a preference to a particular section of the chamber, and so that the directionality of the butterflies could be measured 3 times during each trial without having to disturb the butterflies. An initial trial run was performed to assess whether or not the presence of an observer and rotating the chamber had any effect on butterfly behaviour. Three butterflies, one from each treatment were placed in the flight chamber and the time taken for them to settle in a preferred section of the chamber was recorded. The behaviour of the butterfly was then monitored with the observer positioned at different locations around the chamber to determine if the butterflies would fly into the section of the chamber adjacent to the observer. The chamber was then rotated a number of times, with a 2 minute gap between rotations to evaluate if the movement of the chamber would cause the butterflies to start flying around the chamber. This was then repeated with a further nine butterflies under sunny and cloudy conditions. During this trial, the butterflies showed no reaction to the presence of an observer or the turning of the flight chamber. Butterflies would not fly in clouded conditions or when temperatures were lower than 20°C and so observations were made only on sunny days when the temperature was >20°C. Photographs of the experimental set up are shown in Figs 6.2 and 6.3.

Figure 6.2. Perspex flight chamber with a temperature probe.

Figure 6.3. *Vanessa atalanta* sat on one of the dividing walls within the chamber. The white spot on the right forewing has been marked with red ink to identify it as an individual reared under the spring treatment.

For each observation in the flight chamber, three butterflies of the same sex, one from each treatment, were placed in the chamber at the same time and were allowed 5 minutes to acclimatise and warm up if necessary. Each butterfly was marked with a different colour on the white spot on the forewings according to treatment so that it could be identified from a distance (Fig 6.3). The first direction that each butterfly flew in was recorded as the initial direction and then the chamber was turned through 180 degrees. The butterflies were then allowed five minutes of flight before the second choice of direction was recorded. This was repeated a further two times so that for each individual there were a total of four recorded directions. After each rotation of the flight chamber if an individual did not fly within the next five minutes then the chamber was rotated again and this continued until the individual flew and the direction was then recorded. Observations took place between 11.00 and 16.30 with the choice chamber placed outside in an open garden. A temperature probe was placed within the apparatus (Fig 6.2), and the weather conditions, time of day and temperature were recorded at the start of every observation.

### 6.3.3 **Statistical Analysis:**

The preferred flight direction of individuals was calculated for each treatment group, and was termed migration efficiency. This was calculated as the length of the total vector divided by the number of individuals observed in each treatment, where the total vector represents the sum of all the directions exhibited by individuals in each treatment. This value represents the proportion of individuals in a treatment that had the same flight direction, with values ranging from 0, indicating randomness with respect to direction, to 1 where all individuals exhibit the same flight direction (Scott, 1992; Dingle *et al.*, 1999).

The data on flight direction were analysed using circular statistics. For each individual, the mean angle of four recorded flight directions was calculated and the significance of the flight direction from a random distribution was tested within each treatment group using the Rayleigh test (Batschelet, 1981). Differences in mean flight direction among treatments were examined using a multi-sample Mardia-Watson-Wheeler test (Batschelet, 1981). This test was also used to investigate whether flight direction differed according to adult age and sex. A circular-linear correlation coefficient was calculated in order to test for any correlation between flight direction and temperature. I tested for any effects of turning the flight chamber on the subsequent flight direction for each flight for each individual within the three treatments using a Watson-Williams test.

## 6.4 Results:

A total of 632 adults were reared from the three photoperiod treatments. The mortality rate varied slightly between the treatments (9%, 12% and 14% for spring, summer and autumn treatments respectively), but development times showed little variation (average of 32 (days for spring (SE = 0.34), and 35 days for summer (SE = 0.40) and autumn (SE = 0.44) treatments). From 2nd August to 4th September 2005, a total of 65 spring, 65 summer and 69 autumn adults were flown in the flight chamber. In order to investigate affects of age on flight direction, 68 adults were flown once within five days of emergence, and 32 individuals were flown 2-3 times up to 15 days after emergence..

### 6.4.1 **Flight Direction of the Spring, Summer and Autumn Individuals:**

Spring individuals had a preferred flight direction in a NNW direction, as predicted (Rayleigh test, $p<0.001$, table 6.1). Also as predicted, summer individuals did not exhibit any preferred flight direction (Rayleigh test, $P>0.05$). However, autumn individuals did not exhibit any preferred direction (Rayleigh test, $P>0.05$), which was not what was predicted. Circular histograms of flight directions chosen by individuals from each treatment are shown in figure 6.4. There was no difference in mean flight direction among treatments (Mardia-Watson-Wheeler test, $p = 0.279$).

| Treatment | N | Mean vector | Migration efficiency | Rayleigh test Z | Rayleigh test p value |
|---|---|---|---|---|---|
| Spring | 65 | 327.846 | 0.321 | 6.716 | **0.001** |
| Summer | 65 | 313.798 | 0.167 | 1.805 | 0.165 |
| Autumn | 69 | 293.782 | 0.095 | 0.627 | 0.534 |

Table 6.1. Flight direction of *V. atalanta* individuals reared under spring, summer and autumn photoperiod treatments. Mean vector is a measure of the concentration of flight headings, while the migration efficiency represents the proportion of individuals flying in the same direction, i.e. the mean vector.

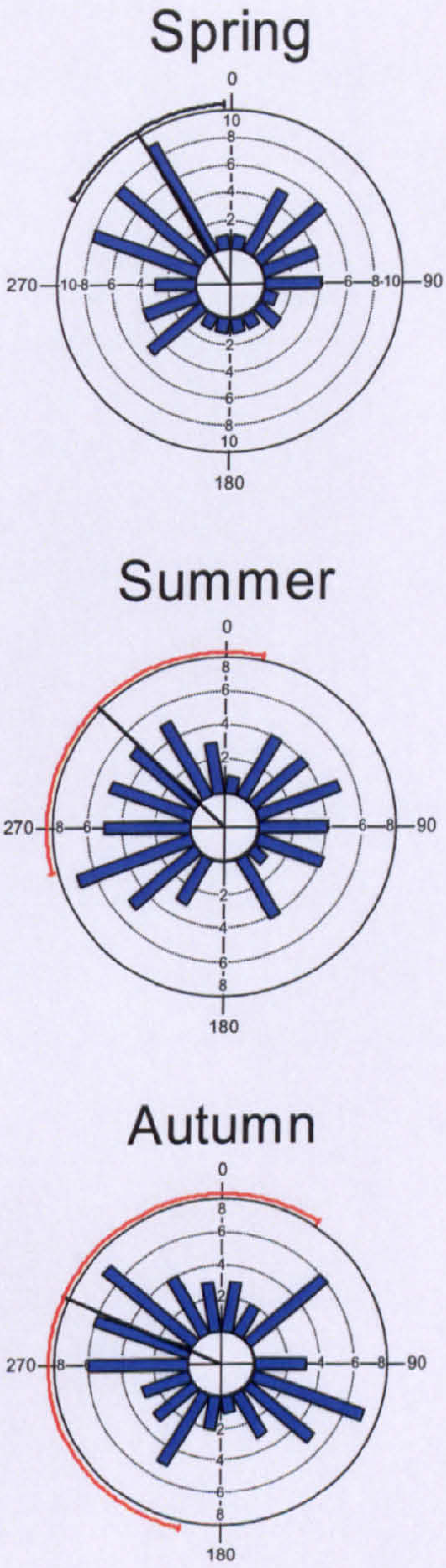


Figure 6.4. Flight direction of butterflies flown under three photoperiod regimes, representing spring, summer and autumn. Each bar represents the frequency of butterflies that flew in that direction. A) Spring individuals (n=65) showed a highly significant NNW flight direction (Rayleigh test, $p<0.001$). B) Summer individuals (n=65) did not show a significant flight direction (Rayleigh test, $p=0.165$). C) Autumn individuals (n=69) also did not show a significant flight direction (Rayleigh test, $p=0.534$). The line around the circumference is the 95% confidence limits of the mean flight direction; a red line indicates that there was an even spread across all directions.

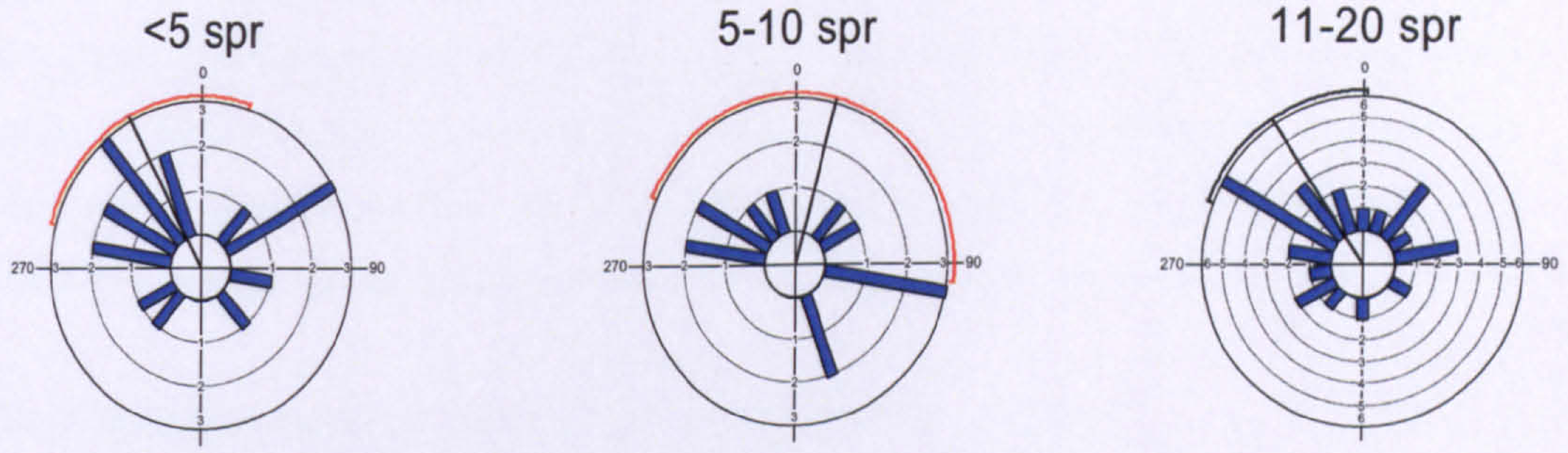
### 6.4.2 Flight Direction and Age:

Adults were tested from 1 day to 15 days post-emergence. Data were assigned to three age groups for analysis: <5days (n = 55), 5-10 days (n = 59) and 11-20 days (n = 61) old. Directionality was detected only in the 11-20 day old spring individuals (Rayleigh test, p=0.008; Fig 6.5). There was no significant difference in flight direction between the age groups for any of the treatments (see table 6.2).

| Treatment | W | *P* |
|---|---|---|
| Spring | 1.374 | 0.849 |
| Summer | 1.822 | 0.768 |
| Autumn | 2.15 | 0.708 |

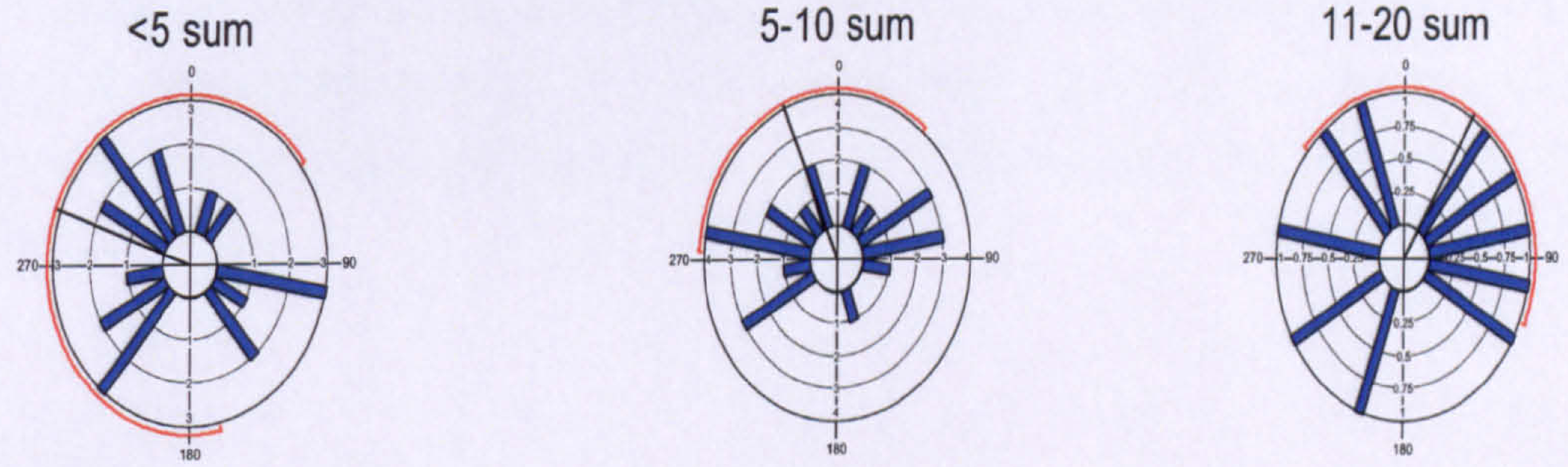
Table 6.2: Comparison of the difference in mean flight direction of *V. atalanta* from different age groups within treatment groups. There was no significant difference among age classes within any treatment group (Mardia-Watson-Wheeler test, p>0.05 for each treatment).

**Spring individuals:**



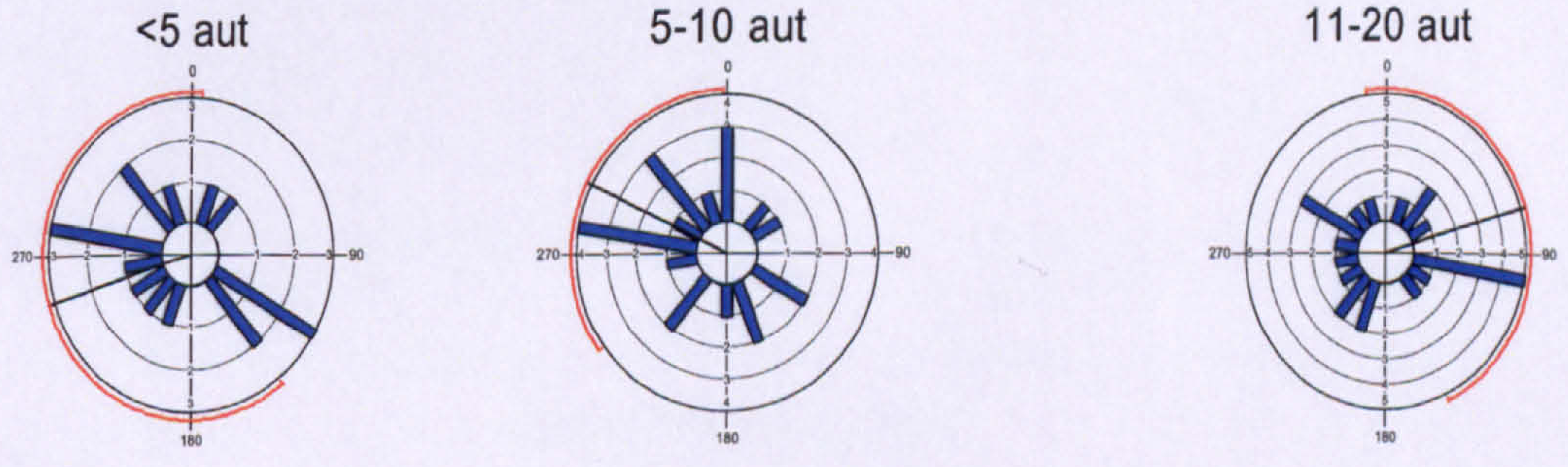
n = 17, $p = 0.064$ n = 13, $p = 0.804$ n = 10, $p = 0.008$

**Summer individuals:**



n = 21, $p = 0.668$ n = 24, $p = 0.217$ n = 10, $p = 0.727$

**Autumn individuals**



n = 17, $p = 0.615$ n = 22, $p = 0.207$ n = 23, $p = 0.99$

Figure 6.5. Circular histograms showing flight directions of individuals from different age groups from the three photoperiod treatment groups. *p* values give significance of Rayleigh tests.

### 6.4.3 Egg Development:

Fifteen females from each treatment were killed on emergence and subsequently dissected to determine their ovarian development. Females in reproductive diapause have small ovarioles, with no ovarian development (Herman, 1973), and as such I investigated the incidence of un-yolked, yolked and mature (chorionated) oocytes. All females in each of the three treatment groups had a large number of mature oocytes present.

### 6.4.4 Further Investigation into Factors Affecting Directionality:

There is no pattern observed in the change of direction demonstrated by individuals from all three treatments, as shown at Fig 6.6. The sun moves across the sky at a rate of 15 degree clockwise per hour. If the butterflies used a sun compass then they would be expected to follow this trajectory. As Fig 6.6 demonstrates there is no similarity in the pattern shown between the movement of the sun, and the change in directionality in *V. atalanta* individuals.

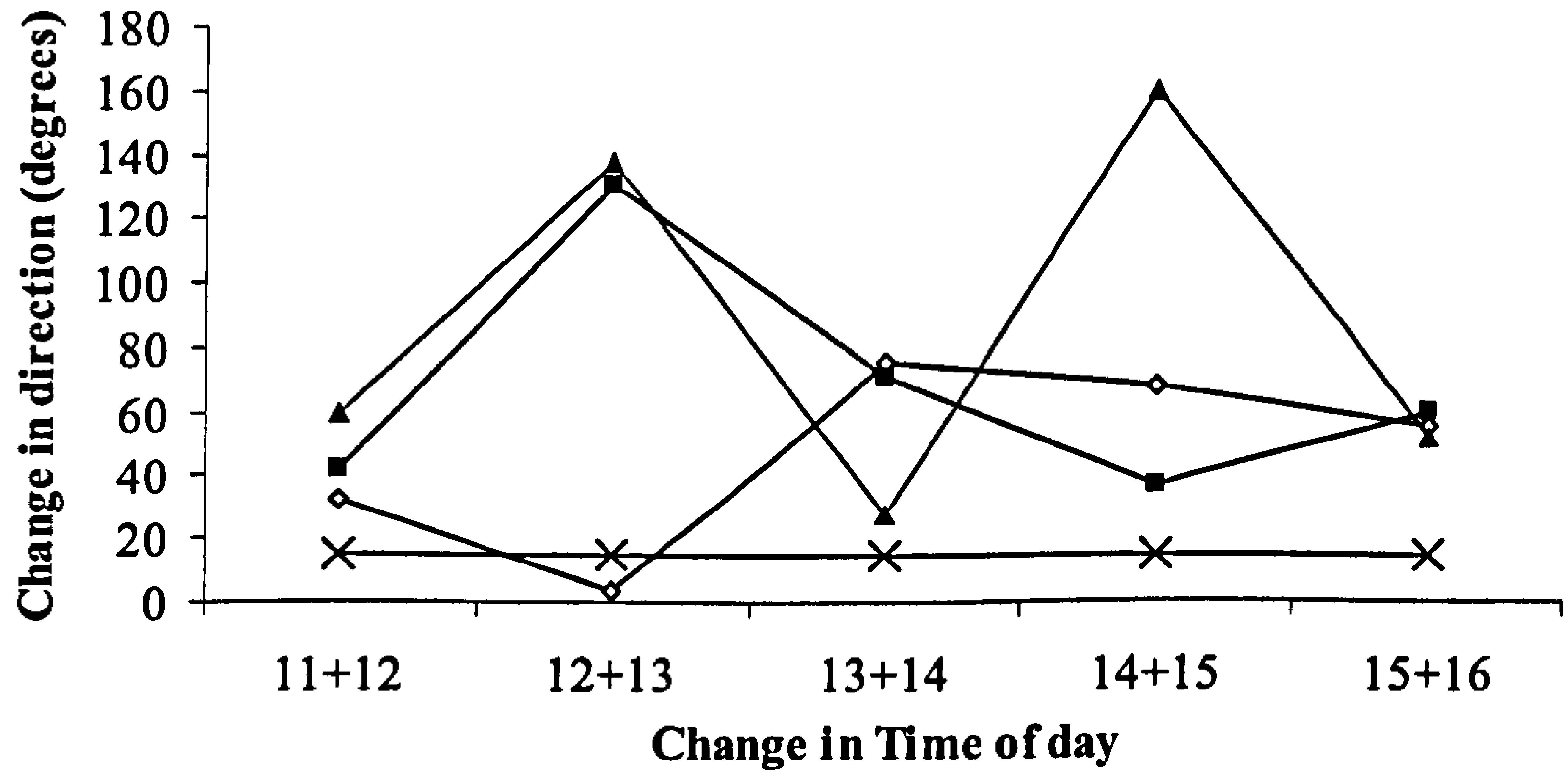


Figure 6.6: The mean change in direction between the hours of the day, starting at 11am and finishing at 4pm for the sun (cross) and the spring (diamond), summer (square) and autumn (triangle) individuals. The sun moves by 15° / hour so if the butterflies had tracked this then you would expect the change in mean direction to change by 15 degree an hour.

## 6.5 Discussion:

### 6.5.1 Treatment Affects:

Spring individuals showed significant directionality, preferring a NNW direction as predicted. These findings support those of other studies that have observed migrant individuals exhibiting a strong preference for a northerly direction during their spring migration, both in the wild-bred *V. atalanta* (Williams, 1951; Benvenuti *et al.*, 1996) and experimentally-bred *Pieris brassicae* (Spieth *et al.*, 1998). Summer individuals showed no preferred flight direction supporting observations of migratory *Vanessa cardui* in Colorado, which were observed to lose unidirectional flight once migration had terminated (Scott, 1992). However, autumn individuals were expected to show a southerly flight direction, but in this study individuals exhibited a random flight direction. This differs from other studies (Benvenuti, *et al.*, 1996; Stefanescu, 2001; Mikkola, 2003). Overall, there were no significant differences in mean flight directions of individuals among treatments, differing from field studies of other migrant butterflies showing that flight path orientation in *D. plexippus* differed significantly between spring, summer and autumn observations (Dingle, 1999).

Migrant insects usually enter reproductive diapause, thus allowing them time to migrate before breeding (Goehling & Oberhauser, 2002; Campos, 2008). Therefore I expected that females in the migrant treatments in this study should be at an earlier stage of reproductive development compared with summer individuals. I measured the ovarian development of females at 1, 3, 6 and 9 days post-emergence, but there was no difference in the sexual maturity of spring and autumn individuals compared with summer individuals. All females were sexually mature, as indicated by the presence of well-developed eggs and the experiment failed to produce diapausing migrants.

Individuals bred under summer conditions in this experiment exhibited no signs of diapause, with mature oocytes present within females, supporting findings by Goehling & Oberhauser (2002), who found similar results in *D. plexippus* kept in an incubator at 21°C under summer photoperiods, namely LD 16:8. However, conflicting results were found for the spring and autumn treatment, with no observations of delayed reproductive development. During larval and pupal development, *V. atalanta* individuals were subjected to increases and decreases in photoperiod at the rate of 10mins day$^{-1}$. This is greater than under natural conditions, where light levels change at 3min day$^{-1}$, and so it would be expected to increase the chances of getting migrants. During the study, although all individuals underwent the same net change in photoperiod, there was an unavoidable delay with some 1st instar larvae not entering the treatment until the 21st July, with the latest on the 8th August. The lack of consistency in the results may suggest that there is a critical threshold in photoperiod that needs to be passed in order to initiate a migration. Therefore the delay in entering the treatment may have meant that individuals missed this 'critical threshold' and as such were not stimulated to enter diapause. A further explanation for the inconsistency, is that within the incubator there was no gradual decrease and increase in light levels, equivalent to sunrise and sunset thus creating an artificial environment. During the experiment a constant temperature of 20°C was maintained and this may be an additional explanation for the lack of consistency in the data. Although photoperiod may be an important cue in initiating a migratory event (Leather *et al.*, 1993; Campos 2008), temperature, also being seasonally variable, may also act in combination with photoperiod as a cue for migration (James, 1983). As such, individuals are more likely to exhibit arrested development when subjected to decreasing photoperiod in combination with fluctuating temperatures (Goehling & Oberhauser, 2002). This highlights that much research is still needed to determine the exact cues behind migration, whether it be photoperiod, temperature or a combination of the two.

### 6.5.2 Age Effects:

There was no effect of age of the directionality of individuals from any of the treatments, while even the youngest females were found to have developed mature eggs. This supports findings observed in *Pieris brassicae*, where butterflies flown for 14 days did not change their preferred flight direction as they got older (Spieth *et al.*, 1998) and in *Agrotis ipsilon* where moths of all ages were found to be capable of undertaking long flights, although 3-day old individuals had the greatest capacity for sustained flight (Sappington & Showers, 1991).

### 6.5.3 External Effects:

Baker (1969) suggested that migrant insects fly at a constant angle to the sun, however there was no evidence of this in my study. For all three treatments, the hourly change in flight direction was very different from the $15^0$ hourly change in the sun's direction (see Fig 6.6) suggesting that butterflies in this study did not track the sun's movement throughout the day. In addition, the time of day at which individuals were flown had no significant affect on mean flight direction for any treatment ($p>0.05$), as found in other studies (Benvenuti *et al.*, 1996, Spieth *et al.*, 1998). These results support the hypothesis that butterflies do not track the sun's movement, but orientate using a time-compensated sun-compass as discussed above.

Temperature had a highly significant effect on flight direction and butterflies increasingly preferred NNW flight direction under warmer temperatures and SEE flights at cooler temperatures. This is contrary to findings by Spieth *et al.* (1998) who found that in *Pieris brassicae*, preferred flight direction was independent of temperature. However field observations in Italy on *V. atalanta* showed that temperature did appear to have an effect, with more easterly directions in cooler temperatures ($15^0$C) and more southerly directions as temperatures increased ($25^0$C). This observed change in direction caused by changes in temperature is similar to trends observed in this experiment, Fig 6.7.

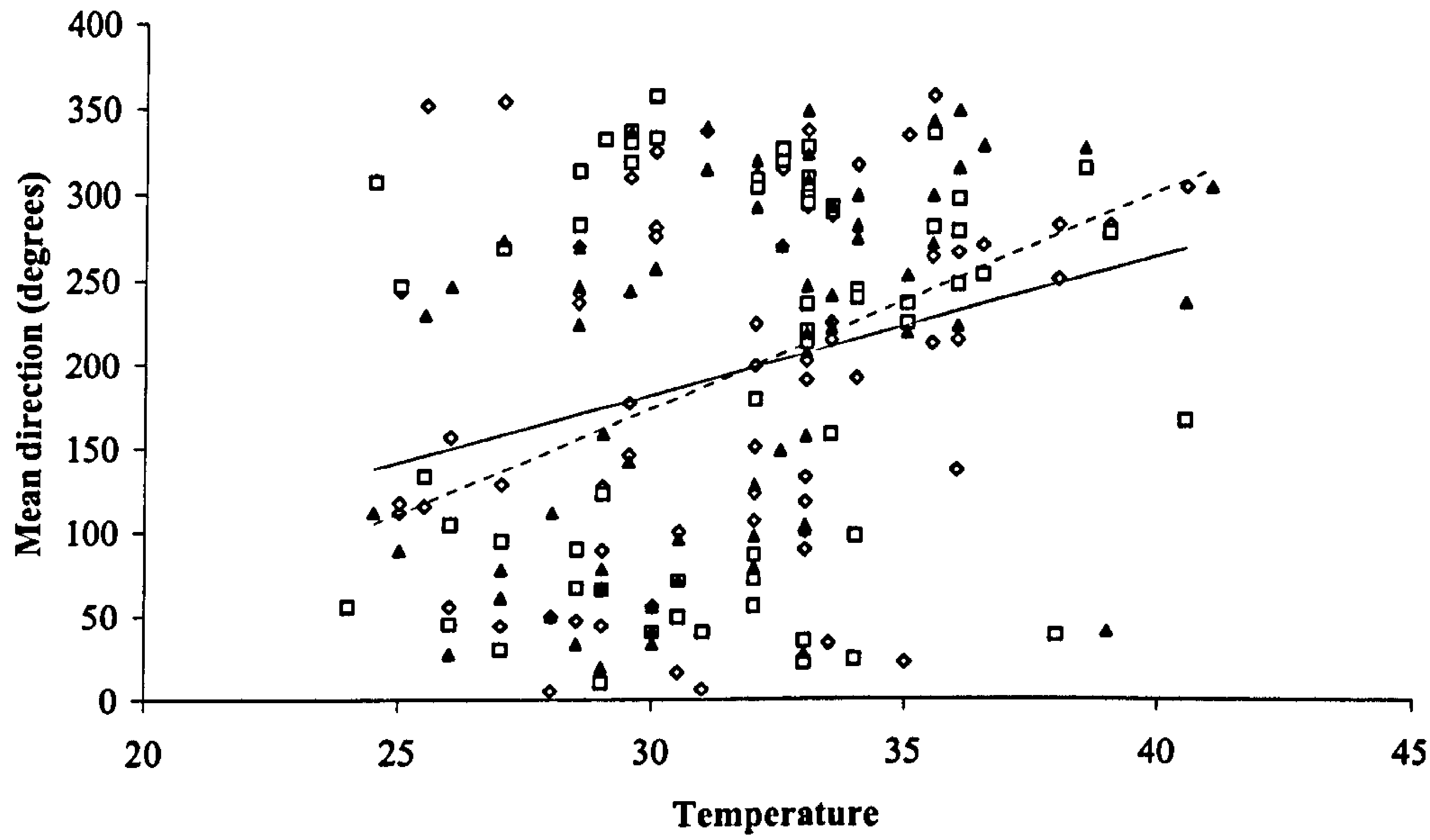


Figure 6.7. Effect of temperature on the mean flight direction of individuals from spring (n = 65), summer (n = 65) and autumn (n = 69) treatments. North northwest directions were preferred at higher temperatures. (Spring = squares; summer = triangles and dashed line; autumn = diamonds and solid line)

## 6.6 Conclusion:

The distribution and abundance of migrant butterflies in Britain has increased over time, however little information is available on the cues that control flight direction during migration. Spring individuals showed significant NNW directionality in their flight direction consistent with predictions for individuals flying northwards from Spain to the UK in spring. Summer individuals showed no flight directionality as predicted in non-migrants. However, autumn individuals also showed no flight directionality, and there was no significant difference between the three treatments in their preferred flight direction. It is likely that the conditions experienced in the incubators were not consistent with those needed to initiate the development of diapausing migrant, particularly by autumn migrants. Further research on the role of photoperiod, particularly in the investigation critical threshold limits and the impact of temperature is needed to fully understand what initiates a migration event.

## Chapter 7

# Tracking butterflies and determining migratory status using hydrogen and oxygen isotopes

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## 7.1 Abstract:

As shown in previous Chapters, migrants are becoming more widespread and numerous in their summer breeding ranges in Britain. In order to understand these migratory events, and to investigate the effect of climate warming on the incidence of overwintering in the UK, I examined whether or not stable isotopes are a useful tool for determining the migratory status of Lepidoptera. I analysed stable isotopes of hydrogen and oxygen to determine the natal origins of wild-caught Lepidoptera (*Vannessa atalanta* and *Autographa gamma*) from four geographical locations in Europe (Gibraltar, Crete, Spain and the UK). My results showed that stable hydrogen isotope analysis could distinguish adults from different geographical locations. These analyses also provided evidence on the migratory status of individuals, although the large amount of variation among individuals and over time complicated the interpretation of these data. Stable oxygen isotope ratios vary little across Europe, and so when analysed alone they lack power to distinguish adults from different sites, however their use in combination with hydrogen isotopes is discussed. Changes in the stable isotope ratios of hydrogen and oxygen have potential as a useful tool for determining the migratory status of Lepidoptera, however further work is needed into improving the reliability and accuracy of the methodology.

## 7.2 Introduction:

### 7.2.1 Stable isotopes:

Stable isotopes are naturally occurring elements that vary in the number of neutrons present, thus changing the atomic mass of the nucleus (see Hobson, 2005). For elements with more than one isotope, the isotope with the least number of neutrons is termed 'light', and the isotope with the most neutrons is 'heavy'. The lighter isotopes are more abundant than the heavier types, and these differences can be measured as the ratio (R) of heavy to light isotopes (Ehleringer & Cerling 2001; Rubenstein & Hobson, 2004; Hobson, 2005).

$$R = \frac{heavy}{light} = \frac{rare}{common} \text{ e.g. } \frac{^{18}O}{^{16}O} \text{ or } \frac{^{2}H}{^{1}H}$$

The fractionation of stable isotopes in the environment is dependent on biological and biogeographical processes, for example, precipitation patterns, temperature, elevation and relative humidity. Fractionation is also affected by anthropogenic processes, for example, climate change (through changing temperature patterns) (Hobson, 1999; Poage & Chamberlain, 2001; Rubenstein & Hobson, 2004). Enriched $^{18}O/^{16}O$ isotope ratios in precipitation have been observed across Europe over the past few years, a period of time associated with anthropogenic climate warming (Rozanski *et al.*, 1992). Bowen & Wilkinson (2002) found a strong spatial correlation between oxygen isotope values and local mean annual temperature, with temperature at particular locations being influenced by altitude and latitude. Oxygen exists in three stable isotopes, $^{16}O$, $^{17}O$ and $^{18}O$, but in environmental studies the ratio of $^{18}O$ to $^{16}O$ has traditionally been used (McCarroll & Loader, 2004). Hydrogen naturally occurs in two stable isotopes, $^{1}H$ and $^{2}H$ (Table 7.1). Carbon and nitrogen also exist in two isotopic forms, $^{12}C$, $^{13}C$, $^{14}N$ and $^{15}N$, and are commonly used in the study of global change and migration.

Carbon isotopes have been used in the study of migrant herbivores, for example in determining the origin of migrating butterflies, *Danaus plexippus* (Wassenaar & Hobson, 1998; Hobson *et al.*, 1999) and birds (Bearhop *et al.*, 2004; Yohannes *et al.*, 2005). The rate at which $^{12}C$ and $^{13}C$ are fixed by plants differs between the photosynthetic pathways $C_3$

(Calvin cycle) and $C_4$ ($C_4$-dicarboxylic acid) (Hobson, 2005) and so it is possible to determine the relative contribution of C3 and C4 plants in an individual's diet from the ratios of the two isotopes in body tissues. Thus, information on the geographical origin of individuals can be determined if a change in diet occurs, moving from C3 plants at one end of their range, to C4 at the other. However, carbon isotopes were not considered in this study because the larval host plants used in both overwintering and summer breeding habitats of the study species are invariably C3 plants, and therefore the isotopic ratio of carbon in adults across their geographical ranges would not be expected to change greatly. Analyses of stable nitrogen isotope ratios are commonly used in determining movements of individuals between marine and terrestrial, benthic to pelagic and xeric to mesic landscapes, and for determining energy flow through food chains (Ostrom *et al.*, 1997; Hobson, 2005), none of which are relevant to the study of migratory Lepidoptera in this chapter, and so nitrogen isotopes were not examined.

| Isotope | Natural abundance (%) |
|---|---|
| $^{16}O$ | 99.759 |
| $^{17}O$ | 0.037 |
| $^{18}O$ | 0.204 |
| $^{1}H$ | 99.985 |
| $^{2}H$ | 0.015 |

Table 7.1: The relative abundance of the three stable isotopes of oxygen and two stable isotopes of hydrogen (Ehleringer & Cerling, 2001).

The direct ratio of heavy to light isotopes is not a very useful index because the absolute values for these ratios are low and geographic variation is very small. Therefore stable isotope ratios are expressed relative to a known standard, to give a delta value (δ). In hydrogen and oxygen isotope studies, the most commonly used standard is mean ocean water (SMOW), which is the average value of the isotopic composition of ocean water. The δ values are expressed as parts per thousand or per mil ($^{0}/_{00}$). The δ values of stable hydrogen isotope ratios are typically negative so that a less negative value can be interpreted as an enrichment of the heavy isotope relative to the lighter one.

$$\delta x = 1000\left(\frac{R_x - R_{s\tan dard}}{R_{s\tan dard}}\right) {}^{0}\!/_{00}$$

Where R is the heavy/light isotope ratio and x represent the particular stable isotope under examination.

Advances in recent years in our understanding of naturally occurring stable isotopes have made it possible to determine the geographic origins of a variety of taxa, including migratory butterflies and birds (Wassenaar & Hobson, 1998; Hobson *et al.*, 2004a). These advances also enable researchers to identify stopover re-fueling sites in migratory birds (Yohannes *et al.*, 2005), to examine the fitness of migratory birds (Bearhop *et al.*, 2004), to examine energy pathways in insects (Ostrom *et al.*, 1997), to investigate nutrient allocation (Hobson *et al*, 2004b), as well as playing a role in wildlife forensics (Kelly *et al.*, 2008). Stable isotopes are taken up into animal tissues directly via the diet, and hydrogen and oxygen isotope ratios found in animal tissues largely reflect values found in surrounding lakes, oceans and groundwater, arising from precipitation (Rubenstein & Hobson, 2004).

### 7.2.3 **Fractionation:**

The physical phenomenon that causes variation in the relative abundance of isotopes is called isotope fractionation. During isotopic fractionation, heavy and light isotopes partition differently because heavier isotopes have stronger bonds and slower reaction rates. There are two types of fractionation:

Kinetic fractionation: Heavier isotopic forms have stronger chemical bonds and therefore are more difficult to break up in chemical reactions (Ehleringer & Cerling, 2001). Substrates containing the lighter isotopic forms have a faster rate of enzymatic reaction than those with the heavier forms, thus causing a difference in the abundances of stable isotopes between the substrate and the product (Ehleringer & Cerling, 2001). This type of fractionation occurs in the evaporation of surface waters and in the majority of biogeochemical processes.

Equilibrium fractionation: Fractionation occurs due to differences in the physical properties of molecules containing the heavier isotopic forms (Ehleringer & Cerling, 2001). During equilibrium reactions, molecules with heavier isotopic forms are more abundant in the lower energy phase (Ehleringer & Cerling, 2001). Although the process is in equilibrium, the rate of exchange (for example water vapour to liquid precipitation) is different, resulting in an enrichment of one of the isotopes.

### 7.2.4 Study species – Lepidoptera:

The exoskeleton of insects contains the structural polysaccharide chitin (Miller *et al.*, 1988), that is a poly amino-sugar which during biosynthesis incorporates hydrogen and oxygen from ingested plant material and water (Grocke *et al.*, 2006). Significant fractionation of isotopes can occur during metabolism and body fluids can become enriched, for example the depletion of $^{1}$H via water-vapour loss through the trachea leads to an increased proportion of $^{2}$H (Grocke *et al.*, 2006). Metabolically active tissues, for example liver and plasma, have higher turnover rates and thus their isotope ratios reflect recent diet whereas tissues with a slower turnover, for example blood, muscle, collagen and chitin, will have isotope signatures reflecting diets over longer time periods (Hobson & Clark, 1993). When adult butterflies emerge from pupae their wings are considered inert, i.e. there is no subsequent tissue turnover, and so an individual's wings retain the isotopic signature of their larval surroundings. The abdomen however, contains reproductive and digestive organs (Karlsson, 1994) that have a relatively constant turnover (approximately 5 days), and thus have isotopic values of recently ingested plant material. Thus substantial differences between wing and abdomen isotope signatures would represent adults that had moved considerable distances from their natal origin.

The movement of oxygen and isotopes through the food chain, from rain water to an individual is shown in Figure 7.1.

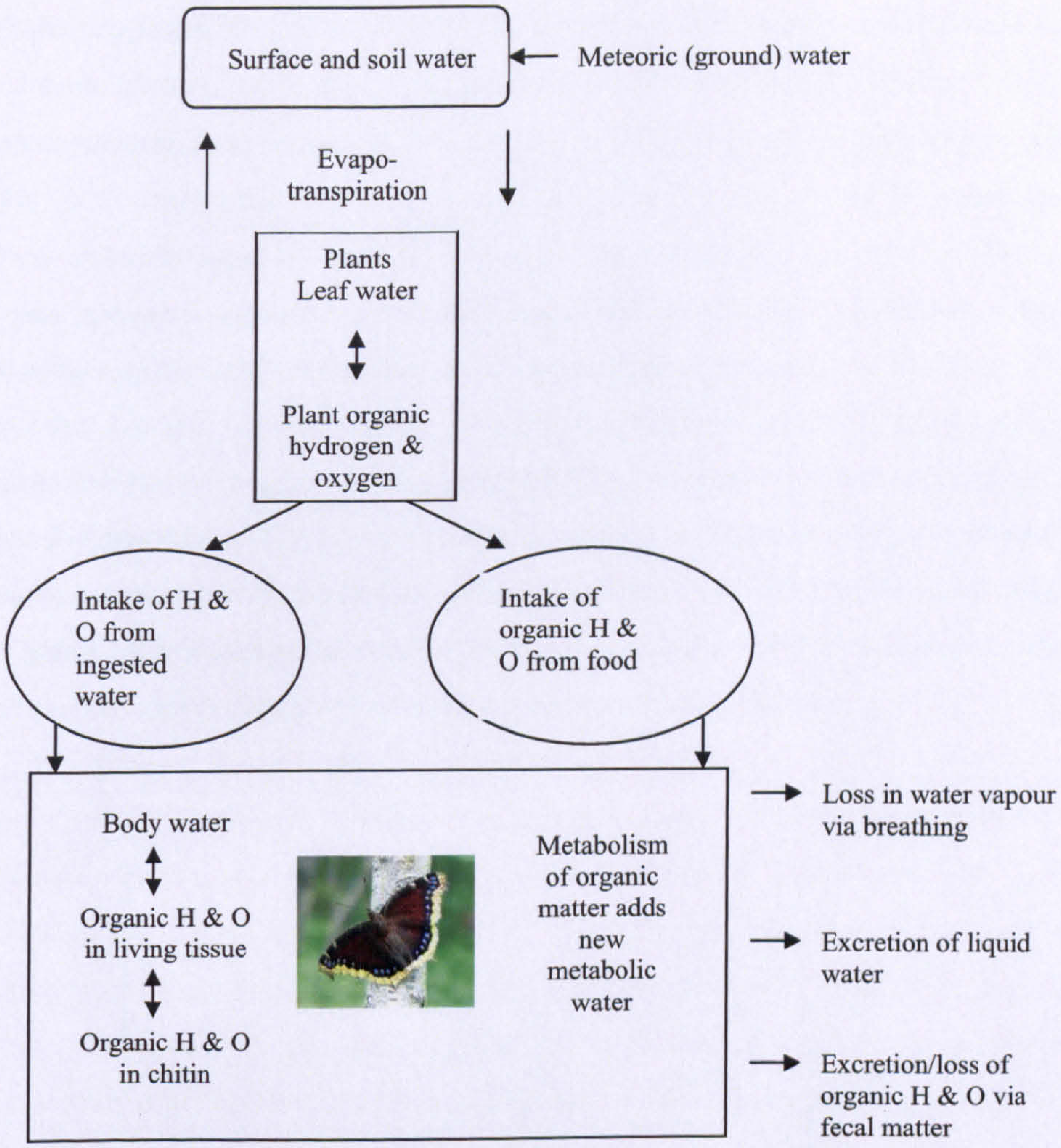

Figure 7.1: Uptake of hydrogen and oxygen atoms into butterfly chitin, each arrow represents fractionation resulting in altered stable isotope ratios (Grocke *et al.*, 2006).

### 7.2.5 **Stable Oxygen and Hydrogen Isotopes:**

Spatial variation in precipitation across the world results in changes in both oxygen and hydrogen isotope composition, as shown in Fig 7.2 for Europe. The effects of latitude, elevation and proximity to oceans on oxygen and hydrogen isotopes are shown in Figure 7.3. δH and δO values decrease from mid-latitudes to the poles, with lower values also occurring at higher elevations and a negative gradient occurring from the coast inland (Bowen & Revenaugh, 2003). This spatial variation in stable isotope ratios has been used to determine geographical origins of individuals. For example, studies on 25 bird species at 35 sites across Europe used stable oxygen isotope ratios of wing tissue to successfully determine geographical origins (Hobson *et al.*, 2004). These authors found that there were positive correlations between δO values in feather material and the mean annual δO values for local ground water ($r^2$ = 0.56), although the correlation was not as strong as for hydrogen isotopes. Hobson *et al.* (2004) suggested that oxygen isotopes are a useful tool for determining the origin of individuals when comparing populations at similar latitudes where other isotopes become less powerful. In comparison, stable hydrogen isotope ratios vary considerably with latitude and so are useful in mapping the north-south migrations of species. For example, δ2H has been used to determine stopover refueling points during long distance migrations in several bird species (Yohannes *et al.*, 2005), and for determining the natal origins of *Danaus plexippus* in North America (Wassenaar & Hobson, 1998) and *Inachis io* (peacock butterfly) in Sweden (Brattström *et al.*, 2008).

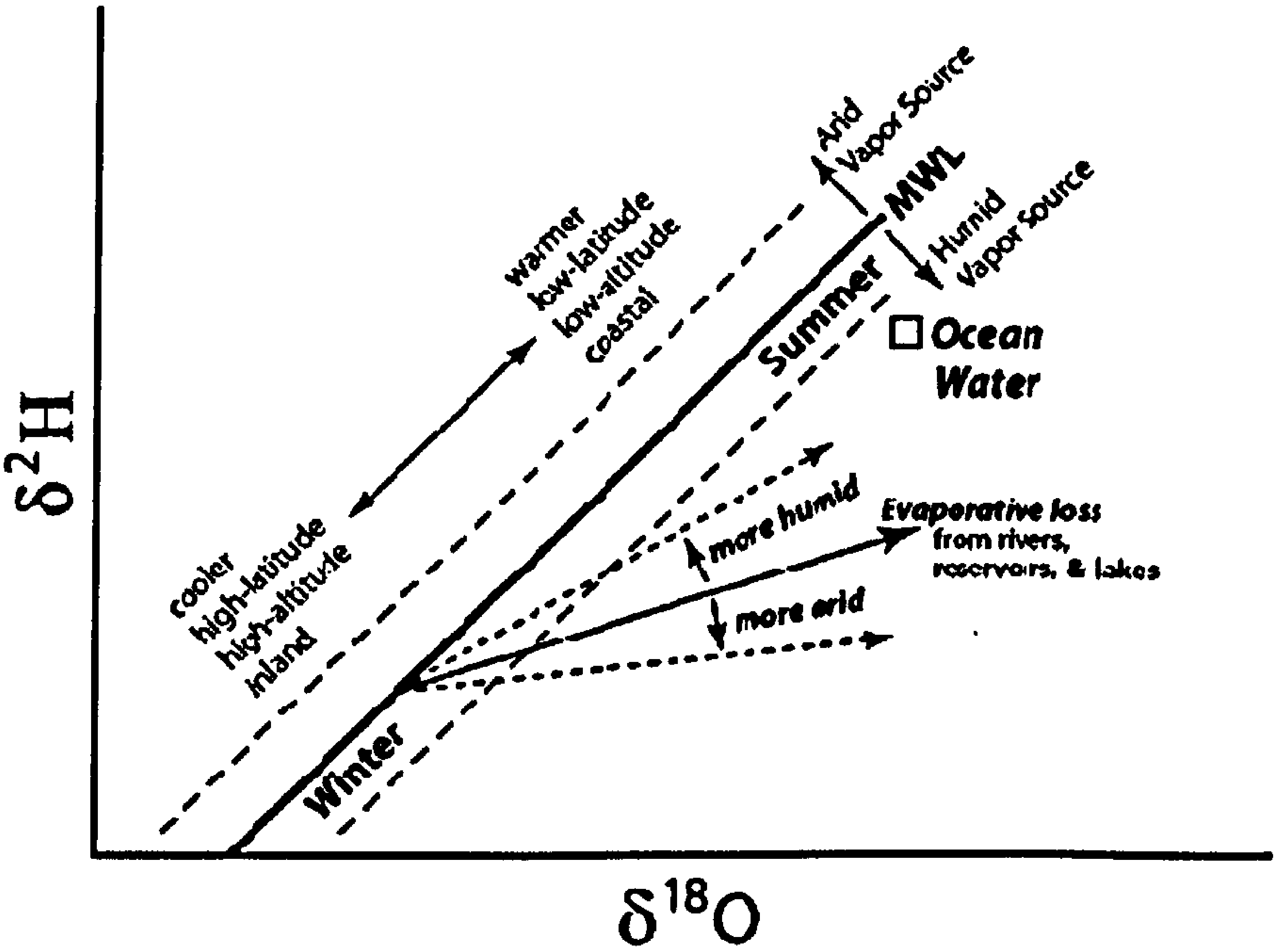

Figure 7.2: The affects of hydrological processes on the oxygen and hydrogen isotopic composition of water. MWL – meteoric water line which expresses the ratio of $^{18}$O to $^{2}$H in meteoric water (Gibson 2005)

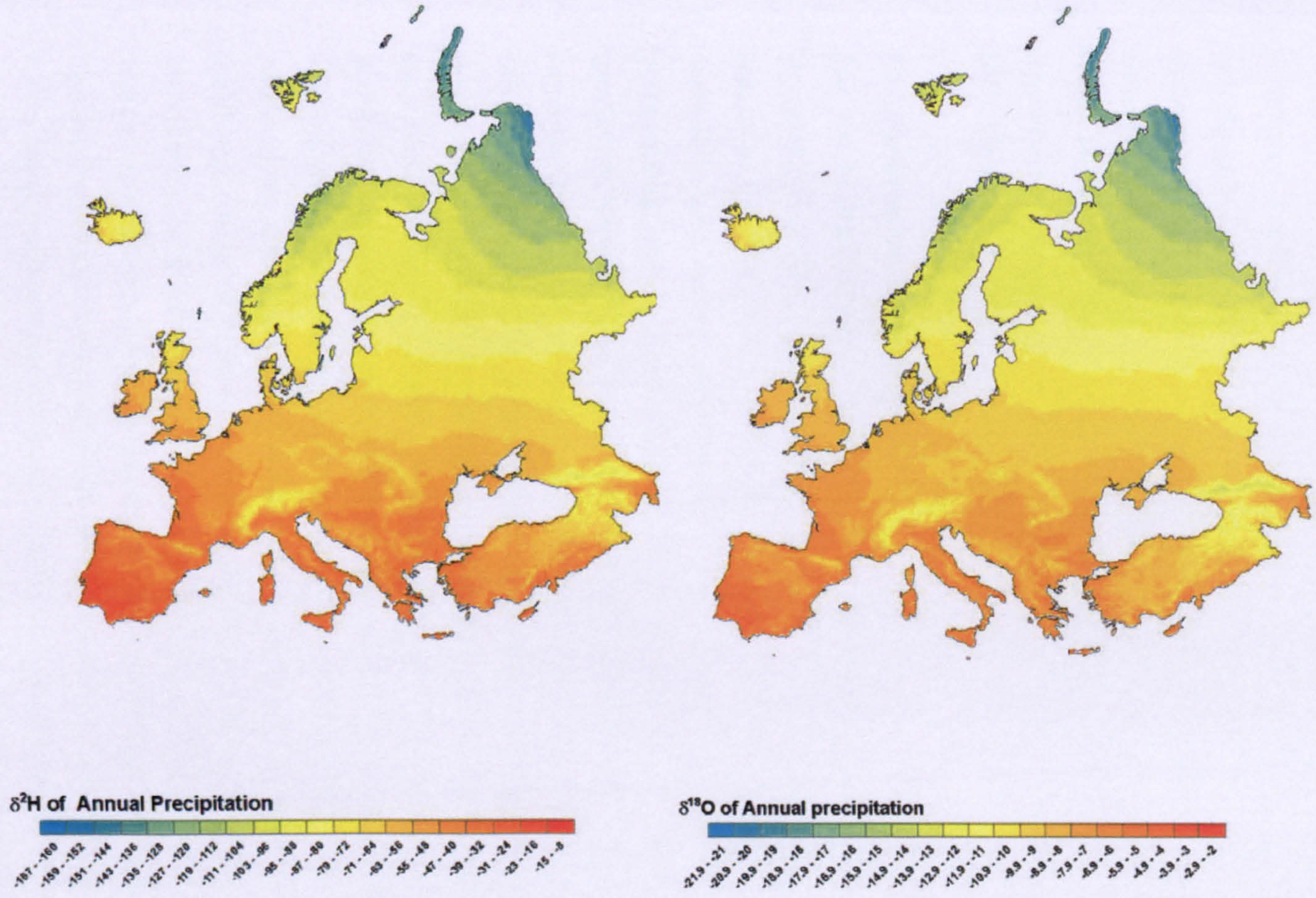

Figure 7.3: Mean annual spatial distribution of $\delta^2H$ and $\delta^{18}O$ in precipitation in Europe. (www.waterisotopes.org)

### 7.2.6 Hydrogen Exchange with Ambient Moisture:

Within insect chitin, 81% of the hydrogen atoms are bound to carbon atoms, leaving 19% that are weakly bonded to either N or O and so readily exchangeable with water vapour (Wassenaar & Hobson, 2000). The non-exchangeable hydrogen is representative of the isotopic values of food and water ingested when the tissue was formed. The remaining hydrogen exchanges continuously over time and so needs to be removed prior to analysis because it will not be a reliable representation of the isotopic composition of water at sites where larvae developed. However, this exchangeable hydrogen can be removed by equilibrating the sample with water vapour of known δD (Wassenaar & Hobson, 2000, 2003; Grocke *et al.*, 2006).

### 7.2.7 Rationale for Further Work:

The use of stable isotopes to track long distance migrations of species is becoming increasingly common. Stable hydrogen and carbon isotope ratios have been used to determine the natal origins of *Danaus plexippus* (monarch butterfly), a species that undertakes long distance migrations each year between Eastern North America and overwintering sites in central Mexico (Wassenaar & Hobson, 1998). A study of the correlations between δH of bird feathers and deuterium patterns in rainfall across Europe suggested that $\delta^2H$ can be used to determine the geographical origins of migratory birds, although the use of $\delta^{18}O$ is less reliable (Hobson *et al.*, 2004, Bowen *et al.*, 2005). However, there has been no study yet considering the possibility of using $\delta^{18}O$ and $\delta^2H$ to track insect movements in Europe. Although we know that these migration events occur, it is difficult to determine the overwintering origins of spring arrivals in to the UK, or to differentiate between migrant and non-migrant individuals in the UK. This information is important for understanding how numbers of migratory species may change in Britain as the climate warms. In order to make more reliable predictions, we need to gain a better understanding of factors driving changes in abundance in the UK, i.e. is the abundance of migrants in the UK controlled primarily by influx of migrants, or by increased summer reproduction rates once the individuals have reached the UK. The isotopic ratios of wing tissues have been examined in migratory Lepidoptera (Hobson *et al.*, 1999), but there is no information comparing wing and abdomen tissues, even though this method could be useful in distinguishing between migratory and non-migratory individuals within a species. The measurement of two or more isotopic elements in tandem is more sensitive than a single isotope measure in defining the geographic origins of individuals (Chamberlain *et al.*, 1997), and in this Chapter, I examine stable oxygen and hydrogen isotope ratios to investigate if they can be used to identify the geographical origins of migrants.

### 7.2.8 General Aims and Predictions of this Chapter:

This chapter investigates whether O and H isotope ratios can be used to determine the natal origin of migratory Lepidoptera and the migratory status of individuals. Wing and abdomen tissues were analysed in one butterfly (*Vanessa atalanta*) and one moth species (*Autographa gamma*) from four locations in Europe. Three sample locations were in potential overwintering sites in southern Europe, whilst the fourth location was in the UK. In addition, the methodology was tested with lab-reared *V. atalanta, V. cardui* and *A. gamma* individuals for which isotopic values are expected to be the same for both wing and abdomen tissue types. This Chapter also compares isotope values among species caught at the same geographical location to determine if species-specific fractionation confounds the results. This chapter has the following objectives:

1. Determine if stable hydrogen and oxygen isotope ratios in wing tissue can be used to identify the natal origins of Lepidoptera caught in the northern and southern parts of their ranges.

2. Examine if the migratory status of individuals can be identified from differences in $\delta^{18}O$ and $\delta^{2}H$ of wing and abdomen tissues. Isotope signatures of these two tissues should differ more in migrants than in resident individuals. Individuals wild-caught in Gibraltar, Spain and Crete are expected to be residents, while those caught in the UK are expected to be migrants.

3. Test the reliability of the methods by comparing wing and abdomen tissues in wild-collected and lab-reared individuals.

## 7.3 Materials and methods:

### 7.3.1 Data Collection:

Wild caught material

Lepidoptera were collected from four locations in Europe. Eight *A. gamma* and eight *V. atalanta* were wild caught in Gibraltar (36°8'N, 5°21'W) during May 2006, seven *V. atalanta* from Crete (35°11'N, 24°23'E) during April 2006, 10 *V. atalanta* from Catalonia (41°41'N, 2°23' E) during late April/early May, nine *A. gamma* (caught May 2006) and 14 *V. atalanta* (caught July 2005/2006) from York, UK (53°57'N, 1°04'W). All the butterflies were caught in flight using a sweep net, killed on capture, dried and stored in an air-tight container.

Lab-reared material

I obtained baseline data on wing and abdomen H and O isotope signatures from Lepidoptera material reared under standard conditions in the UK. I reared offspring from three female *V. atalanta* caught in flight around York (two in Haxby: 27th June 2005, 9th July 2005, grid ref: SE610579 and one in Bishop Wood: 23rd June 2005, grid ref: SE544348). The females were kept in cylindrical cages, 30 cm x 40 cm, and provided with nettle (*Urtica dioica*) stems to encourage egg-laying, and fed with honey (diluted with York tap water). First instar larvae were removed and placed on locally-picked nettles in an incubator at 20°C and 18L:6D photoperiod. After emergence the adults were immediately killed and then dried for 48 hours in an oven at 80°C.

Five *Autographa gamma* adults were collected from a light trap in York (SE 627645) in June 2006. The moths were placed in cylindrical flying chambers and provided with dandelion (*Taraxacum officinale*) leaves to promote egg-laying. The larvae were reared under the same conditions as described above (20°C, 18L:6D), fed on locally-picked dandelion leaves and adults were killed and dried on emergence.

*Vanessa cardui* larvae were obtained from three females caught at Portland Bill, Dorset (grid ref: SY677 684) and reared as above at Rothamsted Research (Hertfordshire), and fed on locally-picked creeping thistle.

### 7.3.2 Sample Preparation:

All insect material was killed and dried immediately to preserve the specimens and transferred to York University for subsequent analysis. Material was stored in air tight containers with silica to minimise any elemental transfer. Lepidoptera were dissected and the abdomen and wings were separated and washed three times in a 2:1 chloroform:methanol solution to dissolve any lipids that could interfere with the isotope measurements of chitin. The body parts were left in a fume hood to allow the solvents to evaporate and then placed in an oven at $80^0$C overnight to ensure that the samples were dry. The dried parts were then ground using a Retsch mill and 0.7 mg of material was weighed out into 3 mm x 5 mm silver cups prior to analysis in a mass spectrometer.

### 7.3.3 Reference Material:

Reference material from throughout the European range of the study species' distributions (North Africa to northern Europe), was required in order to provide a standard with which to compare the sample material. Carabid beetles make a good reference taxon in this respect as species are relatively sedentary and thus likely to represent the isotopes ratios of the surrounding habitat. A West African Carabid (*Tefflus* sp.) was obtained from the Natural History museum in Oxford, beetles were collected from Northern and Southern Spain using pitfall traps set up by Rosa Menendez at Oxford University and further carabids were collected using pitfall trap situated around York University campus. The beetles were dissected and the chitin removed and rinsed three times in 2:1 chloroform:methanol and prepared for analysis as described above.

### 7.3.4 Determination of Isotope Ratios:

The experiment set up of the mass spectrometer is shown at Figs 7.4 and 7.5. The sample passes into the quartz tube from the sample carousel where it undergoes a pyrolysis reaction over hot carbon at 1020°C, converting hydrogen in the sample to $H_2$ and the oxygen into CO. The gases are then passed through the system using helium as a non-interfering inert carrier gas

The gas chromatogram (GC) is used to separate the gas compounds with $H_2$ eluting a few minutes before the CO. In addition $N_2$ (which, with a mass/charge ratio of 28, 29, 30, can cause isobaric interference with CO, with a mass for 28, 30) is also separated by the GC. Within the mass spectrometer, an ion source ionises the gas, which is then accelerated through a high voltage and an electronic lens is used to focus the ions into a narrow beam. The resultant beam is passed through a strong magnetic field which acts as a prism and separates out the different isotopes for detection using Faraday cups.

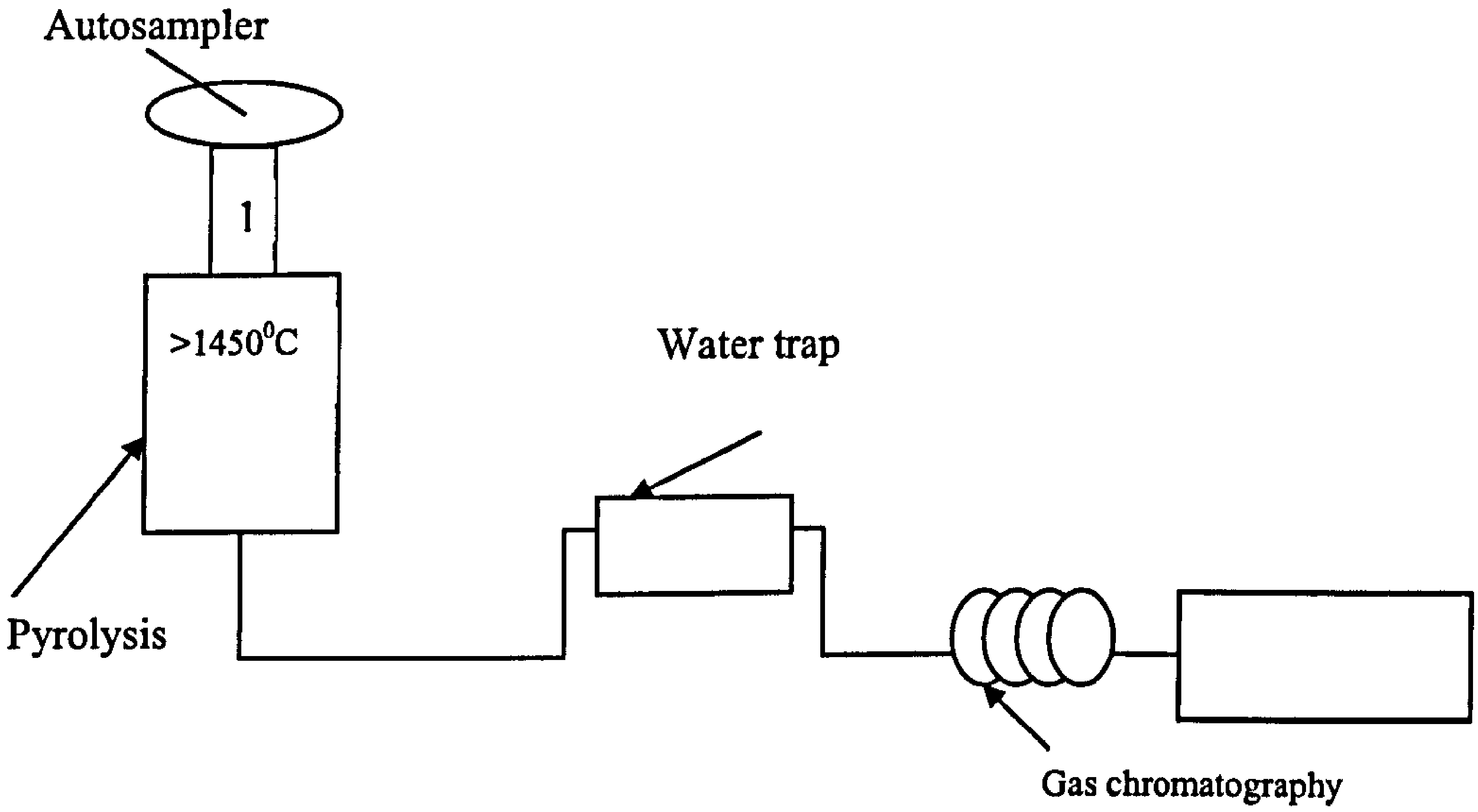

Figure 7.4: The experimental configuration of the continuous flow mass spectrometer. The configuration of the pyrolysis system is shown below in Figure 7.5.

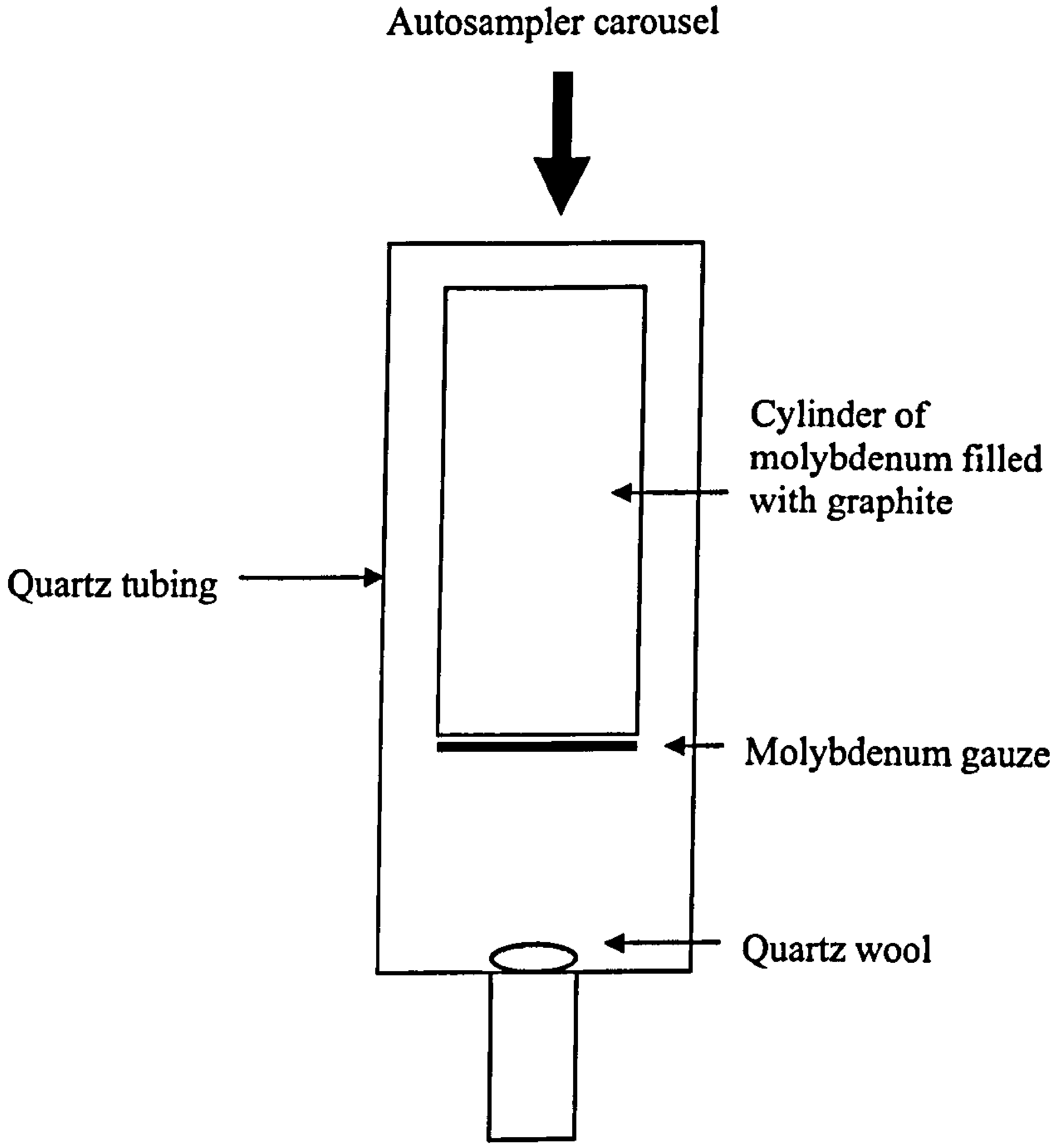

Figure 7.5: Pyrolysis system.

### 7.3.5 Statistical Analyses:

The results were analysed using the statistical software package SPSS 15.0. Data were tested for normality using Kolmogorov-Smirnov tests. Paired *t* tests, and ANOVA were used to compare wing and abdomen tissues, and for distinguishing among species and geographical locations.

## 7.4 Results:

### 7.4.1 Comparing Isotope Ratios in Wing Tissues of Individuals from Four Sites in Europe:

There was a significant difference in stable hydrogen isotope ratios of wing material among *V. atalanta* individuals from UK, Spain, Crete and Gibralter (ANOVA, F=2.9, df=3,37, p=0.045), with individuals from Gibraltar having the least negative delta value and UK individuals have the greatest. However, there was no significant difference in the hydrogen isotope ratio in wing tissue of *A. gamma* from the UK and Gibralter (*t*-test, df=28 p=0.199). There was also no difference in oxygen isotope ratios between locations for either species (*V. atala*nta, ANOVA, F=0.162, df=3,37 p=0.770; *A. gamma*, *t*-test, t=-0.371, df=13, p=0.717). I also carried out a 2-way ANOVA to examine differences between *V. atalanta* and *A. gamma* individuals at the two sites where they were both sampled (Gibraltar and the UK). There was a significant effect of site on the stable hydrogen isotope ratio of the wing material (2 way ANOVA of isotope value by site and species, F=4.861, df=1,38, p=0.034), but there was no difference between the species (ANOVA, F=0.986, df=1,38, p=0.332), and no interaction effects (ANOVA, F=0.562, df=1,38, p=0.458). There was no significant differences in stable oxygen isotope ratios between sites (2-way ANOVA, F= 0.038, df=1,38, p=0.846) or between species (ANOVA, F=0.816, df=1,38, p=0.373).

Data for $\delta^{18}O$ and $\delta^{2}H$ of wing tissues from the four sites for the two species (Figs. 7.6, 7.7, 7.8) show considerable overlap across sites, although UK individuals generally are distinct from the remaining locations, which are grouped together.

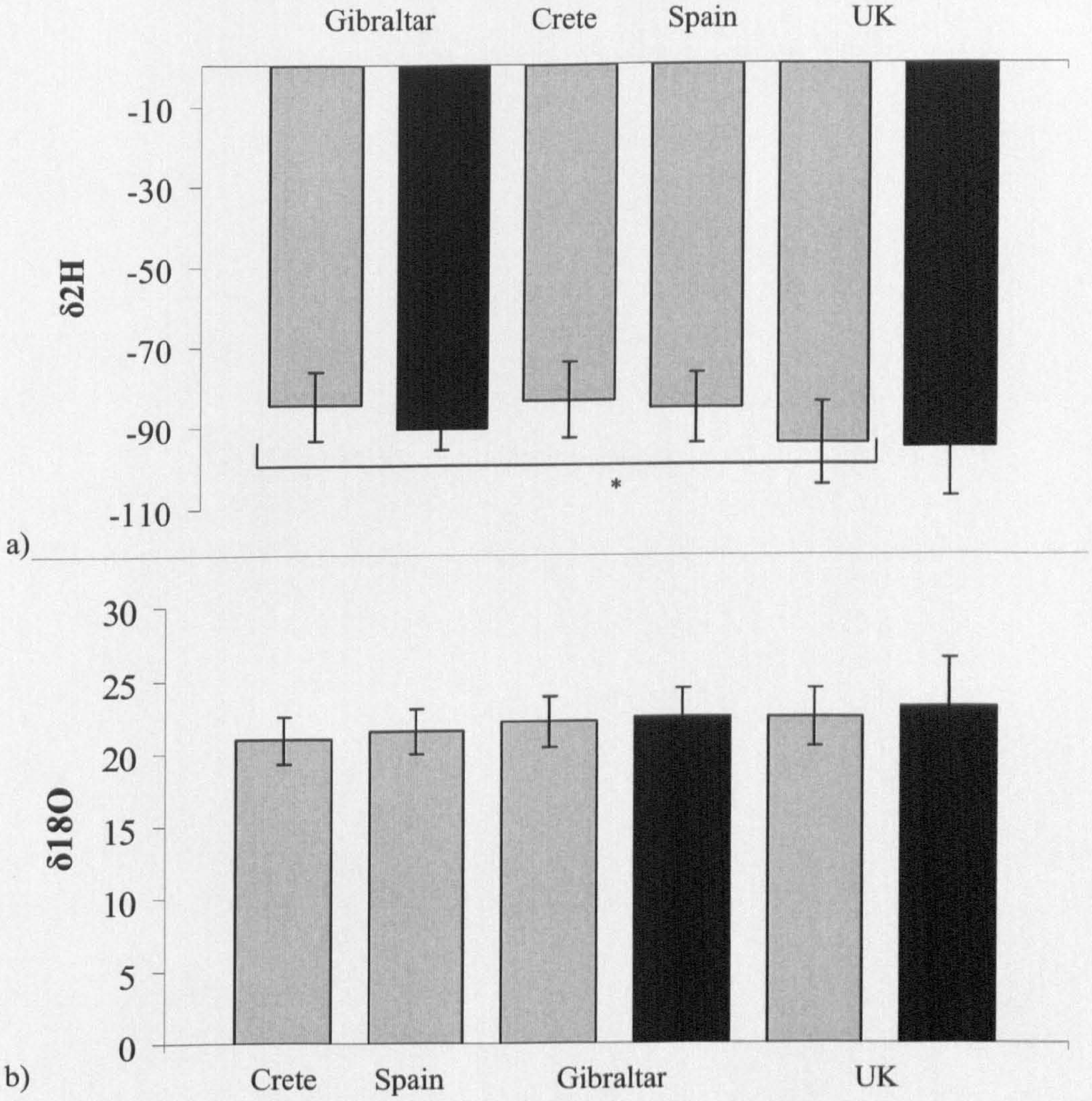

Figure 7.6: Comparison of a) $\delta^2$H and b) $\delta^{18}$O in wing material of wild-caught *V. atalanta* (grey) and *A .gamma* wing (black) from Gibraltar, Crete, Spain and the UK. Means and standard deviations shown. The sites are in the order in which the isotope values vary naturally in precipitation, by latitude for hydrogen, and by longitude for oxygen.

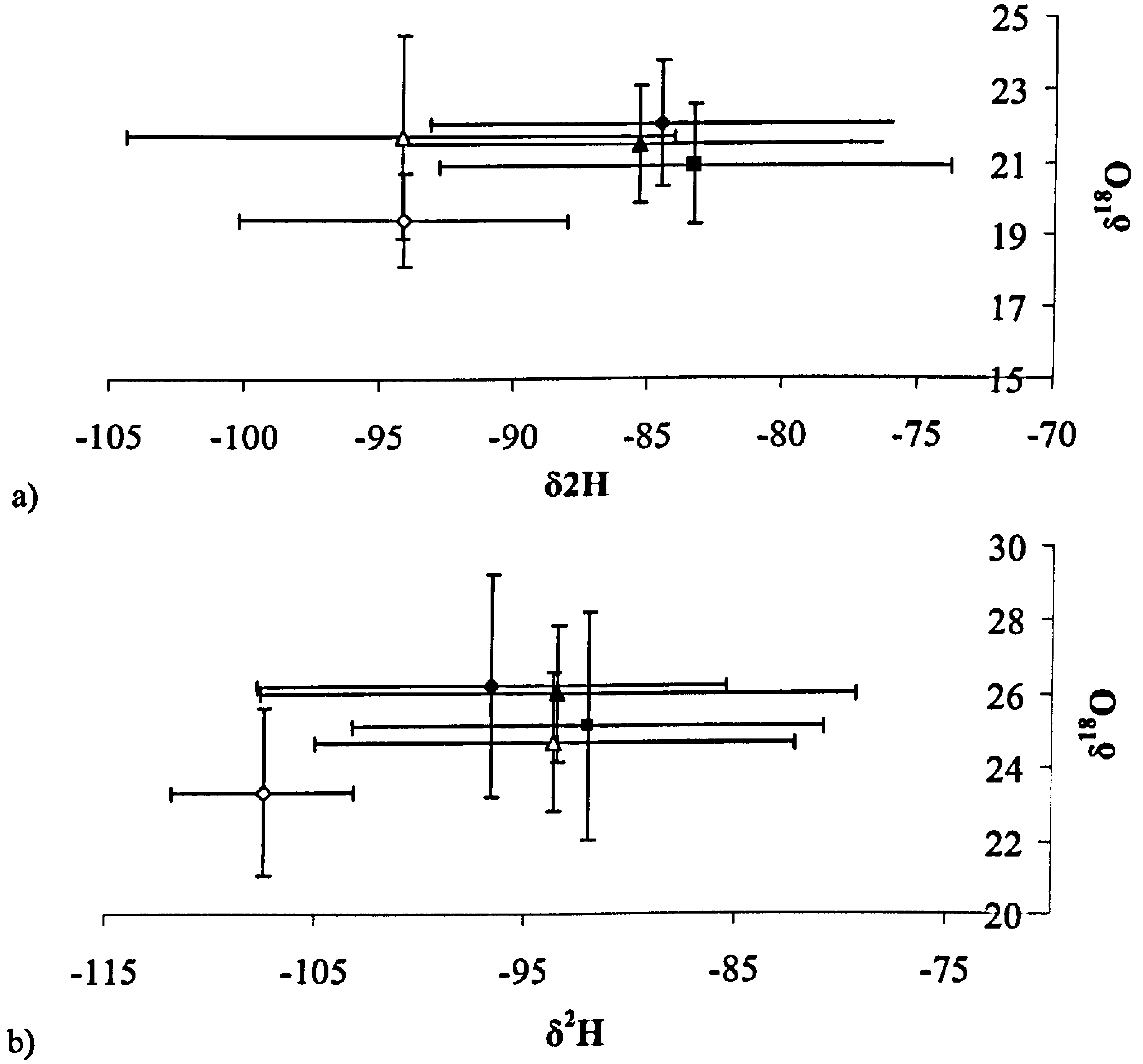

Figure 7.7: 2D plot of $\delta^2H$ against $\delta^{18}O$ in a) wings and b) abdomen from *V. atalanta* caught in Gibraltar (black diamond, n=10), Crete (black square, n=6), Spain (black triangle, n=10) and the UK (hollow triangle, n=14) and lab-reared in the UK (hollow diamond, n=10). Means and standard deviations are shown.

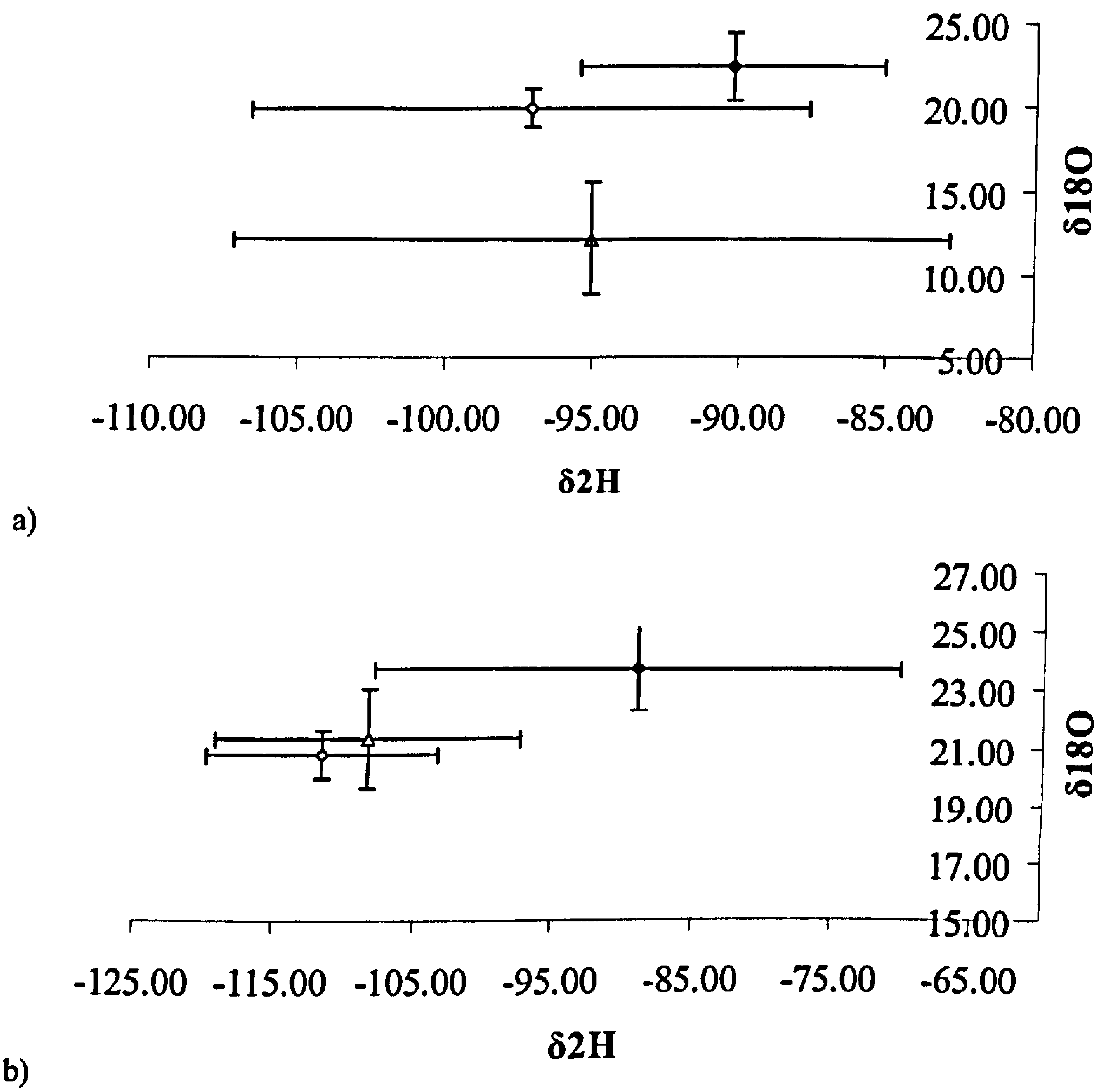

Figure 7.8: 2D plot of $\delta^2$H against $\delta^{18}$O in a) wings and b) abdomen from *A. gamma* caught in Gibraltar (black diamond, n=6), the UK (hollow triangle, n=9) and lab-reared in the UK (hollow diamond, n=8). Means and standard deviations are shown.

### 7.4.2. Determining the Migratory Status of Wild-caught Individuals:

The $\delta^2$H and $\delta^{18}$O values from wing and abdomen tissue of wild-caught individuals were compared in order to attempt to distinguish between migrants and residents, with a larger difference between the two tissue types expected for migrants. There was no clear pattern in the change in isotopic ratio between the two tissues from individuals from the four locations, with much variance around the mean. In order to examine this further I computed the difference in isotopic ratio of wings and abdomen tissues at the four sites and for the two species as (abdomen value – wing value)/wing value. There was no significant difference in this value between sites for the two species for hydrogen (2-way ANOVA, F=0.0, df=1,38, p=0.986) or oxygen (2-way ANOVA, F=4.070, df=1,38, p=0.051), see Fig. 7.9.

### 7.4.3 Validating the Methodology with Lab-reared Material:

I reared *V. atalanta*, *V. cardui* and *A. gamma* in the laboratory in order to produce adults of known origin (non-migrants) that were predicted to have the similar isotopic values for both wing and abdomen tissues. However, Figures 7.10 and 7.11 show that there were significant differences between the wing and abdomen tissue in all three species, *V. atalanta* (paired *t*-test, hydrogen, t=5.515, df=9, p=<0.001; oxygen, t=-7.324, df=9, p<0.001), *A. gamma* (paired *t*-test; hydrogen, t=6.779, df=7, p<0.001; oxygen, t=-5.292, df=7, p=0.001) and *V. cardui* (paired *t*-test; hydrogen, t=3.888, df=9, p=0.04; oxygen, t=-4.458, df=9, p=0.02).

I also compared $\delta^2H$ and $\delta^{18}O$ of wing and abdomen tissue among the three lab-reared species. Species did not differ in $\delta^2H$ of wings (ANOVA, F=2.0, df=2,25 p=0.156), but there were significant differences in $\delta^2H$ of abdomens (ANOVA, F=2799.7, df=2,27, p<0.001) and in $\delta^{18}O$ of both tissues (wing, ANOVA, F=2003.2, df=2,27, p<0.001; abdomen, ANOVA, F=14.9, df=2,27, p<0.001).

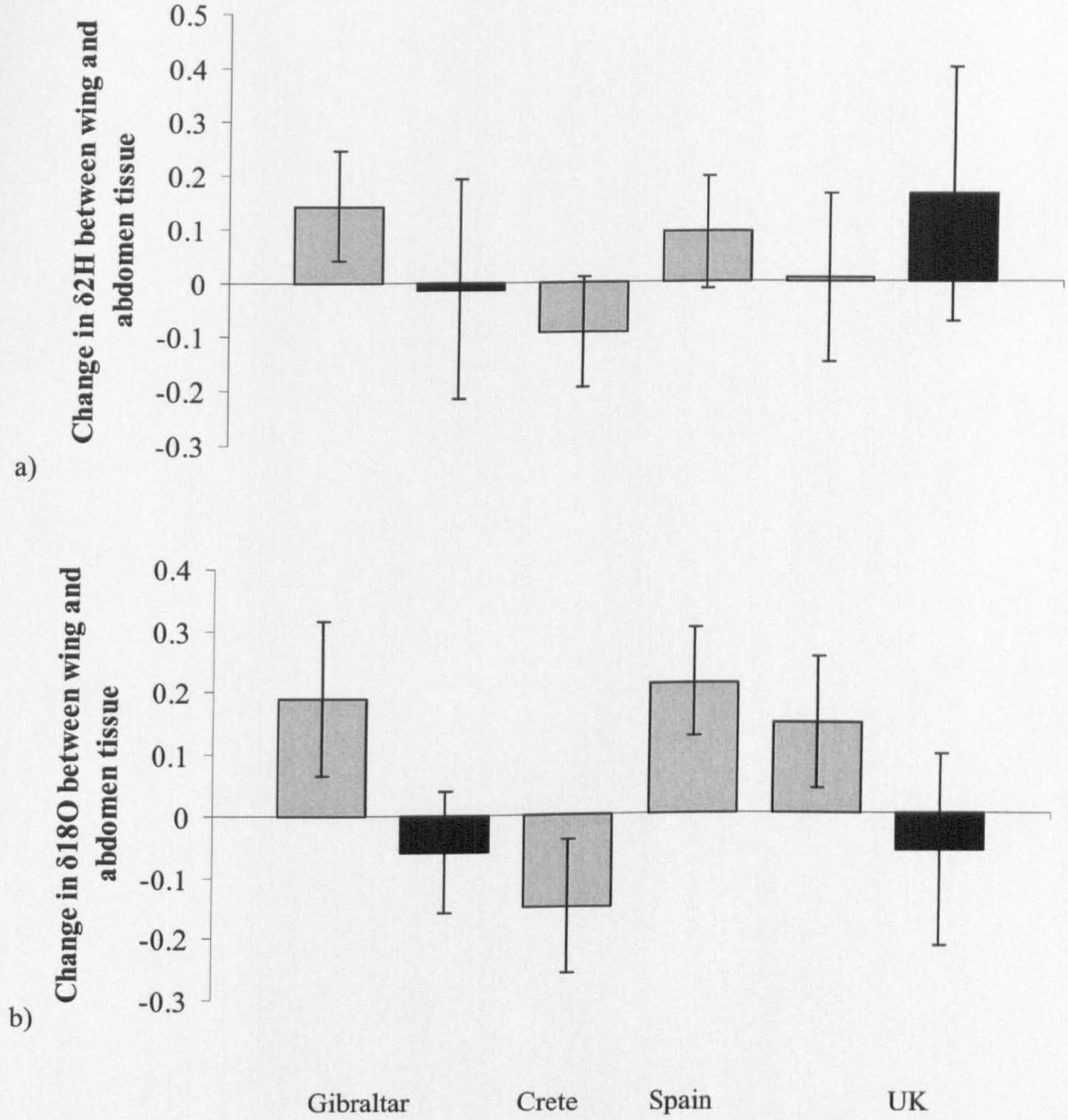

Figure 7.9: Comparison of the change a) $\delta^2$H and b) $\delta^{18}$O between wing and abdomen tissue for wild-caught *V. atalanta* (grey) and *A .gamma* wing (black) in Gibraltar, Crete, Spain and the UK. Means and standard deviations shown. A positive value means that the wing tissue represent an area more enriched in the heavier isotopic form than the abdomen tissue. If a species was a resident, no change could be expected, whereas a large difference would be obtained for migrant that had developed in a geographically distinct place from where it was caught.

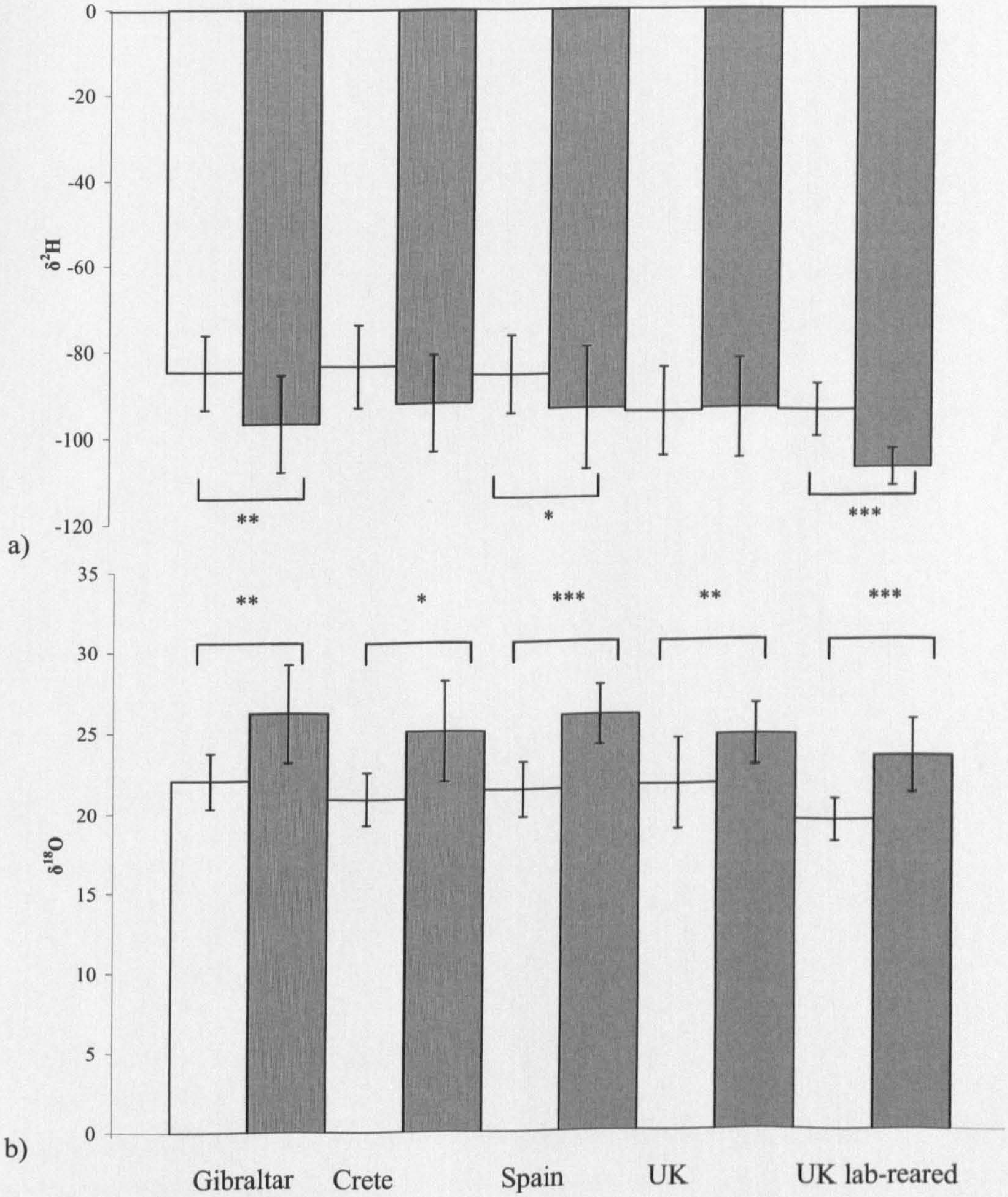

Figure 7.10: Comparison of a) $\delta^2$H and b) $\delta^{18}$O for wing (white) and abdomen (grey) in *V. atalanta* wild-caught at Gibraltar (n=10), Crete (n=6), Spain (n=10), UK (n=14) and lab-reared in the UK (n=14). Means and standard deviations are shown. Significance is based on paired t-tests between wing and abdomen for each geographical location: *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

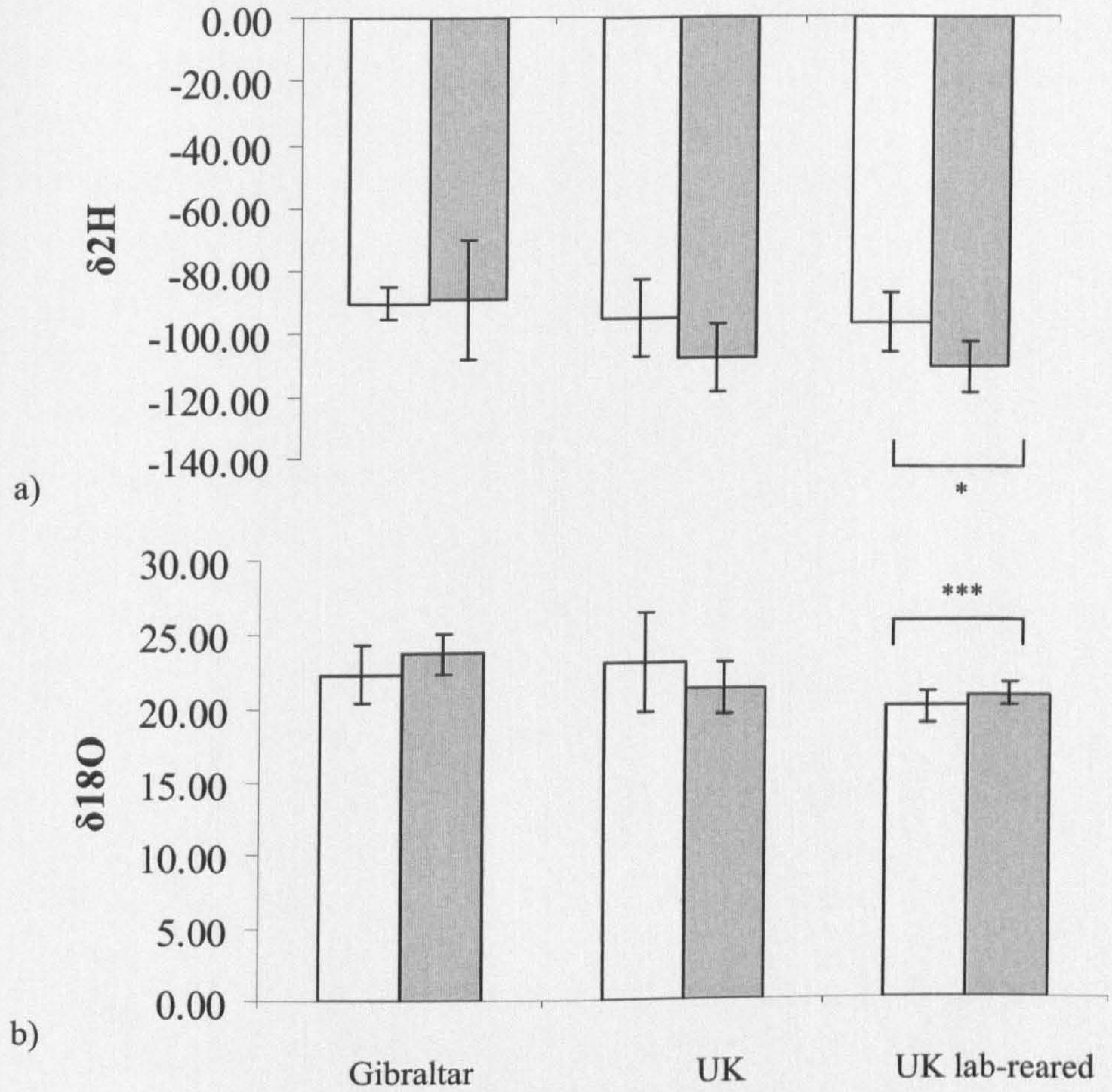

Figure 7.11: Comparison of a) $\delta^{2}H$ and b) $\delta^{18}O$ for wing (white) and abdomen (grey) in *A. gamma* wild-caught at Gibraltar (n=6), UK (n=9) and lab-reared in the UK (n=8). Means and standard deviations are shown. Significance is based on paired t-tests between wing and abdomen for each geographical location: *** $p<0.001$, * $p<0.05$.

### 7.4.4 **External Effects on Isotope Ratios**:

Figure 7.12 shows mean monthly values for $\delta^{2}H$ and $\delta^{18}O$ in precipitation at the four sample locations illustrating variability in these values over the year. It show that UK values are most different from other sites, but that $\delta^{18}O$ values in the UK in summer are very similar to those in Spain during the winter months. This will make it hard to distinguish among insect material that even though it was from distant sites, was from different times of the year when isotope signatures are similar.

It is apparent from Fig 7.12 that climatic variables affect hydrogen and oxygen isotope ratios in precipitation. Samples from Gibraltar, Crete and Spain were all collected over a relatively short period of time, however *V. atalanta* and *A. gamma* individuals from the UK were caught over a relatively long period of time. I analysed the effect of collection time of on $\delta^2H$ and $\delta^{18}O$ values of wing and abdomen tissue. There was a significant positive relationship between the collection time of year and both $\delta^2H$ (Regression, $R^2=0.254$, $F=7.494$, $b=0.323$, $df=23$, $p=0.012$) and $\delta^{18}O$ (Regression, $F=12.926$, $R2=0.370$, $B=0.072$ $p=0.002$) of abdomens (but not wings), indicating that adults ingest plant material that is constantly varying in $\delta^2H$ and $\delta^{18}O$ due to the effect climate has on hydrogen and oxygen ratios in precipitation.

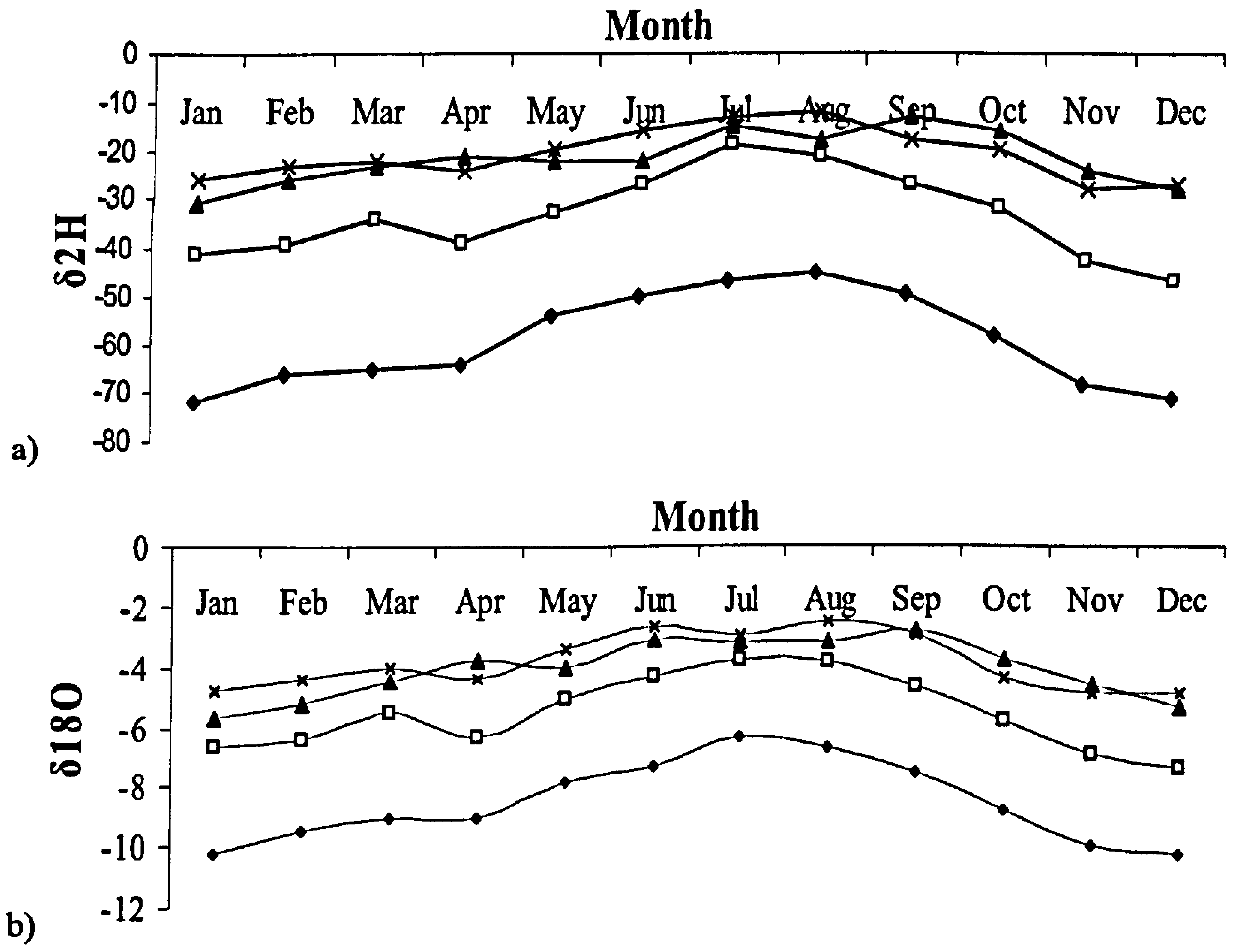

Figure 7.12: Monthly a) $\delta^2H$ and b) $\delta^{18}O$ (obtained from OIPC, available at www.waterisotopes.org) for the four European locations from which Lepidoptera material was collected, Gibraltar (cross), Crete (triangle), Spain (square) and the UK (circle).

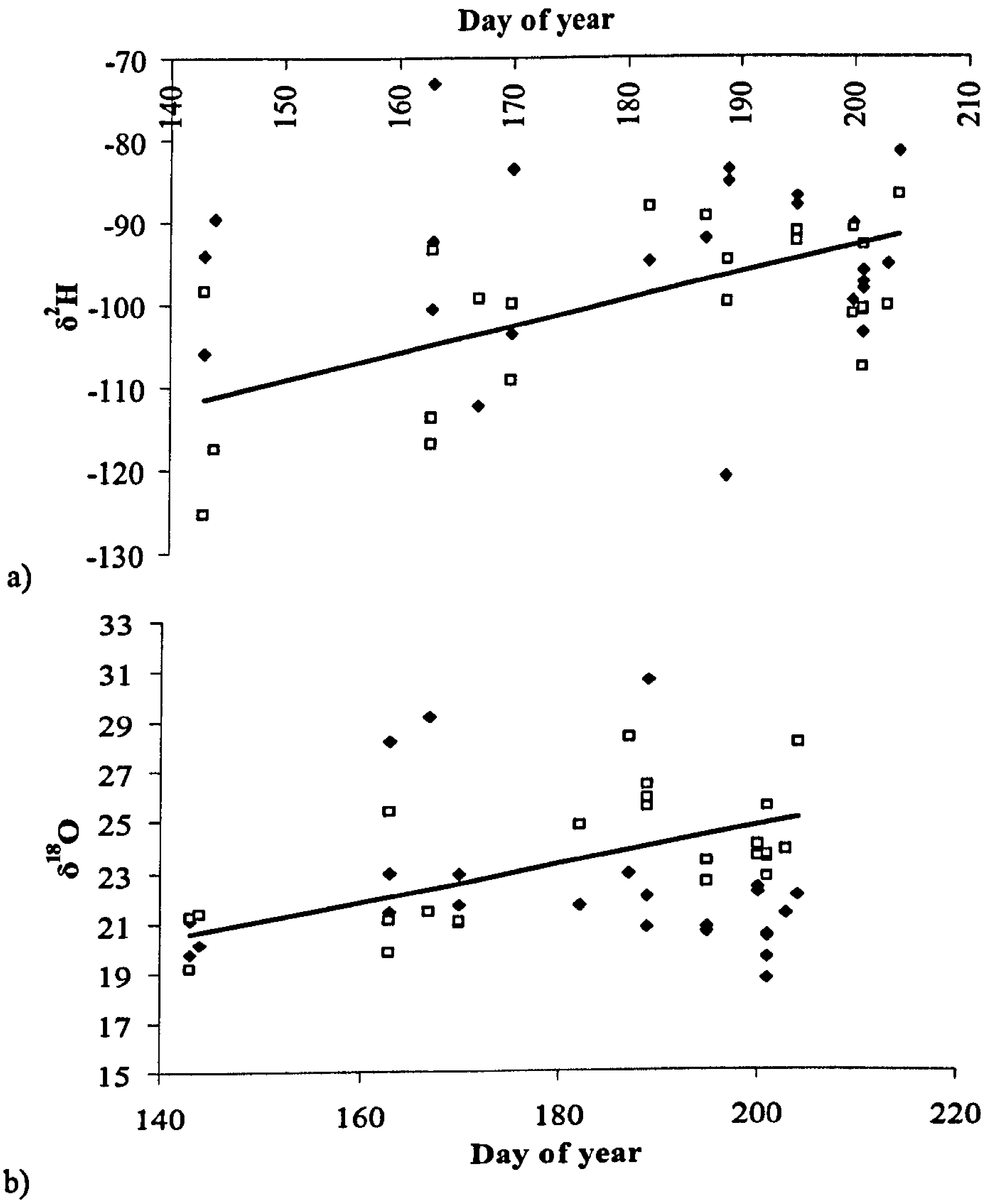

Figure 7.13: Effect of time of year when individual was caught and a) $\delta^2$H and b) $\delta^{18}$O of wings (diamond and black line) and abdomens (square) tissue. Only significant regressions are plotted.

## 7.5. Discussion:

### 7.5.1 Pattern of Isotope Ratios in Wing Tissue of Wild-caught Individuals:

In order to establish if stable isotopes can be used to distinguish between Lepidoptera caught at different locations through Europe, I examined the hydrogen and oxygen isotope ratios in the wing tissue of *V. atalanta* wild-caught in Gibraltar, Crete, Spain and the UK, as well as *A. gamma* caught in Gibraltar and the UK. There was a significant difference in $\delta^2H$ among sites for *V. atalanta* individuals, suggesting that these individuals were from distinct natal populations. However there was no difference in $\delta^2H$ between sites in *A. gamma* suggesting that individuals sampled in the UK may have originated from a geographical location close to Gibraltar. There was greater variation around mean $\delta^2H$ for UK individuals, than that for Gibraltar individuals, suggesting that the individuals caught in the UK had come from a variety of overwintering sites, while the Gibraltar individuals came form similar natal sites. This result was strengthened by the fact that $\delta^2H$ differed between Gibraltar and the UK for both *A. gamma* and *V. atalanta.* The ratio of heavy to light hydrogen isotopes in precipitation varies considerably with latitude, as shown at Fig 7.12, with an average difference between Gibraltar and the UK of approximately 46 ‰. By contrast, spatial variation in $\delta^{18}O$ is much less (Fig 7.12), with differences between Gibraltar and the UK of only approximately 5‰. Thus, distinguishing between populations based on $\delta^{18}O$ is a difficult task, as conformed by the non-significant results for $\delta^{18}O$ in wing tissues in this study, with no significant differences between any of the sites.

The plots of $\delta^2H$ against $\delta^{18}O$ for both wing and abdomen tissue (Figs 7.7 and 7.8) show that data for both *V. atalanta* and *A. gamma* overlapped and no distinct populations were identified. Nonetheless, *V. atalanta* specimens from the UK were most distinct from the other three locations suggesting that these possibly form a distinct group, but further work on a larger sample size is required. It may indicate, however, that individuals caught in the UK did not have the same natal origin as those caught in southern Europe and thus may have developed in the UK, this is further confirmed when comparing wing and abdomen, as discussed below. This pattern seems different for *A. gamma* where abdomen isotope values of UK individuals were more distinct from Gibralter populations than were wing tissues, suggesting that the natal origin of *A. gamma* sampled in the UK was a geographical location close to Gibraltar. This implies that UK individuals of *A. gamma* were migrants, with their wing tissue representing isotopic values of their natal origin (i.e. southern Europe), and their abdomen tissue reflecting where they have been feeding recently (i.e. the UK).

### 7.5.2 Determining the Migratory Status of Wild-caught Individuals:

I calculated the difference in $\delta^2$H and $\delta^{18}$O values for wing and abdomen tissue and compared them between wild caught *V. atalanta* and *A. gamma* at different geographical locations. It was predicted that migrants have a greater difference in $\delta^2$H and $\delta^{18}$O values between wing and abdomen tissues than do locally bred non-migrants. The Lepidoptera studied in this Chapter follow a north-south migration route, and so UK spring migrants would be expected that $\delta^2$H of abdomens would be more negative than $\delta^2$H of wings, representing the enrichment of the lighter isotopic form that is more abundant at higher latitudes. However, no clear pattern is apparent in Fig. 7.9. Nonetheless, there were very small differences in $\delta^2$H between wing and tissue for *A. gamma* in Gibraltar, indicating that these individuals developed near where they were sampled, while larger differences between tissue types of individuals caught in the UK suggests that these *A. gamma* had migrated into the UK from overwintering sites further south.

Data for *V. atalanta* individuals caught in the UK showed similar isotopic signatures of wing and abdomen tissues, indicating these individuals were likely to be resident and had potentially overwintered in the UK Thus data provide contrasting results for *A. gamma* (appearing to be migratory), and *V. atalanta* (appearing to be resident) in the UK. The time of year that individuals of these two species were caught differed, with the majority of *A. gamma* caught during May and June, whereas *V. atalanta* individuals were caught during July, with 10 individuals caught after the migrant/resident cut off point used in Chapter 5. An explanation for the differences observed could be that I missed the spring arrival of *V. atalanta* migrants, and that the individuals caught were in fact the progeny of the spring immigrants which had developed within the UK, thus possessing similar $\delta^2$H values for both wing and abdomen tissue. However, results from analyses of *V. atalanta* from Crete and Spain also suggest these individuals were migratory, which conflicts with observations in the literature that *V. atalanta* is known to overwinter in these locations (Stefansecu, 2001). All these analyses had large variation occurring about the mean, as shown at Fig 7.12, and so in order to determine if this method could be used as a reliable tool further study would be needed with larger sample sizes.

### 7.5.3 Validating the Methodology with Lab-reared Material:

I reared *V. atalanta, V. cardui* and *A. gamma* in the lab, and because individuals had been reared in the same geographical location, and fed on locally sourced plant material it was expected that the $\delta^2$H and $\delta^{18}$O values for wing and abdomen tissue should be comparable. However, this was not the case, with significant differences present between wing and abdomen tissue in all three species. In the methodology it was assumed that abdomen chitin is structurally similar to wing chitin. Twenty per cent of the hydrogen in butterfly wings is exchangeable and is was presumed that this was the same for abdomen chitin, finding no evidence for the contrary. Further investigation into the amount of exchangeable hydrogen in abdomen chitin may provide answers into the discrepancy between the $\delta^2$H values in wing and abdomen tissues. Further error in the experiment may have come from the use of chloroform:methanol to dissolve out the lipid within the abdominal cavity. Individuals were dried on capture such that the internal organs could not be separated from the abdominal wall so the fat was dissolved using chloroform:methanol. This may have caused the differences observed in wing and abdomen tissue of lab-reared individuals. A recent study by Chambellant *et al* (pers comm.) examining the efficiency of chloroform:methanol produced poor results, with only 22% of fat dissolved from liver tissue, and 12-19% from muscle tissue. In addition, the authors found that using chloroform:methanol resulted in the removal of membrane proteins, with a subsequent increase in the carbon isotope ratios. Wing material has negligible fat content such that washing this material in chloroform:methanol is likely to have little effect, however the abdominal cavity contains a lot of fat, and it is possible that the lipid extraction method was responsible for producing the significantly different values for $\delta^2$H and $\delta^{18}$O as observed.

The validity of using hydrogen isotopes to determine the natal origins of migrating Lepidoptera using wing tissue was tested using the lab-reared individuals. Comparisons between the three species showed few differences in $\delta^2$H of wing tissues, suggesting that this is a reliable method for comparing across different Lepidoptera species, and gives further assurance that this method may be useful for distinguishing between geographically distinct populations. However, it must be noted that caution must be taken when using this approach. This study has demonstrated that there is a large amount of variation between the isotope ratios of individuals caught within the same geographic area, a finding also recorded in migratory song bird populations in North America (Langin *et al.*, 2007), and as such large sample sizes are required to give this analysis more power. Isotopic ratios of oxygen vary little across the study area, and thus have little power to differentiate between geographically distinct populations, which supports previous work (Hobson *et al.*, 2004). Nonetheless, the use of $\delta^{18}O$ in combination with other isotopes has proved more useful in separating distinct populations.

### 7.5.4 External Effects on Isotopes:

As shown in Fig 7.12, $\delta^{2}H$ and $\delta^{18}O$ values vary over the year due to changes in e.g. temperatures. This provides additional complications when comparing migrants sampled at different times of the year. This is particularly the case for oxygen, and to a lesser extent for hydrogen, with precipitation levels in the UK during summer months being very similar to precipitation level in Spain during the winter. Thus a migrant in the UK might have similar wing and abdomen $\delta^{18}O$ and $\delta^{2}H$ values, not because it had developed in the UK but because wing values represent isotope signatures in Spain during winter development, and the abdomen representing the isotope values in the UK during summer feeding. This problem of intra-annual variation has been highlighted in previous studies where intra-annual differences at sites have been found to be greater than differences between years (Brattström *et al.*, 2008). This finding is supported by Figure 7.13 which suggests that seasonal changes in temperatures may have the most significant effects on $\delta^{2}H$ and $\delta^{18}O$.

The $\delta^{2}H$ and $\delta^{18}O$ values obtained in this study vary greatly from the values in precipitation as shown at Fig 7.12. This reflects the fractionation that has occurred through the food chain, from rainfall through into the plant material and larva. In this study, I found that $\delta^{2}H$ in wing tissue varied from $\delta^{2}H$ of precipitation by approximately 70 per mil, which is similar to that found in *D. plexippus* in North America (Wassenaar & Hobson, 1998) and *I. io* in Sweden (Brattström *et al.*, 2008). No such comparison is available for Lepidoptera and $\delta^{18}O$ fractionation, however Hobson *et al.* (2004), found that $\delta^{18}O$ in bird feathers was approximately 15 per mil different from that of precipitation, while I found differences between Lepidoptera tissue and precipitation of approximately 15-20 per mil. This suggests that fractionation rates between the same trophic levels may be similar and would be an interesting further study.

## 7.6 Conclusion:

Measures of $\delta^2$H and $\delta^{18}$O were used to attempt to distinguish between the natal origins of wild-caught Lepidoptera, as well as determining the migratory status of individuals by comparing wing and abdomen tissues. $\delta^2$H proved to be a more sensitive measure in this study, although the time of year at which individuals were caught confounded results. The spatial distribution of oxygen isotopes in Europe shows little variation and so lacks power in determining the natal origin and migratory status of Lepidoptera in Europe. However it's potential in combination with hydrogen is something that needs to be studied further; it may be a valuable tool for investigating changes in the occurrence of overwintering of migrants in UK as the climate warms. However, further work is needed to increase the reliability of this method.

# Chapter 8

# General Discussion

## 8.1 Thesis Aims:

The overall aim of my thesis was to study the responses of migratory insects to climate change in terms of changes in their distribution and abundance and migratory behaviour. I aimed to compare these responses not only between different migrant taxa, but also between migrant and resident species. I also focussed on the migratory behaviour of butterflies, in terms of directionality of migratory flight and arrival patterns into Britain.

## 8.2 Thesis Findings:

- In Chapter 3, I studied distributional changes in migrant butterflies, hoverflies and dragonflies over time, and quantified shifts in their northern range margins in Britain. I developed a range of methods for analysing distribution changes that proved reliable and easy to interpret such that the methods could be used for determining differences in the response of migrants and resident species in Chapter 4. From the analyses in this Chapter, I concluded that migrant species from the three taxonomic groups are all responding similarly to climate warming by increasing their distribution extents in Britain. All study species show shifts in their northern range limits at rates comparable with shifts in isotherms.

- In Chapter 4, I applied the methods developed in Chapter 3 to determine if migrant and resident butterflies have shown similar responses to climate warming in relation to changes in distribution and abundance changes over time. I found that migrant butterflies are successfully tracking climate change, and have increased in their abundance over time. These responses by migrants were generally greater than those of generalist and specialist resident species.

- In Chapter 5, I examined distribution and abundance changes in three migratory butterflies to determine factors affecting changes in their abundance in Britain. I examined changes in spring (migrant) and summer (resident) populations over time, and also examined changes in arrival patterns at transect sites over time. I found that increased abundance of migrants in Britain is associated with both increased immigration rates (spring abundance) as well as increased reproductive success of migrants in Britain resulting in larger summer populations. For *V. atalanta,* changes in abundance were positively related to spring temperatures in Britain, but not for the other two species. My analyses suggest that these butterflies are not restricted to migrating within their flight boundary layer, because there was no evidence indicating that coastal sites were colonised before inland sites. However it is apparent that the three butterflies follow different migratory pathways on their journey northward from southern Europe and North Africa.

- In Chapter 6, I examined the role of photoperiod on the directionality of *V. atalanta* by comparing the flight directions of individuals reared under increasing, constant and decreasing photoperiods. I found that individuals reared under increasing photoperiods' typical of photoperiods in spring in Spain' had a preferred NNW flight direction, consistent with that expected for spring migrants. Butterflies reared under constant light and decreasing light treatments showed randomness in their flight direction. Further investigation into the ovarian development of females showed that individuals from all three photoperiod treatments were sexually mature and there was no evidence that decreasing and increasing photoperiod treatments had produced reproductively diapausing 'migrant' individuals.

- In Chapter 7, I examined the use of oxygen and hydrogen stable isotopes as a tool for determining the natal origin and migratory status of Lepidoptera. I found that analysis of $\delta^2$H can be used to distinguish between individuals from different geographic locations in Europe, and differences in $\delta^2$H of wing and abdomen tissues provided some evidence of the migratory status of individuals. By contrast, analysis of $\delta^{18}$O failed to distinguish individuals from different sampling locations.

## 8.3 Tackling the problems of changes in recorder effort:

As discussed within Chapters 2, 3 and 4, variability in recorder effort over time presents a considerable problem when analysing distribution data, because increased recorder effort over time may potentially result in over-estimating distribution changes. Accounting for changes in recorder effort (both spatially and temporally) is a complex problem that has no clear solution. A number of methods have been developed, for example Warren *et al.* (2001), Telfer *et al.* (2002), Thomas *et al.* (2004), Hickling *et al.* (2006) and Fox *et al.* (2006) that use various sub-sampling techniques to minimize bias in the results caused by increased recording over time. However, no single method is likely to be able to completely compensate for increasing recorder effort.

Within Chapter 3 and 4, I adopted the technique used by Hickling *et al.* (2006), whereby the data were sub-sampled to include only well-studied squares, based on the recorded species richness within each 10 km grid square. This resulted in the inclusion of the best recorded squares, and excluded areas which were apparently poorly recorded. Although this is a crude method, it enabled a simple and easy comparison to be made between different taxonomic groups. However, it does have limitations. There is a negative relationship between species richness and latitude, and so selecting grid squares with high species richness will tend to disproportionately exclude northern squares. Thus estimates of range shifts may be conservative, especially those species whose range boundary is in northern Britain, as is the case for a number of the migrants studied in this thesis. In Chapter 3, the large difference between the estimated northward shifts for 'no-control' and for '25% species richness' control demonstrates this and highlights the caution needed when interpreting output from this method. However, this method could be developed in the future to include a species richness control value that varied across the UK in relation to latitude, rather than setting a constant value.

Focusing on butterflies in Chapters 4 and 5 allowed me to compare distribution data with abundance data from the UKBMS, a standardized method of data collection which is free from many of the recorder effort problems associated with the distribution data. A number of analyses were undertaken using both distribution and abundance data resulting in similar trends observed for both data sets. This gives further strength and validity to the results from Chapter 3.

A large range of methods described in the literature could be applied to the distribution data in an attempt to control for recorder effort, with each having their own limitations. However, due to time constraints and the data available I feel that the approach used by

Hickling *et al.* (2006) gives robust and reliable results, with similar trends observed at all levels of recorder effort control, and allows an easy comparison not only between taxonomic groups but also within groups. Comparisons of the distribution data with the more standardized abundance data set gives further confidence into the reliability of using distribution data to examine climate driven changes in distribution extent and range shifts.

## 8.4 Do Butterflies Migrate Above Their Flight Boundary Layer?

Much debate exists in the literature as to the flight strategy undertaken by large migrating insects. While field observations, particularly of migrating butterflies, have presented strong evidence that these larger day-flying insects migrate close to the ground within their flight boundary layer, new evidence is coming to light that suggests that they are capable of rising out of their flight boundary layer, taking advantage of fast-flowing air-streams at high altitude.

Within this study, I analysed abundance data in an attempt to tackle this question, by examining the arrival patterns in the UK of three migratory butterflies, *Vanessa atalanta, Vanessa cardui* and *Colias croceus*. Although this is a rather crude method for studying migratory behaviour, it was clear from the results that all three species appear capable of colonising southern Britain (below OS grid line 450N) almost simultaneously, with no evidence that coastal sites are colonized before inland sites. To reach Britain, individuals have to cross large expanses of sea, of varying distances dependent on their migratory route, and as such might be expected to have to re-fuel immediately they reach land if they are flying within their flight boundary layer. This hypothesis was not supported by my findings, highlighting the possibility of individuals exploiting wind currents by migrating above their flight boundary layer. The lack of any pattern of arrival within and between years suggests that individuals may be exploiting favourable fast flowing winds at high altitude.

The technique of using radar to identify species flying at high-altitude is becoming increasingly advanced and has provided insights into the migratory behaviour of moths (Chapmen *et al.*, 2008a,b), dragonflies (Feng *et al.*, 2006) and carabids (Feng *et al.*, 2007). Another technique investigates associations between the arrivals of spring immigrants with the occurrence of high-altitude winds. For *V. cardui,* close associations between arrival times in Spain and high altitude North African winds were discovered, allowing the authors to use back trajectories to establish the natal origins of individuals (Stefanescu *et al.*, 2007).

Butterflies lend themselves well to radar techniques, with all migratory species being sufficiently large to be distinguishable from most other day-flying insect groups. As such, it would be interesting to use radar to confirm whether or not butterflies fly above their flight boundary layer. If indeed they do exploit wind currents, then knowledge of the altitude at which they migrate will enable back trajectories to establish where the migrants have originated from. It would also be interesting to examine mechanisms that allow individuals to select the most appropriate wind currents.

### 8.5 Driving Forces Behind Migration:

In order to fully understand the effects that future climate warming will have on migratory insects, a greater understanding of the mechanisms behind migratory events is required, thus allowing more reliable predictions to be made. The effect of three climate variables, Spanish mean winter temperature, UK mean winter temperature and UK mean spring temperatures, on the migrant and resident abundance of three migratory butterflies were examined in Chapter 5. These climatic variables appear to have little impact on butterfly abundance in the UK, with the only significant correlation arising between UK mean spring temperature and the abundance of resident *Vanessa atalanta*. It is apparent, therefore, that other variables are likely to be driving these observed changes. Being thermophillic, it was expected that temperature would play an important role in affecting the population dynamics of migratory insects. However, my results show that climate variables other than temperature are important. Determining the environmental variables affecting immigration of individuals into Britain is a complicated task, taking into consideration not only climate variables in migrants summer ranges in Britain, but also at overwintering sites and along their migratory route. My results suggest that within species, spring migrant abundance has increased more than summer resident populations, and it would be interesting to investigate effects of climate at overwintering sites. *Vanessa atalanta* overwinters in Spain (Stefanescu, 2001), but population fluctuations in Britain did not correlate with Spanish temperatures during the winter larval development period. Precipitation is another important climate variable associated with Lepidoptera population dynamics. Damp conditions can increase the risk of disease and larval mortality, and rain may hinder migration events, although high rainfall may promote larval host plant quality, especially in arid regions of the Mediterranean. This topic would be a very interesting further area of research.

## 8.6 Implications of my Findings:

Climate warming has not only resulted in migrants increasing their distribution and abundance in Britain, but has led to an increasing number of migrant species arriving in Britain each year (Sparks *et al.*, 2007). With a vast proportion of migrants being pests, for example locusts, aphids and a number of moth species, their migratory behaviour has large implications for human welfare in terms of loss of crops and the spread of disease (Holland *et al.*, 2006). A further implication of these findings is on species assemblages. I have shown in Chapter 4 that migrant butterflies generally have shown greater responses to climate warming than have resident species. This may have a significant effect on species assemblages, with migrants coming to dominate communities and out-competing less mobile, habitat specialists which have already been shown to responding negatively to climate warming (Warren *et al.*, 2001). As indicated throughout these Chapters, we are still a long way from understanding the exact mechanisms controlling insect migration, and considering the implications discussed above it is important that further research is undertaken if the full impacts of increased migration are to be understood.

## 8.7 Overall Conclusions:

All of the migratory insects examined in this study have shown positive responses to climate warming, both by increasing their distribution extent and by shifting their range margins northwards at rates comparable with shifting isotherms. Increased abundance has been observed in migratory butterflies, with greater increases observed in Britain in spring arrivals compared with summer resident populations.

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