University of Southampton

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Ocean and Earth Science

Disentangling the effects of ecology and life history on ectothermic temperature-size responses

by

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Abstract

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Body size is considered to be one of the most important traits of an organism due to its strong links to many ecological and life history traits, and adult body size is often an important indicator of fitness. Recent research suggests that a decrease in body size is a common response to climate warming, particularly in ectotherms. However, my research shows that this is not the case for all species, with some showing the reverse response and others showing no response to temperature at all. My thesis shows that ecological and life history factors are important to consider when assessing the direction and strength of temperature-size responses across ectotherms. The overall aim of this study was to determine which ecological and life history factors may be important for determining ectotherm temperature-size responses using two representative taxa, marine gastropods and butterflies. This was achieved by using a combination of historical museum specimens (covering a temporal range of over 100 years) and modern field data. The results from the study show that factors such as taxon, developmental stage, sex and trophic level and habitat type are important for predicting temperature-size responses in ectotherms. Many species of terrestrial ectotherms, particularly insects, have been shown to increase in size with increasing temperatures. Yet, this study shows that when size is studied in relation to temperatures during each immature stage, that temperature-size responses can vary between life stages. Thus, overall changes in size will rely upon during which stage the individuals are experiencing warm or cool temperatures. Conversely, most aquatic species are expected to decrease in size as temperatures continue to rise. The results presented here, however, show that not all aquatic species respond in this way. None of the species studied decreased in size over time, but size and shape vary spatially suggesting that the results may be a product of local conditions and not climate warming. Additionally, the work in this thesis highlights the importance of using museum collections in ecological research and the potential future applications given the technological advances such as digital collections and computer vision software.

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List of Accompanying Materials

The data from Chapter 2 is available from the Dryad Digital Repository (DOI: 10.5061/dryad.f5cf448).

The data from Chapter 3 is available on Zenodo (DOI: 10.5281/ZENODO.3732132).

The other datasets from this thesis are available in the University of Southampton online repository - PURE (DOI: 10.5258/SOTON/D1799).

Research Thesis: Declaration of Authorship

Print name: Rebecca Jayne Wilson

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temperature-size responses

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

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Wilson, R. J., Brooks, S. J. and Fenberg, P. B. (2019) 'The influence of ecological and life history factors on ectothermic temperature–size responses: Analysis of three Lycaenidae butterflies (Lepidoptera)', *Ecology and Evolution*, 9, pp. 10305–10316. doi: 10.1002/ece3.5550.

Signature: Rebecca Wilson..... Date: 28/12/2020.....

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Continuation of declaration - information pertaining to point 6 on the previous page.

Work conducted jointly with or partly by others:

Chapter 2 - The influence of ecological and life history factors on ectothermic temperature-size responses: analysis of three Lycaenidae butterflies (Lepidoptera)

The manual measurements and statistical analyses for this chapter were completed by R. Wilson. This work has been published in full in Ecology and Evolution (see Wilson, Brooks and Fenberg, 2019).

Chapter 3 - Temperature-size responses in British butterflies using computer vision applied to natural history collections

The computer vision pipeline used in this chapter was created by Feng *et al.* (2020). P. Fenberg ran the images through the pipeline, and the subsequent output images were analysed by R. Wilson, with some help from G. Fisher and G. Wilson. R. Wilson also completed the manual measurements for the pipeline testing, with the exception of *Hesperia comma*, where the data came from previously published research (Fenberg *et al.*, 2016). The statistical analyses were completed by R. Wilson. This work is in review at the Journal of Animal Ecology.

Chapter 4 - Temporal and spatial trends in the size and shape of rocky shore gastropods across the UK

Field work was carried out by R. Wilson, with the help of P. Fenberg, M. MacLean, R. Huggett and C. Quick. Museum specimens were measured by R. Wilson, with the help of P. Fenberg and M. MacLean. Statistical analyses were carried out by R. Wilson.

Chapter 5 - Natural history collections as proxies for spatial and seasonal abundance patterns: a test using butterflies and snails

Butterfly survey data were provided by UKBMS, and museum data was provided by B. Price. Part of the gastropod field data was provided by P. Fenberg. Statistical analyses were completed by R. Wilson.

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List of Abbreviations

AIC ..... Akaike Information Criterion

ANOVA ..... Analysis of Variance

CET ..... Central England Temperature

CI ..... Confidence interval

IT ..... Information theoretic

HSV ..... Hue, Saturation, Value

K-S test ..... Kolmogorov-Smirnov test

MARINe ..... Multi-Agency Rocky Intertidal Network

NHC ..... Natural History Collection

NHM ..... Natural History Museum (London)

NMW ..... National Museum Wales

NMS ..... National Museum Scotland

OMNH ..... Oxford University Museum of Natural History

SE ..... Standard error

SSD ..... Sexual size dimorphism

TBT ..... Tributyltin

TSR ..... Temperature-size rule

VIF ..... Variance inflation factor

UKBMS ..... United Kingdom Butterfly Monitoring Scheme

Chapter 1 Introduction

Body size is considered to be one of the most important traits of an organism due to its strong links to many ecological and life history traits (Bowden *et al.*, 2015; Horne, Hirst and Atkinson, 2015; McCauley *et al.*, 2015; Baar *et al.*, 2018). Adult body size is often an important indicator of fitness, with larger individuals being fitter than smaller ones. This is especially true in females, where size is linked to fecundity (Garcia-Barros, 2000; Fischer, Klockmann and Reim, 2014; Bowden *et al.*, 2015; Forrest, 2016). However, reduction in body size is often cited as the third "universal" response to climate warming, along with changes in distributional range and phenology (Daufresne, Lengfellner and Sommer, 2009; Gardner *et al.*, 2011; Sheridan and Bickford, 2011). These size decreases are supported by ecological theories such as the temperature-size rule (TSR) and Bergmann's rule (latitude-size clines), which have been shown for many species, and suggest that often species will decrease in size in response to increasing temperatures (Daufresne, Lengfellner and Sommer, 2009; Gardner *et al.*, 2011; Sheridan and Bickford, 2011; Ghosh, Testa and Shingleton, 2013; Irie, Morimoto and Fischer, 2013; Ohlberger, 2013; Horne, Hirst and Atkinson, 2015; Classen *et al.*, 2017; Taylor-Cox *et al.*, 2020). Yet, recent studies have shown that some species exhibit a reverse-Bergmann cline or increase in size with rising temperature, whereas some species appear to show no temperature-size response at all (Høye *et al.*, 2009; Shelomi, 2012; Scriber *et al.*, 2014; Horne, Hirst and Atkinson, 2015; Fenberg *et al.*, 2016; Upton *et al.*, 2016; Classen *et al.*, 2017; Wonglersak *et al.*, 2020). Thus, it is now clear that a reduction in body size is not a "universal" response to temperature warming. Therefore, the next challenge is to determine what factors affect species' temperature-size responses so that the direction and strength of a species' response to temperature warming can be predicted more accurately.

Below, I summarise the theoretical predictions and empirical results from the literature to highlight how ecological and life history traits can affect temperature-size responses.

1.1 Endotherms versus ectotherms

Long-term data sets are useful for research of temperature-size responses as they allow for size to be studied over a wide range of temperatures. Until recently, however, research on body size response to warming using long-term datasets has focused on endotherms (Gardner *et al.*, 2011; Sheridan and Bickford, 2011). Research on ectotherms, however, is often based on experiments

or predictive modelling rather than on long-term field data or comparisons of historical data (e.g. museum collections) with modern measurements, although recent studies are starting to do this (Roy *et al.*, 2003; Ortega *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017; Baar *et al.*, 2018; Tseng *et al.*, 2018; Audzijonyte *et al.*, 2020; Polidori *et al.*, 2020; Wonglersak *et al.*, 2020). The internal temperature of ectotherms is closely linked to that of their external environment, while endotherms actively maintain a high internal temperature (Ohlberger, 2013). As a result, changes in environmental temperature are likely to have a larger effect on the metabolic rate of ectotherms. An increase in temperature will result in an increased metabolic rate and therefore a higher food intake will be required to compensate for this, otherwise size will decrease (Sheridan and Bickford, 2011). Furthermore, increased size at lower temperatures may be due to an increased efficiency of converting food for growth, which, along with an increased intake of food, would overcome the higher metabolic rate at higher temperatures (Karl and Fischer, 2008).

For endotherms, size is linked with the amount of energy required to maintain a constant body temperature in their thermal environment, and therefore, under certain conditions smaller size will be favoured, such as if there is an increase in annual mean temperature (Gardner *et al.*, 2011). Originally Bergmann's rule specifically described responses of endotherms, such that lower surface-area to volume ratios are more favourable at higher latitudes due to lower heat loss compared with smaller individuals. The rule, however, was also later applied to ectotherms (Ohlberger, 2013). It has also been suggested that endotherm size declines are likely an indirect response to climate change through changes in diet and available nutrition, whereas ectotherm size declines are thought to be more directly related to climate change (Bickford, Sheridan and Howard, 2011). The two opposing theories for endotherm temperature-size responses (i.e. i) having a higher surface-area to volume ratio in warmer climates and ii) changes in food quantity or quality as temperature changes affecting growth rate), however, are not mutually exclusive (Yom-Tov and Geffen, 2011; Naya, Naya and Cook, 2017).

1.2 Aquatic versus terrestrial

Meta-analyses based on experimental studies or latitudinal gradients by Horne, Hirst and Atkinson (2015), suggested that marine ectotherms are likely to decrease in size with warming temperatures whereas some terrestrial species may increase in size with warming temperatures. Some studies using long-term data sets are also in agreement with this pattern (Babin-Fenske, Anand and Alarie, 2008; Fenberg *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017), but other studies are more equivocal. For example, a study of 335 Australian reef fish species found

that there was a relatively even split in species that increased in size with increasing temperatures and those that decreased in size (Audzijonyte *et al.*, 2020). Endotherms have also shown different responses to warming temperatures between aquatic and terrestrial species. A recent meta-analysis of long-term size changes in birds and mammals found that not only was there variation in the direction of size changes across species (positive, negative and no change), but the aquatic species were generally increasing or showing no change in size, whereas the terrestrial species were decreasing in size over time (Naya, Naya and Cook, 2017), the reverse of what is found in ectotherms.

One of the main mechanisms hypothesised to be responsible for ectotherm body size declines is oxygen limitation reducing growth at higher temperatures (Verberk *et al.*, 2011; Forster, Hirst and Atkinson, 2012; Ghosh, Testa and Shingleton, 2013; Ohlberger, 2013; Horne, Hirst and Atkinson, 2015). Additionally, the body size at which oxygen becomes a limiting factor for growth decreases with increasing temperature (Ghosh, Testa and Shingleton, 2013). This, however, is more likely to be a limitation for aquatic species, as oxygen solubility declines as water temperature increases, and the diffusion rate of oxygen is slower and more costly in water than in air (Verberk *et al.*, 2011; Forster, Hirst and Atkinson, 2012; Horne, Hirst and Atkinson, 2015). Furthermore, the diffusion rate slows with increasing temperature (Forster, Hirst and Atkinson, 2012; Ohlberger, 2013). Therefore, as a consequence of reduced oxygen solubility and slower diffusion rates under warm conditions, it is hard for aquatic species to maintain large body sizes (Forster, Hirst and Atkinson, 2012; Horne, Hirst and Atkinson, 2015). Another suggested mechanism behind size declines is that some species are unable to compensate for increased costs of metabolism at higher temperatures (Bowden *et al.*, 2015); increased food intake is required to accommodate this increased metabolic rate, otherwise size may decline (Bowden *et al.*, 2015). This possibly explains the size decline of the sandy beach clam *Amarilladesma mactroides* (Ortega *et al.*, 2016). Additionally, experiments have shown that optimum temperature for growth may be lower when food quantity is a limiting factor as the increased metabolism under warmer conditions require a higher food intake (Ohlberger, 2013); yet, it remains to be seen if this is the case in the natural environment. This could be particularly problematic in aquatic conditions, where oxygen may also be limiting at higher temperatures. These issues do not however apply to aquatic endotherms, which breathe above the water surface. Furthermore, the temperature of water is not affected as greatly as that of air during extreme weather events and so aquatic endotherms are not impacted as greatly as terrestrial endotherms. Additionally, water temperature is thought to be below optimal for thermoregulation in most aquatic endotherms, so an increase in temperature would reduce the

energy needed for thermoregulation and thereby increase the amount available for growth (Naya, Naya and Cook, 2017).

1.3 Taxonomic differences

There are also differences in how species respond to temperatures between and within taxonomic groupings at various levels (e.g. Horne, Hirst and Atkinson, 2015; Baar *et al.*, 2018; Tseng *et al.*, 2018; Wonglersak *et al.*, 2020). For example, Horne, Hirst and Atkinson (2015) analysed the changes in size per °C increase for orders within the phylum Arthropoda. Their analysis showed that there was a wide variation both between and within arthropod orders, particularly within terrestrial orders such as Lepidoptera and Coleoptera, which ranged from large decreases in size to large increases in size (Horne, Hirst and Atkinson, 2015). These variations in responses may be largely down to differences between aquatic and terrestrial taxa (see section 1.2), and between species with different voltinism (see section 1.6.1; Horne, Hirst and Atkinson, 2015). Responses to temperature and changes in size over time have been shown to vary between species from eight families of Coleoptera, but generally, responses were not family-specific (Baar *et al.*, 2018). A study of Carabidae, one family of Coleoptera, using museum collections found that large-bodied species decreased in size over time, while other species stayed the same or increased in size. Additionally, decreases in size over time could be related to increases in autumn temperatures and decreases in spring temperatures over that period (Tseng *et al.*, 2018). This may be due to an increase in resource availability for growth as spring temperatures increase (Tseng *et al.*, 2018). Another study compared the temperature-size responses of species of British Odonata; in the suborder Zygoptera, species were more responsive to temperature than species in the suborder Anisoptera (Wonglersak *et al.*, 2020). The difference in responses may be due to male Anisoptera species being highly territorial and, therefore, larger size will be strongly selected for (Wonglersak *et al.*, 2020).

1.4 Tropical vs. extra-tropical

Tropical species are thought to be more sensitive to temperature changes than temperate species as the former may already be living at their upper thermal limit (Kingsolver *et al.*, 2011; Fischer, Klockmann and Reim, 2014; Klockmann *et al.*, 2016). In a study on the tropical butterfly, *Bicyclus anynana,* individuals were exposed to short periods of increased temperature, which decreased

fitness and mass, and increased development times. Increasing the mean temperature permanently by 3°C, however, did not have as much impact; these individuals had the highest growth rate but their final mass was lower than the control group (Fischer, Klockmann and Reim, 2014). At the other geographic extreme, two Arctic butterfly species (*Boloria chariclea and Colias hecla*) were found to decrease in size with warmer temperatures (Bowden *et al.*, 2015). Conversely, a study of Arctic spider *Pardosa glacialis* found that adult body size was larger in warmer years (Høye *et al.*, 2009). These studies show that species living at high or low temperatures do not always respond in the same way, and that increased temperatures in these regions will not always result in decreased body size.

Species that experience seasonality (i.e. those in extra-tropical regions) may show more variation in size between seasons. Extra-tropical locations should experience greater temperature variability year to year than tropical locations. Therefore, while tropical species may exhibit long-term trends in response to long-term temperature increases, extra-tropical species are likely to exhibit comparatively more seasonal/inter-annual variation, although underlying long-term trends should still be visible in response to long-term climate warming. However, in order to test this hypothesis, studies should focus on phylogenetically and ecologically similar groups found in both tropical and temperate regions and compare inter-annual variation in body size, which has not yet been done. Furthermore, the observed impact of climate change on size may depend on which season the study takes place (Ohlberger, 2013). A study of multivoltine aquatic and terrestrial arthropods found that in a large proportion of species studied, adults were smaller in warmer seasons (Horne, Hirst and Atkinson, 2017). Temperature was also found to be the most important variable for explaining seasonal body size clines in copepod species, and that feeding strategy may determine the strength of the cline in those species (Horne *et al.*, 2016). Research on the fruit fly *Drosophila subobscura* found that in the north-western USA there was a negative relationship between wing length and temperature, resulting in seasonal size differences and shorter wing lengths during the summer (Kari and Huey, 2000).

1.5 Trophic level and food availability

Species interactions, such as predation, competition, and trophic level, can have an impact on the strength of temperature-size responses (Ohlberger, 2013). Predators, for example, may be more vulnerable to size declines as metabolic rate increases with temperature. Given their higher trophic level, food is naturally more limiting for predators than it is for species within lower trophic levels. Thus, in order to maintain their size with increasing metabolic rates, they must

compensate by eating more, which may not always be possible. This was suggested to be the case for the predatory intertidal gastropod *Nucella lapillus*, where the maximum size in the southern UK has decreased over time as climate has warmed (Wilson-Brodie, MacLean and Fenberg, 2017).

A study of phytoplankton communities found a shift towards smaller sizes at higher temperatures as smaller individuals have the competitive advantage for nutrient assimilation (Reuman, Holt and Yvon-Durocher, 2014). This is in contrast to a study of bee assemblages on Kilimanjaro, which found a loss of larger species and decrease of species richness with increasing elevation (and therefore decreasing temperature), most likely due to limited resource availability (Classen *et al.*, 2017). Furthermore, ecological rules, such as the TSR, may not apply under stressful environmental conditions, as shown by the moth (Lepidoptera) *Manduca sexta*, which had a reversed temperature-size response under low quality food conditions (Diamond and Kingsolver, 2010). Therefore, it is also important to consider attributes that make an environment stressful, such as decreased food quality and quantity, in response to anthropogenic impacts (Angiletta, Steury and Sears, 2004; Gardner *et al.*, 2011; Reiskind and Zarrabi, 2012). A study of copepods found generally larger adults at lower temperatures, unless food quantity decreased, which resulted in smaller adults. Additionally, the strength of the temperature-size response was related to the feeding strategy, with predators having the strongest response (Horne *et al.*, 2016). Food quality, however, does not affect size in all species. When two dragonfly species were reared experimentally under two temperature conditions and two feeding regimes, food quality did not affect growth or survival and temperature affected survival but not growth (Chavez *et al.*, 2015). This is not always the case for this taxon, as other studies have found that dragonfly larvae increase growth rate as food quantity and quality increases (Suhling, Suhling and Richter, 2015).

1.6 Life history traits

Life history traits such as voltinism and phenology, and their variation across the geographic ranges of species may have interactive effects on the direction and strength of response of body size to warming (Gardner *et al.*, 2011; Zeuss, Brunzel and Brandl, 2016). Warming induced changes in the timing of spring emergence of holometabolous insects or hibernation of endotherms and may be associated with changes in adult body size (e.g. Ozgul *et al.*, 2010; Fenberg *et al.*, 2016; MacLean, Kingsolver and Buckley, 2016). For example, shorter hibernation season of marmots in North America has prolonged the period of time they can forage for food, which ultimately increases body size (Ozgul *et al.*, 2010). Certain life history traits in some species may lead to poor adaptability when the local environment changes in response to climate

warming. For example, the clam *Amarilladesma mactroides,* decreased in size and abundance, and developed an increased number of body abnormalities with increasing temperatures, which is thought to be due to it being a cold-water species, a habitat specialist and having limited movement (Ortega *et al.*, 2016). The roles of some life history traits in the temperature-size response are discussed below.

1.6.1 Voltinism

How voltinism (i.e., the number of generations per year) relates to temperature-size response has been a focus of recent research, particularly in insects. An increase in temperature can lead to a longer growing season for many insect species (Forrest, 2016). It is thought that obligate univoltine species (restricted to one generation per year) delay emergence and thus experience a prolonged larval growth period and therefore, size at emergence increases with increasing temperature (Horne, Hirst and Atkinson, 2015). The univoltine butterfly *Hesperia comma*, increased in adult size with increasing temperatures during the late larval period, which may be due to improved food quality during warmer years (Fenberg *et al.*, 2016). Conversely, *Dolichovespula sylvestris*, a univoltine wasp species, was found to decrease in size with increasing temperature (Polidori *et al.*, 2020). Some species, however, are able to increase voltinism and squeeze in an extra generation at higher temperatures, resulting in a decrease in size due to a trade-off between a prolonged single generation growth period and producing more generations with less time for growth in each generation (Horne, Hirst and Atkinson, 2015; Van Dyck *et al.*, 2015; Forrest, 2016; Zeuss, Brunzel and Brandl, 2016). A study of the multivoltine butterfly *Pararge aegeria*, which can have more generations per year at lower latitudes than high latitudes, found that adult size increased with latitude (following Bergmann's rule). This species also had a positive, but weak, relationship between development temperature and forewing length (i.e., a reverse TSR response; Taylor-Cox *et al.*, 2020). Some species may also not be able to increase voltinism if development is limited by another factor, such as daylight length (Forrest, 2016). Alternatively, the facultative univoltine zygopteran species *Coenagrion puella* extends its development time in cooler years by changing to a semivoltine cycle (i.e. decreasing the number of generations per year), resulting in larger individuals in cooler years (Wonglersak *et al.*, 2020).

A meta-analysis of arthropod species (based on laboratory and spatial studies) found that some of the variation in temperature-size responses is likely due to voltinism, with univoltine species generally increasing in size with temperature whilst multivoltine species decrease in size (Horne, Hirst and Atkinson, 2015). In this study, however, species were considered without regard to each generation or sex. Additionally, a study of Lepidoptera and Odonata species found that voltinism decreases with increasing latitude (Zeuss, Brunzel and Brandl, 2016) and, as

temperature generally decreases with increasing latitude, this suggests that individuals living in warmer environments generally have more generations per year. The two taxa showed differing responses of body size in relation to latitude however. Lepidoptera have more large species at low latitudes and Odonata generally have more large species at high latitudes (Zeuss, Brunzel and Brandl, 2016). Thus far, it is unclear whether multivoltine species or obligate bivoltine species, which cannot change their voltinism, will increase or decrease in size with warming temperature (but see chapters 2 and 3). Furthermore, for multivoltine species which can increase voltinism, it is unknown which generations will be affected in terms of temperature-size responses.

1.6.2 Life stages

Different life stages may also respond differently to changes in temperature, especially if they grow in different environmental conditions (Kingsolver *et al.*, 2011) or if one particular stage is more important for determining size at maturity. For example, in the moth *Manduca sexta,* early larval growth is likely impacted by leaf temperature of the host plant, but size of later larval instars are more influenced by air temperatures. The pupal stage, however, lives buried in the soil, and therefore, development rate is affected by soil temperatures (Kingsolver *et al.*, 2011). Generally, larval growth is thought to increase in warmer temperatures (Suhling, Suhling and Richter, 2015; Klockmann *et al.*, 2016; Upton *et al.*, 2016), as shown by the temperate butterfly *Lycaena tityrus* (Klockmann *et al.*, 2016). At cooler temperatures, larval growth can be prolonged, sometimes leading to a larger adult size compared to those developing in warmer temperatures (Angiletta, Steury and Sears, 2004). Immature stages in marine gastropods have also been found to be affected by temperature. When the cowrie *Monetaria annulus* was kept experimentally at two temperatures, individuals at higher temperatures were smaller at the end of the juvenile stage as they had completed development more quickly than those reared at lower temperatures, which grew larger over a longer period of time (Irie, Morimoto and Fischer, 2013).

In butterflies, a study of *H. comma* found that June temperatures are most important for predicting final adult size. June is when individuals are likely to be in their final larval stage and temperature during this stage is most important for determining adult size (Fenberg *et al.*, 2016). Similarly, a study of *L. tityrus* found that increased food intake and assimilation efficiency in the final larval instar appeared to be responsible for increasing adult size, albeit in this case at lower temperatures (Karl and Fischer, 2008). In contrast, a study of *Anthocharis cardamines* found that increasing temperatures during the pupal stage resulted in adults with larger wing size, which was suggested to be due to the impact of temperature on metabolic processes (Davies, 2019). This species has a pupal diapause over winter, which means that they are not time constrained in relation to pupation, so it is thought that temperatures during this stage may be more important

for influencing adult size than temperatures during the larval period (Davies, 2019). For species without a pupal diapause, which are seasonally time constrained, it may also be important to consider changes in size alongside any changes in phenology (as in Fenberg *et al.*, 2016). Changes in phenology are also thought to be a response to climate change (Sheridan and Bickford, 2011), and therefore, if individuals are emerging earlier as adults, they may be experiencing a shortened development period in warmer years (Fenberg *et al.*, 2016). If this is the case, then changes in body size will be determined by whether shortened development time can be overcome by an increased growth rate in warmer temperatures.

1.7 Sexual size dimorphism (SSD)

A recent study using museum collections and historical monthly temperature records of a British population of the univoltine butterfly species, *Hesperia comma*, found that adult size increased with increasing temperature; this, however, was only the case for males and not females, which showed no significant change in size (Fenberg *et al.*, 2016). The females of this species are larger than males and therefore, these results suggest a shift away from sexual size dimorphism (SSD). This differs from research on Arctic spiders *Pardosa glacialis,* which found that females increased in size with earlier snowmelt and the species became more sexually dimorphic in size (Høye *et al.*, 2009), and research on two Arctic butterflies, *Boloria chariclea and Colias hecla*, which found that size was negatively correlated with temperature in both males and females despite the difference in size between the sexes (Bowden *et al.*, 2015). Similarly, the wing size of both male and female *Calopteryx splendens* have a positive relationship with mean winter temperature (Upton *et al.*, 2016), and other Odonata species have also been found to have similar temperature-size responses in both males and females (Wonglersak *et al.*, 2020). Hirst, Horne and Atkinson (2015), suggested that the degree of SSD is not temperature dependent and for 85 arthropod species, males and females have, on average, a similar temperature-size response.

Rensch's Rule predicts that in closely related species, the degree of SSD increases with increasing body size when males are the larger sex, but the degree of SSD decreases with increasing size when females are the larger sex (Dale *et al.*, 2007; Lengkeek *et al.*, 2008). A study of three blenny species (fish) found that two of the species conformed to Rensch's Rule and the degree of SSD varied more between populations than between species, and one species showed a full range of SSD from females being slightly larger to males being much larger (Lengkeek *et al.*, 2008). A study of British Zygoptera (damselflies), where females are the larger sex, found that both sexes decreased in size with increasing temperature at a similar rate, and therefore in this

suborder, the degree of SSD does not change with decreasing size (Wonglersak *et al.*, 2020). Additionally, in insect species where SSD is present, the larger sex generally has a longer development time (Teder, 2014). A study of the butterfly *L. tityrus* found that both sexes followed the temperature size rule. It is thought the males increased growth rate with increasing temperatures but development time decreased, whilst in females assimilation and overall food intake increased due to a longer development period than the males (Karl and Fischer, 2008). This led to females of this species becoming larger than the males, and therefore a higher degree of SSD, at lower temperatures (Karl and Fischer, 2008). These studies highlight both the importance of studying males and females separately, and that each species (and sex) may have different responses to climate change rather than following a set rule.

1.8 Current research

As can be seen from the above review, decreasing size with increasing temperature is not a "universal" response to climate change. Many species go against this in terms of temperature-size trends and therefore it is unrealistic to use the TSR as a baseline rule. I propose that instead, a more flexible approach should be used for predicting temperature-size responses, which incorporates life history and ecological traits of the species (or population) in question. This would allow important factors such as voltinism, sex, trophic level and environment to be included in temperature-size response predictions.

In order to determine if a species is following the TSR, its size must be studied over a range of temperatures. Often this is done experimentally as temperatures can be manipulated easily. Occasionally, studies look at natural populations over time, but these are limited in their temporal scale, and therefore, only include a limited range of temperatures. Museum collections provide a potential solution to this problem as a time series covering a 100+ years can be available if a species is well collected (Fenberg *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017; Baar *et al.*, 2018; Tseng *et al.*, 2018; Polidori *et al.*, 2020). These data can be compared to modern field data and temperature records to give an indication of how species may respond to changing temperatures (Lister *et al.*, 2011).

The overall aim of this thesis is to determine which ecological and life history factors may be important for determining ectotherm temperature-size responses using two representative taxa, marine gastropods and butterflies. To do this, I will be using a combination of field studies and collecting data from museum collections. Both of the selected taxa are readily available in museum collections and therefore, a large amount of data can be obtained to create a time series

of data. Additionally, good study species for this type of research are ones in which males and females, and in some cases separate generations, can easily be identified and appropriate temperature records or other climate data are readily available for analysis. Together these taxa cover a wide range of factors to be studied including both aquatic and terrestrial environments, life stage, voltinism, trophic level, family and sex.

Furthermore, the two taxa studied exhibit different growth types. Determinate growers, such as insects, do not continue to grow as adults. Therefore, adult size of individuals cannot change and only conditions during the pre-adult stages need to be considered in analyses. Indeterminate growers, on the other hand, continue to grow as an adult, until they become limited by physiological restrictions, and so conditions through all life stages may be important. This is also important to consider when using museum collections to assess historical size change given that, for indeterminate growers, the individual may not have reached maximum size at time of collection. Therefore, it is only possible to tell from modern field sampling whether the larger size classes have become smaller or if there is no change, but not whether they have become bigger over time (Roy *et al.*, 2003; Wilson-Brodie, MacLean and Fenberg, 2017). This is not an issue for determinate growing species as the adult specimens in the collections are at maximum size already and therefore can be compared over time.

Below I outline each chapter and how it links into the overall study aim.

Chapter 2. The influence of ecological and life history factors on ectothermic temperature-size responses: analysis of three Lycaenidae butterflies (Lepidoptera)

In this chapter, I manually measure wing lengths of three British Lepidoptera from the Natural History Museum, London (NHM) iCollections and compare the data to monthly air temperature values for the year of collection. In all three species, sex could be determined so this was included in the analyses as a factor, and one species was bivoltine so generations were studied separately. By using monthly temperature records, I can equate the temperature-size responses in each month to a development stage for each species. Although the species are from the same taxa, other ecological factors such as habitat and overwintering stage can be considered. I found that for all three species, adult male size increases in relation to temperatures experienced during the late larval stage but decreased in size in relation to temperatures in the early larval stage. The female responses, however, vary between species. For the bivoltine species, only the first generation showed a temperature-size response, but in both sexes. These

results show that sexes and developmental stages can have different responses to temperature and should be studied separately.

Chapter 3. Temperature-size responses in British butterflies using computer vision applied to natural history collections

In this chapter, I use a recently produced computer vision pipeline to automatically measure wing lengths of 21 species of British butterflies from the NHM iCollections. This data is then compared to temperature, as in chapter 2, and then a multi-species analysis is used to determine what factors are most important for predicting temperature-size responses during the growth stages. The factors considered are family, habitat type, sex, overwintering stage and relative wing size. The testing of the pipeline shows that it can reliably measure wing lengths for some species and is a promising tool for the future. As with chapter 2, the most consistent result is that there is an increase in adult size in relation to increasing temperature during the late larval stage. The responses to temperature during the early larval and pupal stages were more variable. Family and sex are important factors in predicting responses, and there are also some differences between species living in grassland and woodland habitats. This highlights the importance of considering a variety of ecological and life history factors when predicting and studying temperature-size responses.

Chapter 4. Temporal and spatial trends in the size and shape of rocky shore gastropods across the UK

In this chapter, I compare modern size and shape measurements of seven rocky intertidal gastropods, taken in the field across UK shores, to measurements taken from museum specimens. Data are compared temporally and spatially to determine how morphology varies across those parameters. The species included in this chapter have indeterminate growth and are aquatic, and therefore, provide a direct contrast to the butterflies studied in chapters 2 and 3. This chapter also follows similar methods used in Wilson-Brodie, MacLean and Fenberg (2017), and so results can be compared to those of the predatory gastropod *N. lapillus*. As all species studied in this chapter are grazers, this provides a comparison between trophic levels. For all seven species, and in contrast to *N. lapillus*, size is not decreasing over time. This may be due to the lower trophic level of these species and there being a high abundance of food available for them. Additionally, size and shape vary spatially, suggesting that local conditions may be more important in

predicting morphology than temperature. Furthermore, this shows that aquatic species do not always follow the TSR.

Chapter 5. Natural history collections as proxies for spatial and seasonal abundance patterns: a test using butterflies and snails

While this chapter does not directly look at size responses of ectotherms, it gives support to the use of natural history collections in ecological research. I compare museum specimen locality data to monitoring survey data for marine gastropods and butterflies to determine whether museum collections can be used as a proxy for abundance based on field data. I found that the relative abundance patterns (as density plots) using museum data generally matched well to the patterns shown by the field data for both butterflies and gastropods. Therefore, museum specimen counts (per location or date) can be used as a proxy for spatial and seasonal abundance patterns. Furthermore, this research shows that when used cautiously that museum data is reliable and therefore, is a useful tool for ecological research.

Chapter 6. General Discussion

In this chapter, I discuss the results from chapters 2-5 in relation to the environmental and biological factors that have an impact upon temperature-size responses, and how the results presented in the thesis have added to current knowledge. The factors that appear to be particularly important for predicting temperature-size responses include taxon, developmental stage, sex and trophic level. Habitat type and local environmental conditions may also influence the response, but for ectotherms, it is not as simple as stating that aquatic species follow the TSR and terrestrial species show the reverse response. Additionally, the work in this thesis highlights the importance of using museum collections in ecological research and the potential future applications given the technological advances such as digital collections and computer vision software.

Chapter 2 The influence of ecological and life history factors on ectothermic temperature-size responses: analysis of three Lycaenidae butterflies (Lepidoptera)

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2.1 Abstract

Body size has been shown to decrease with increasing temperature in many species, prompting the suggestion that it is a universal ecological response. However, species with complex life cycles, such as holometabolous insects, may have correspondingly complicated temperature-size responses. Recent research suggests that life history and ecological traits may be important for determining the direction and strength of temperature-size responses. Yet, these factors are rarely included in analyses. Here, I aim to determine if the size of the bivoltine butterfly, *Polyommatus bellargus*, and the univoltine butterflies, *Plebejus argus* and *Polyommatus coridon*, change in response to temperature and whether these responses differ between the sexes, and for *P. bellargus*, between generations. Forewing length was measured using digital specimens from the Natural History Museum, London (NHM), from one locality in the UK per species. The data were initially compared to annual and seasonal temperature values, without consideration of life history factors. Sex and generation of the individuals and mean monthly temperatures, which cover the growing period for each species, were then included in analyses. When compared to annual or seasonal temperatures only, size was not related to temperature for *P. bellargus* and *P. argus*, but there was a negative relationship between size and temperature for *P. coridon*. When sex, generation and monthly temperatures were included, male adult size decreased as temperature increased in the early larval stages, and increased as temperature increased during the late larval stages. Results were similar but less consistent for females, while second generation *P. bellargus* showed no temperature-size response. In *P. coridon*, size decreased as

temperature increased during the pupal stage. These results highlight the importance of including life history factors, sex and monthly temperature data when studying temperature-size responses for species with complex life cycles.

2.2 Introduction

Body size is considered to be one of the most important traits of an organism due to its strong links to ecology and life history (Fenberg and Roy, 2008; Bowden *et al.*, 2015; Horne, Hirst and Atkinson, 2015; McCauley *et al.*, 2015; Baar *et al.*, 2018). Ecological rules such as the temperature-size rule (TSR) and Bergmann's rule (latitude-size clines) have led to the prediction that body size declines will be a "universal" response to climate change (Daufresne, Lengfellner and Sommer, 2009; Gardner *et al.*, 2011). Specifically, the TSR predicts that individuals developing in cool conditions will be larger as adults than those developing in warm conditions, and this has been shown to be the case for many species (Daufresne, Lengfellner and Sommer, 2009; Gardner *et al.*, 2011; Sheridan and Bickford, 2011; Ghosh, Testa and Shingleton, 2013; Irie, Morimoto and Fischer, 2013; Ohlberger, 2013; Horne, Hirst and Atkinson, 2015; Tseng *et al.*, 2018). However, it has also recently been shown that some species increase in size with increasing temperature, whereas some species appear to show no temperature-size response (Høye *et al.*, 2009; Shelomi, 2012; Scriber *et al.*, 2014; Horne, Hirst and Atkinson, 2015; Fenberg *et al.*, 2016; Upton *et al.*, 2016; Classen *et al.*, 2017). Thus, it is now clear that a reduction in body size is not a "universal" response to warming temperatures.

Recent studies have provided insights into the many ways ecological and life history factors are likely to affect the direction and strength of temperature-size responses (but a full synthesis is needed). These factors include, but are not limited to, sex, voltinism, trophic level, immature stage, and habitat type. For example, aquatic ectotherms exhibit a decrease in size with warming temperatures but the strength of response may vary depending on trophic level (Forster, Hirst and Atkinson, 2012; Wilson-Brodie, MacLean and Fenberg, 2017). In contrast, some terrestrial species increase in size with warming temperatures, but response can vary based on sex and voltinism (Horne, Hirst and Atkinson, 2015; Fenberg *et al.*, 2016). In some holometabolous insects, univoltine species increase in size with temperature whilst multivoltine species decrease in size, whereas bivoltine species appear to show no change (Horne, Hirst and Atkinson, 2015). While these are important findings, approaches thus far have not generally considered whether individual generations and/or sexes have varying responses. This can be significant because individuals from each generation will experience different temperatures. Fenberg *et al.*, (2016)

showed this is particularly important during growth of the final larval instar. Sex can also be an important factor especially if there is sexual size dimorphism (SSD), as males and females do not always respond in the same way to temperature (e.g. Fenberg et al., 2016; Høye et al., 2009). Thus, omission of such ecological and life history factors when conducting analyses may oversimplify the study system and/or cause some temperature-size responses to be masked.

Climate warming potentially provides a longer growing season for some insect species, particularly for temperate species emerging early in the season (Forrest, 2016). This is especially significant for obligate univoltine species (restricted to one generation per year) which often attain a larger size at maturation with increasing temperature (Horne, Hirst and Atkinson, 2015). Some species, however, are able to increase voltinism and produce an extra generation per year at higher temperatures, resulting in a decrease in adult size due to a trade-off between increased single generation growth and producing more generations with smaller individuals (Horne, Hirst and Atkinson, 2015; Van Dyck *et al.*, 2015; Forrest, 2016; Zeuss, Brunzel and Brandl, 2016). Thus far, it is unclear how each generation of multivoltine species (more than two generations in one year) or obligate bivoltine species, which are restricted to two generations each year, will respond to increasing temperatures.

A recent study using museum collections and monthly temperature records over a 100-year period of a British population of the univoltine butterfly species, *Hesperia comma*, found that adult size increased with increasing temperature; this, however, was only the case for males and not females, which showed no significant change in size (Fenberg *et al.*, 2016). The females of this species are larger than males and therefore these results suggest SSD decreases with increasing temperature. This differs from field research on the Arctic spider *Pardosa glacialis,* which found that females (but not males) increased in size with earlier snowmelt and the species became more sexually dimorphic in size (Høye *et al.*, 2009), and from field research on two Arctic butterflies, *Boloria chariclea* and *Colias hecla*, which found that size was negatively correlated with temperature in both males and females despite the difference in size between the sexes (Bowden *et al.*, 2015). These studies highlight both the importance of studying males and females separately, and that species may have different responses to climate change, reflecting different life history strategies, rather than following a particular rule.

Museum collections are a useful resource for studying historical size changes and can be used to create a time series which can be compared to climate variables over the same period (Lister *et al.*, 2011). Furthermore, these collections often cover a wide range of taxa over large spatial areas and temporal periods (Johnson *et al.*, 2011). There are, however, some potential problems in using museum collections as metadata are often incomplete or missing altogether,

which reduces the number of useable specimens (Johnson *et al.*, 2011; Lister *et al.*, 2011), and specimens are usually collected opportunistically rather than systematically as part of long term projects (Kharouba *et al.*, 2018). Additionally, museum specimens often do not provide an ecological context, such as other species present or abiotic environmental factors, and therefore, the drivers of change may not be apparent (MacLean *et al.*, 2018). When species are well represented in collections, however, they provide a unique opportunity to study changes in species body size over a long time-period (decades to centuries; Kharouba et al., 2018). As long as specimens are used critically (i.e. by disregarding those without sufficient metadata or that are in poor condition) and paired with appropriate temperature records, these collections can provide important insights into recent historical size changes, and may prove useful in predicting future changes.

Butterfly collections provide a useful resource for studying the effect of various factors on temperature-size responses. Good study species for this type of research are ones in which males and females, and in some cases separate generations, can easily be identified and climate data are readily available for analyses. This approach was used in previous research on *H. comma* (Fenberg *et al.*, 2016) in which monthly temperature records were compared to adult size, rather than a single annual or seasonal temperature value. This has the advantage of assessing which monthly temperature has the most impact on adult size and therefore, in which life stage growth is most affected by temperature. Laboratory experiments often use a constant, single temperature per replicate (for example studies used for meta-analysis in Horne *et al*. (2015)), but in the natural environment, temperature fluctuates and individuals may experience a wide range of temperatures during the growth period and from one year to the next as shown in field research by Bowden *et al*. (2015) and in historical data used by Fenberg *et al*. (2016).

In contrast to previous studies, which have not considered generational differences in temperature-size responses, I have chosen to examine how temperature affects the adult size of each generation in a bivoltine species, the Adonis Blue butterfly (*Polyommatus bellargus*), compared to the response of two univoltine species, one from the same habitat (the Chalkhill Blue butterfly *Polyommatus coridon*) and one from a different habitat type as *P. bellargus* (the Silver-studded Blue butterfly *Plebejus argus*). *Polyommatus bellargus* has two temporally distinct generations (separated by approximately two weeks) and for all three species, the sexes are easily identifiable, and are present in large numbers in the collections of the Natural History Museum (NHM; London), making them ideal study species to investigate temperature-size responses over many decades.

Based on the meta-analysis by Horne *et al*. (2015), I hypothesise that the univoltine species, *P. coridon* and *P. argus*, will increase in size with temperature and, as a bivoltine species, the size of *P. bellargus* will not change in response to temperature warming. Horne *et al*. (2015) did not include the effect of generation or sex in their analysis; however, life history traits, such as timing of the final larval stage and sex, can influence whether there is a response to temperature (Fenberg *et al.*, 2016). Therefore, when sexes and generations are analysed separately I may find that a subset of the population is responding differently to temperature changes. If this is the case, I predict that for *P. bellargus*, generation one will show a greater size response to temperature than generation two due to the extended growing period available to generation one in warm years and the less favourable growing conditions during cool springs (Thomas, 1983; Thomas and Lewington, 2014). In particular I would expect May temperatures to be important for generation one as this is when the species is in the final larval stage (Thomas and Lewington, 2014). For the two univoltine species, I would expect a similar response as *Hesperia comma* (Fenberg *et al.*, 2016); therefore, I predict that the size *P. argus* and *P. coridon* will increase in size with increasing temperature for the month corresponding to the final larval stage (June for both species (Thomas and Lewington, 2014)), and there will be a greater size change for the smaller sex.

2.3 Material and Methods

2.3.1 Study system

The three study species are European Lepidoptera in the Lycaenidae with northern range edges in the UK. These species have different life history and ecological traits, which are summarised in Table 2.1. Further details about the species are given below.

In Britain, *Polyommatus bellargus* and *P. coridon* are restricted to calcareous grasslands on the south-facing slopes of chalk hills, and populations are discrete (Thomas, 1983; Harper, Maclean and Goulson, 2006; Brereton *et al.*, 2008). *Plebejus argus* occurs on lowland heaths, mosses, calcareous grasslands and sand dunes; this species has rapidly declined across the UK in the last century, and its stronghold is now on the New Forest heaths in Hampshire (Thomas, 1985; Thomas and Lewington, 2014). *Polyommatus bellargus* is obligatorily bivoltine, with the first generation emerging in May and June, and the second in August and September (Thomas, 1983). This makes *P. bellargus* an interesting study species as it will not respond to temperature by attempting to fit in an extra generation. In contrast to this, *P. coridon* and *P. argus* are both

univoltine and adults are on the wing from mid-July to early September and late June to August, respectively (Thomas, 1985; Thomas and Lewington, 2014). Despite laying their eggs on the same plant species, there is limited direct competition between larvae of *P. bellargus* and *P. coridon* as there is little overlap between the timings of the larval stages (Thomas, 1983). Like many European Lycaenidae species, *P. bellargus, P. coridon* and *P. argus* are all associated with certain ant species, including *Lasius alumius*, *L. niger* and *Myrmica sabuleti* (Kitching and Luke, 1985; Fiedler, 1989). The butterfly larvae produce secretions, and are 'milked' by ants. The ants nurture the larvae and bury them in earth cells when they are inactive or moulting (Thomas, 1983; Thomas and Lewington, 2014).

Table 2.1 Some traits and characteristics of the three lycaenid study species (Thomas, 1983, 1985; Brereton *et al.*, 2008; Thomas and Lewington, 2014).

Species	No. of generations	Larger sex	Habitat type	Overwinter stage	Relationship with ants	Larval food plant	Larval activity
P. bellargus	2	Males	Chalk hill grasslands	Larva	From second larval instar to pupal stage, March-October	*Hippocrepis comosa*	Diurnal
P. coridon	1	Males	Chalk hill grasslands	Egg	As above, from May-August	*Hippocrepis comosa*	Nocturnal
P. argus	1	Males	Heathland (also mosses, grassland and sand dunes)	Egg	All stages (egg to adult)	A variety of heath plants e.g. heathers or gorse	Nocturnal

2.3.2 Climate variables

Temperature records were obtained from the UK Meteorological Office (http://www.metoffice.gov.uk/climate/uk/summaries/datasets). Regional records of annual, seasonal (spring-summer average) and monthly mean temperatures were used from the South East and South-central England (for *P. bellargus* and *P. argus*) and the East Anglia regions (for *P. coridon*), as these areas included my study sites. These data cover the years from 1910 to the present day and provided appropriate coverage for the majority of my butterfly data set.

2.3.3 Image analysis

The NHM has recently digitised their entire British butterfly and moth collections (Paterson *et al.*, 2016). A large number of specimens of *P. bellargus* with accompanying collection date and locality metadata were present in the collections from Folkestone (51°4'53.05"N, 1°10'10.20"E; 1396 out of 4814 specimens for that species). The most common site in the collections for *P. coridon* was Therfield Heath (52°2'24.00''N, 0°1'12.00''W; 2300 out of 3097 specimens). For *P. argus*, many specimens were collected from the New Forest in Hampshire (centred around 50°52'12.00''N, 1°37'48.00"W; 1049 out of 2121 specimens). No other single area had as high a density of specimens for those species and therefore I focussed on those locations to remove any potential effects of locality on the results.

Images of these specimens were checked for usability and separated into year groups and by generation. The generations of *P. bellargus* were separated following the methods used in Brooks *et al*. (2017). To be usable, the images needed to be sharp (i.e. not blurred) and in dorsal view with undamaged forewings. For *P. coridon,* some years had an excessive number of specimens so 50 usable specimens were selected at random for those years. The number of specimens used in analyses is given in Table 2.2, along with the years covered by the specimens. It should be noted that for *P. argus*, 48 specimens were not included as they were collected prior to 1910 (when local temperature records began). For *P. coridon*, males in 1919 were all considerably smaller than males in other years and therefore were excluded from analyses as outliers.

Table 2.2 The number of specimens used in data analyses for each species (total, males and females), the temporal range covered by those specimens, and the number of years included in the analysis within that range.

Species	Total number	No. of Males	No. of Females	Date Range	No. of Years
***P. bellargus* (generation 1)**	190	65	125	1912 – 1953	12
***P. bellargus* (generation 2)**	532	203	329	1911 - 1956	18
P. coridon	417	133	284	1910 - 1931	15
P. argus	412	281	131	1910 – 1953	16

For each image, the sex of the specimen was noted and the length of the forewings were measured using ImageJ software. First, the scale was set in ImageJ for each individual image using the scale bar imbedded in each image to measure out a known distance e.g. 10mm. Each forewing

was measured from the point at which the wing meets the thorax to the apex of the wing (not including the scales at the very edge of the wing as these were often absent), and an average forewing length was calculated for each individual. Forewing length of museum specimens has been found to correlate strongly with wing surface area (Fenberg *et al.*, 2016) and has been used in previous studies as a proxy for overall body size (Bowden *et al.*, 2015; Fenberg *et al.*, 2016). Temperature data were added to the data sets for the corresponding years of collection.

2.3.4 Statistical analysis

Within each species (or generation where applicable), specimens were only included in the statistical analyses if there were three or more individuals for each sex per year. For each species, average forewing length was compared to mean annual temperature; all data were used in this model without taking into account generation or sex, which is consistent with methods used in previous studies and meta-analyses of temperature-size responses (e.g. Baar *et al*., 2018; Horne *et al*., 2015). This was repeated using mean spring-summer temperatures, which were an average of March to August temperatures to include the main growing periods for all species.

Data were then analysed using R statistical packages MASS and MuMMIn to run multiple linear regressions using two methods; stepwise regression in both directions to select variables for the final model, and information theoretic (IT) model selection with model averaging based on Akaike Information Criterion (AIC) as described in Fenberg *et al.* (2016). The linear models were used to determine whether there was a relationship between mean forewing length of individuals and monthly mean temperatures (for *P. bellargus*, each month from March to May for generation 1 and June to August for generation 2; for *P. coridon* and *P. argus*, each month from March to July). These months were selected to cover the entire post-winter larval growth period and included the pupal phase as well (Thomas and Lewington, 2014). The data were analysed separately for males and females, and for generations one and two in *P. bellargus*.

Additionally, where there were significant correlations between size and monthly temperatures, a percentage change in size per °C change in temperature was calculated for the most important months for predicting adult size so that change could be compared between species and sexes. Percentage wing size change was calculated using the formula $(\exp^{(slope)}-1)*100$; slopes were calculated using the natural log of wing lengths to account for any scaling effects that may have resulted from the differences in size between species and sexes (Forster, Hirst and Atkinson, 2012; Fenberg *et al.*, 2016).

2.4 Results

2.4.1 Sexual size dimorphism

For *P. bellargus*, generation one adults were significantly larger than generation two adults ($F_{1,718}$=25.278, p<0.001) but there was no overall difference in size between sexes ($F_{1,718}$=1.286, p=0.257). The males of generation one were larger than females in generation one (t=-4.536, p<0.001; male average size = 14.772 mm, female average size = 14.378 mm; Fig. 2.1), but there was no significant difference in size between generation two males and females (t=-0.882, p=0.378; male average size = 14.292 mm, female average size = 14.241 mm). Therefore, the SSD was limited to generation one, as a result of males being larger in this generation. For *P. coridon,* males were significantly larger than females (t=-3.7492, p<0.001; male average size = 15.414 mm, female average size = 14.951 mm; Fig. 2.1). This was also the case for *P. argus* (t=-7.995, p<0.001; male average size = 12.231 mm, female average size = 11.683 mm; Fig. 2.1).

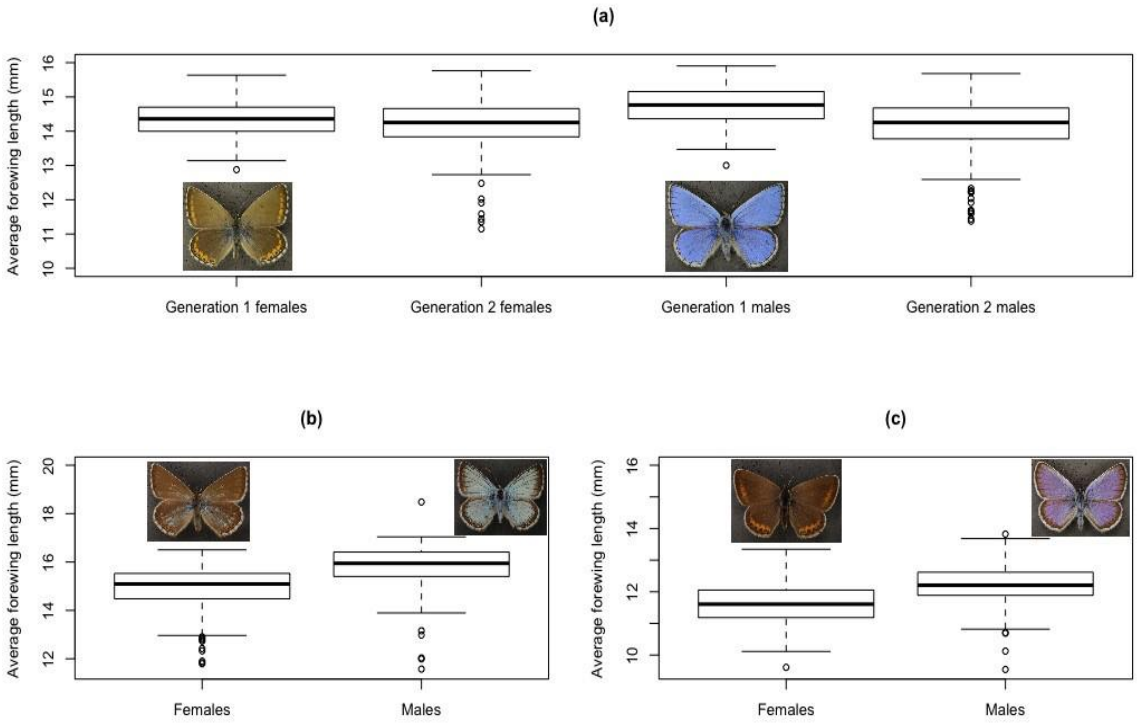

Figure 2.1 Boxplots of average forewing length of (a) *P. bellargus* specimens according to generation and sex, (b) *P. coridon* specimens according to sex, and (c) *P. argus* specimens according to sex. The box represents the interquartile range, the line in the box represents the median value, the whiskers show the 5th and 95th quantiles, and the circles represent outliers. Images of each species are shown next to the relevant boxplots for both sexes.

2.4.2 Annual and seasonal temperature

Average forewing length was first compared to annual mean temperature using a linear model, irrespective of sex and generation, for each species. The model was not significant for *P. bellargus* (adjusted R^2=0.00224, F=2.617, df=1 and 720, p=0.106) or *P. argus* (adjusted R^2=0.0045, F=2.857, df=1 and 410, p=0.0917) but was significant for *P. coridon* (adjusted R^2=0.00904, F=4.796, df=1 and 415, p=0.0291), which showed a negative relationship between size and annual temperature (slope estimate=-0.220). Forewing length was also compared with mean spring-summer temperature (an average of March to August temperatures), but was not significant for *P. bellargus* (adjusted R^2=-0.000949, F=0.316, df=1 and 720, p=0.574) or *P. argus* (adjusted R^2=0.00656, F=3.716, df=1 and 410, p=0.0546). The model was, however, significant for *P. coridon* (adjusted R^2=0.0123, F=6.159, df=1 and 415, p=0.0135), showing a negative relationship between spring-summer temperature and size (slope estimate=-0.244). However, these correlations for *P. coridon* may have been influenced by the significant correlations with July mean temperatures (r=0.609, p=0.0160; r=0.677, p=0.00560), which have a negative relationship with both male and female forewing length (see below), and to a lesser extent April temperature, which had a negative effect on male forewing length (r=0.629, p=0.0120; r=0.614, p=0.0147).

2.4.3 Monthly mean temperature

2.4.3.1 *Polyommatus bellargus*

Multiple linear regression of the *P. bellargus* size data using mean monthly temperatures for males and females in both generations showed that the model was significant for generation one males (Table 2.3), with mean March, April and May temperatures as significant variables; these variables were included in the final model after stepwise regression (Table 2.4i). For generation one females, the model was also significant (Table 2.3); May was the only significant month but March was also included in the final model after stepwise regression (Table 2.4i). In both cases, May temperature (and, for males, April temperature) had a positive relationship with forewing length (Table 2.4i, Fig. 2.2a-b, Fig. 2.3), but March temperature had a negative relationship with forewing length as shown by the slope estimates (Table 2.4i). These results imply that adult size decreases as temperature increases during early larval stages, but adult size increases as temperature increases during late larval stages (Table 2.5). With increasing May temperatures, there was a 1.09% increase in adult size per °C for generation one males and a 0.97% increase for generation one females. For males, this positive relationship appears strongest during years when

May temperatures are cool to moderate (~10-11.5°C; Fig. 2.3). In addition, for males there was also a 3.03% increase in size with April temperatures and a 2.02% decrease with March temperatures. The models were not significant for generation two males or females (Table 2.3).

Table 2.3 Results of the linear models for predicting average forewing length of *P. bellargus* (both generations)*, P. coridon* and *P. argus* using mean monthly temperatures as variables; values are adjusted R^2 (AR^2), the F statistic (F), degrees of freedom (df) and the p-value (p). Results are for models analysing males and females separately.

Species	**Males**				**Females**			
	AR^2	**F**	**df**	**p**	**AR^2**	**F**	**df**	**p**
***P. bellargus* generation 1**	0.147	4.67	3, 61	0.00529	0.101	5.62	3, 121	0.00122
***P. bellargus* generation 2**	0.00534	1.36	3, 199	0.256	-0.00820	0.111	3, 325	0.954
P. coridon	0.0624	2.76	5, 127	0.0213	0.0431	3.55	5, 278	0.00397
P. argus	0.0408	3.38	5, 275	0.00555	0.0466	2.27	5, 125	0.0514

2.4.3.2 *Polyommatus coridon*

For *P. coridon*, multiple linear regression models with mean monthly temperatures were significant for both males and females. For males, mean June temperature was the only significant variable, but all variables were included in the final model after stepwise regression (Table 2.4ii). For females, mean July and May temperatures were significant variables and the only variables included in the final model after stepwise regression (Table 2.4ii). The results imply that adult size in both males and females decreases in size as temperature increases during early larval stages, and adult males increase in size as temperature increases during late larval stages (Table 2.5). Additionally, the results suggest that both males and females decrease in size when temperatures are higher during the pupal stage. For males, there was a 1.93% increase in adult size per °C increase in June temperatures (Fig. 2.2c) compared to a 1.10% decrease in adult size of females per °C increase in July temperatures (Fig. 2.2d).

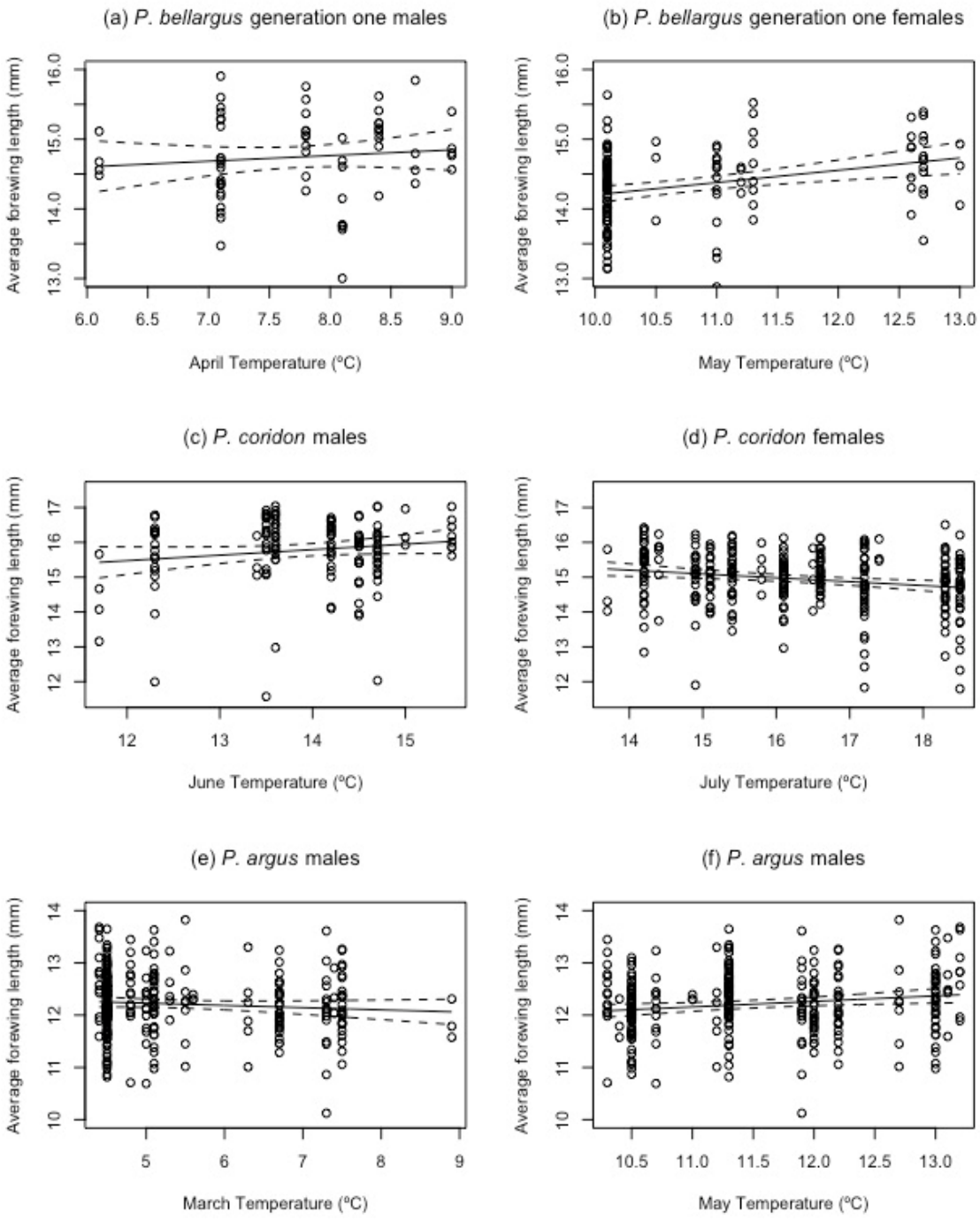

Figure 2.2 Average forewing length (mm) compared to mean monthly temperatures for (a) generation one *P. bellargus* males versus April temperature, (b) generation one *P. bellargus* females versus May temperature, (c) *P. coridon* males versus June temperature, (d*) P. coridon* females versus July temperature, (e) *P. argus* males versus March temperature, and (f) *P. argus* males versus May temperature. Circles represent individual specimens, solid lines are the predicted values given by the linear models, and the dashed lines represent two standard errors above and below the predicted values. The months shown in the plots are those which were the most important for predicting adult size in the multiple linear regression analysis.

Table 2.4.i Outputs for variables included in linear models for *P. bellargus* generation one males and females using mean monthly temperatures; t-value and significance (p) for each variable, slope estimate, standard error and upper and lower confidence intervals (CI), importance scores, variance inflation factors (VIF) and whether the variable was selected for the final model after stepwise regression in both directions. Slope estimates, standard error and confidence intervals are based on an average of all candidate models (using the IT-AIC approach), and variables in bold are those retained after nested models were removed. Importance scores are based on the number of candidate models the variable was present in, with a score of 1.0 indicating that the variable was present in all candidate models.

		t-value	**p**	**Slope estimate**	**Standard error**	**Lower CI**	**Upper CI**	**Importance**	**VIF**	**Final model (Y/N)**
Males	**March**	-3.178	0.00254	-0.291	0.0956	-0.482	-0.100	0.94	2.286	Y
	April	3.105	0.00288	0.425	0.143	0.139	0.711	0.94	2.331	Y
	May	2.275	0.0264	0.157	0.0703	0.016	0.297	0.80	1.043	Y
Females	**March**	-1.663	0.0989	-0.117	0.0754	-0.266	0.0325	0.55	1.536	Y
	April	0.720	0.473	0.0394	0.0956	-0.150	0.228	0.29	1.285	N
	May	2.660	0.00886	0.159	0.0523	0.0550	0.262	0.97	1.232	Y

Table 2.4.ii Outputs for variables included in linear models for *P. coridon* males and females using mean monthly temperatures; t-value and significance (p) for each variable, slope estimate, standard error and upper and lower confidence intervals (CI), importance scores, variance inflation factors (VIF) and whether the variable was selected for the final model after stepwise regression in both directions. Slope estimates, standard error and confidence intervals are based on an average of all candidate models (using the IT-AIC approach), and variables in bold are those retained after nested models were removed. Importance scores are based on the number of candidate models the variable was present in, with a score of 1.0 indicating that the variable was present in all candidate models.

		t-value	**p**	**Slope estimate**	**Standard error**	**Lower CI**	**Upper CI**	**Importance**	**VIF**	**Final model (Y/N)**
Males	**March**	1.627	0.106	0.202	0.110	-0.0160	0.419	0.66	1.814	Y
	April	-1.396	0.165	-0.102	0.0995	-0.299	0.0942	0.39	1.600	Y
	May	-1.806	0.0733	-0.217	0.162	-0.536	0.103	0.52	3.459	Y
	June	2.495	0.0139	0.240	0.116	0.0110	0.469	0.77	1.573	Y
	July	-1.870	0.0638	-0.164	0.0972	-0.356	0.0278	0.62	1.871	Y
Females	March	0.761	0.447	0.0311	0.0453	-0.0581	0.120	0.31	1.650	N
	April	-0.662	0.509	-0.0229	0.0617	-0.144	0.0985	0.28	1.637	N
	May	-2.232	0.0264	-0.131	0.0564	-0.242	-0.0200	0.86	1.729	Y
	June	0.048	0.962	0.00555	0.0580	-0.108	0.120	0.26	1.330	N
	July	-3.932	<0.001	-0.150	0.0405	-0.230	-0.0706	1.00	1.533	Y

Table 2.4.iii Outputs for variables included in linear models for *P. argus* males using mean monthly temperatures; t-value and significance (p) for each variable, slope estimate, standard error and upper and lower confidence intervals (CI), importance scores, variance inflation factors (VIF) and whether the variable was selected for the final model after stepwise regression in both directions. Slope estimates, standard error and confidence intervals are based on an average of all candidate models (using the IT-AIC approach), and variables in bold are those retained after nested models were removed. Importance scores are based on the number of candidate models the variable was present in, with a score of 1.0 indicating that the variable was present in all candidate models.

		t-value	**p**	**Slope estimate**	**Standard error**	**Lower CI**	**Upper CI**	**Importance**	**VIF**	**Final model (Y/N)**
Males	**March**	-2.664	0.00817	-0.0898	0.0403	-0.169	-0.0105	0.86	1.518	Y
	April	1.397	0.163	0.0634	0.0500	-0.350	0.167	0.46	1.476	N
	May	2.336	0.0202	0.124	0.0505	0.0251	0.224	0.89	1.606	Y
	June	1.412	0.159	0.142	0.0886	-0.0319	0.317	0.58	1.312	Y
	July	-0.361	0.718	-0.00468	0.0369	-0.0755	0.0678	0.27	1.253	N

2.4.3.3 *Plebejus argus*

The multiple linear regression was significant for male, but not female *P. argus* (Table 2.3). For the males, mean March and May temperatures were significant and were included in the final model after stepwise regression along with June temperatures (Table 2.4iii). March temperatures had a negative relationship with forewing length (Table 2.4iii, Fig. 2.2e), but May and June temperatures had a positive relationship with forewing length (Table 2.4iii, Fig. 2.2f). This trend is the same as in the other two species resulting in a decrease in adult male size as temperature increases during early larval stages and an increase in size as temperature increases during late larval stages (Table 2.5). There was a 0.85% decrease in male adult size per °C increase in March temperatures compared to a 0.95% increase in male adult size per °C increase in May temperatures. Female *P. argus*, however, do not show this trend (Table 2.5).

Table 2.5 Stage-specific temperature-size trends in three lycaenid species, and *Hesperia comma* (Hesperiidae; results from Fenberg *et al*., 2016). Yes (Y) and no (N) indicates whether males (M) and females (F) of each species follow the stage-specific trends (second column) appearing in the linear models (Table 4i-iii) for the effect of temperature on adult size. The influence of temperature on adult size during the pupal stage in *Polyommatus bellargus* could not be assessed due to an overlap in timing with the final larval instar.

Life stage	**Effect of increasing temperature on size**	***P. bellargus* (G1)**		***P. coridon***		***P. argus***		***H. comma***	
		M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Early instar larvae	Decrease in size	Y	Y	Y	Y	Y	N	Y	N
Late instar larvae	Increase in size	Y	Y	Y	N	Y	N	Y	N
Pupae	Decrease in size	?	?	Y	Y	N	N	N	N

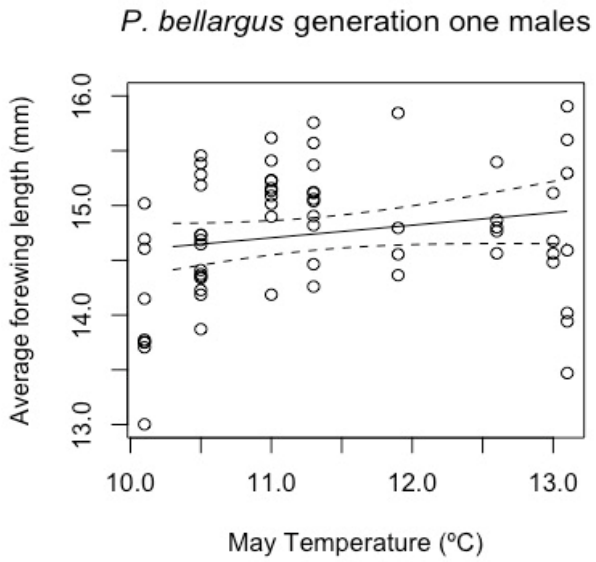

Figure 2.3 Plot of average forewing length (mm) versus May temperature for generation one *P. bellargus* males. Circles represent individual specimens, the solid line is the predicted values given by the linear model, and the dashed lines represent two standard errors above and below the predicted values. Note, the increasing trend during years covering cool to moderate May temperatures (10-11.5°C).

2.5 Discussion

Many insect species have seasonally complex life cycles (Kingsolver *et al.*, 2011). As a result, individuals at different stages within each cycle can respond to seasonal temperatures in different ways; either by varying growth rates (during the larval phase), emergence time (during the pupal stage), and/or dispersal distances (during the adult stage; Fenberg *et al*., 2016). Moreover, responses to temperature can vary by sex, and, for those species with more than one generation per year, by generation. Although rarely studied, untangling how and why species with complex life cycles respond to seasonal temperature during each life cycle stage requires an approach that takes into consideration multiple ecological and life history factors. Here, I have taken such an approach by exploring one facet of temperature responses (body size changes) in three species with complex life cycles.

When data were analysed with no consideration of sex or generation and only using one temperature measure for each year (annual or spring-summer averages), there was no significant relationship between size and temperature for the bivoltine species, *P. bellargus*. This is consistent with results shown in Horne *et al*. (2015). In contrast to those results, however, the

univoltine species studied here did not increase in size when compared to a single temperature value; *P. argus* showed no response to temperature and *P. coridon* decreased in size with increasing temperature. Often these annual and seasonal values may be the only climate data available but they are biologically less meaningful than using the growth period monthly averages for short-lived species (such as those studied here). As I show, the temperature-size relationship of an organism can also vary between and within life stages, which would not be detected unless temperature records for the appropriate months are included in analyses. This is important, since any significant effect obtained with annual or seasonal temperatures could result from a correlation with temperatures during one or more months during the life cycle, in which case the full complexity of the response to temperature will be missed. Furthermore, for species with more than one generation in a year, each generation may experience different environmental conditions (e.g. climate and food quality and quantity), which can differentially affect size between generations (Horne, Hirst and Atkinson, 2017). If generational differences are not taken into account (e.g. by averaging wing length of all individuals regardless of generation), or if there is a bias towards a particular generation in the data set, especially if that generation is not responsive to temperature, any effects of temperature on size may be masked.

When growth period monthly temperatures, sex and generation are included separately in the models for the three species, there is a significant relationship between size and temperature, apart from female *P. argus* and generation two *P. bellargus*, which showed no response to temperature. While there are varying responses within a species between males and females, generations and larval stages, temperatures during the late larval stages were generally found to be the most important for predicting adult size. This is consistent with previous results for other butterfly species (Fenberg *et al.*, 2016; MacLean, Kingsolver and Buckley, 2016).

Although temperatures during late and final larval stages are most predictive of adult size, I also find a general trend of decreasing adult size with increasing temperature during early larval stages (in line with the TSR). Previous research on the univoltine butterfly species *Hesperia comma,* also found changes in male size with temperature which followed these trends (Table 2.5; Fenberg *et al.*, 2016). For *P. coridon,* there is also a decrease in adult size when temperatures increase during the pupal stage. It was not possible to test the influence of temperature on the pupal stage in *P. bellargus* as some specimens may be in the pupal stage during May and others may be in the late larval stage. To a lesser extent, this may also be the case in *P. coridon* and *P. argus*. A decrease in size with increasing temperature during early larval stages may be due to a trade-off between using energy for growth and energy for producing secretions to attract ants, which are more active during warmer conditions (Thomas and Lewington, 2014). Another possible explanation is that ecdysis (moulting) takes place more slowly and at a higher metabolic cost at

lower temperatures (which is more likely earlier in the season), and therefore, early stage larvae in cool conditions take in more food over a longer period at a higher efficiency, and are able to grow to a larger size during early development (Karl and Fischer, 2008).

The increasing size in males following increasing temperature (i.e. showing reverse TSR) during late larval stages of each species studied here (and *H. comma*; Fenberg *et al.*, 2016) may be the result of reproductive pressure. Males that emerge earlier have a competitive advantage for access to females. At cooler temperatures there is a trade-off between reaching a large size and emerging earlier. Therefore, males tend to be smaller during years when cooler conditions coincide with the late larval stages (Hirst, Horne and Atkinson, 2015). Males must also actively compete with other males for females (in *P. bellargus* males swarm around freshly emerged females; Thomas and Lewington, 2014), so large males are at an advantage. However, emerging in time to compete for females may be more important for males than growing to a larger size, hence they tend to be smaller in years with lower temperatures during late larval stages (e.g. Fig. 2.3). For females, only *P. bellargus* generation one showed an increase in size following an increase in temperature during late larval stages. In bivoltine (and multivoltine) species, females in the first generation may be under time-pressure to emerge and lay eggs. Therefore, in cooler years, when growth is slower, females emerge at a smaller size.

Conversely, in univoltine species, females are under less time pressure to develop so may prioritise growth, to increase fecundity, and therefore female size may be less affected by temperature. Nevertheless, for female *P. coridon*, my results showed a decrease in size with increasing May temperatures, although it was smaller than the decrease in size shown by the males. May is towards the end of the period occupied by early larval stages in this species. A possible explanation is that the relationship with ants starts in May and, therefore, the larvae use some energy for producing the secretions that are attractive to ants, and this activity increases under warmer conditions (Thomas and Lewington, 2014). This may also be the reason for the decrease in adult size when higher temperatures occur during the pupal stage, because the pupa of this species also produces secretions and sounds to attract ants (Thomas and Lewington, 2014). This contrasts with results in a study of the univoltine butterfly *Anthocharis cardamines*, which is not associated with ants in the larval or pupal stages, which found that an increase in adult body size was correlated with temperature increases during the pupal stage of development, and that both sexes responded in the same way (Davies, 2019).

My findings suggest that a reversal of the TSR occurs only during late larval instars for males in univoltine species and in both sexes in the first generation of bivoltine species. It is likely that the second generation of bivoltine species (and any further generations in multivoltine species) do

not respond to temperature by changing size due to the limited time available for growth and conditions being more favourable for growth later in the season. For example, in second generation *P. bellargus*, the larval foodplant covers a larger area, the plants are taller, and the larvae are not restricted to the warmest short vetches (Thomas and Lewington, 2014). This is reflected in the greater abundance of individuals in generation two in the museum collections and in population monitoring data (Thomas, 1983; Thomas and Lewington, 2014; UKBMS, 2018). A phenological study of British butterfly species found that in warmer years, both generations one and two of *P. bellargus* emerge earlier than in cooler years (Brooks *et al.*, 2017). Therefore, it is possible that any increase in growth rate due to increased temperature is counteracted by an earlier emergence time leading to no overall change in average size between years for generation two.

In contrast to my results, a meta-analysis of a large number of arthropod species found that, in general, there was no difference in the strength and direction of the temperature-size responses in males and females of the same species, and therefore, SSD did not change with temperature (Hirst, Horne and Atkinson, 2015). Yet in agreement with other previous studies, my results show that temperature can have variable effects on the sizes of each sex, resulting in a shift away or towards SSD (Høye *et al.*, 2009; Fenberg *et al.*, 2016). Unlike most butterfly species, in which females are the larger sex and have a longer development time (Wiklund and Kaitala, 1995; Teder, 2014), the males in all three of my study species are larger, despite males emerging earlier than females (Thomas, 1985). Therefore, an increase in female size (as the smaller sex) with increasing temperature was predicted. Yet, for *P. argus* and *P. coridon*, males increased in size with increasing temperatures during the late larval stages, while the females did not (Table 2.5). A similar trend was found in *H. comma* (Table 2.5; Fenberg *et al.*, 2016), despite males being smaller in that species, which suggests that sex-specific life history constraints are more important than the direction of SSD for predicting which of the sexes will respond to temperature. Furthermore, for generation one *P. bellargus* the percentage increases in male size with increasing April and May temperatures are larger than the increase in female size with increasing May temperatures. On the other hand, in *P. argus* and *H. comma*, males decreased in size as temperature increased during the early larval stages, whereas females did not (Table 2.5; Fenberg *et al*., 2016). Clearly, the sizes of both sexes can respond differently to temperature, which will have variable effects on SSD. This will partly depend on which months during the life cycle have temperatures that are higher or lower than average, and on the initial strength and direction of the SSD. As a future response to climate warming (when all months during larval stages are predicted to be warmer), if the growth rate during late larval stages is greater than the growth

rate during early larval stages, SSD will become increasingly biased towards males, at least among four British butterfly species (including *H. comma*; Fenberg *et al*., 2016).

As my data were derived from museum specimens, not all ecological and life history traits could be considered in my analyses. An earlier study of the relationship between size and traits of multiple butterfly species found low (but significant) correlations between adult body size and several life history traits, including the proportion of the year adults are present, larval development time and type of foodplant (Garcia-Barros, 2000). *Plebejus argus,* for example, has some flexibility in food and habitat preference, which may be why the temperature-size responses in this species are not as strong as those of *P. bellargus* and *P. coridon*. If quality or quantity of food plant species were affected by changes in temperature or other environmental conditions, this would be less problematic for *P. argus*, which could switch plants and therefore show a weaker size response to temperature. I was also unable to investigate how the relationship between butterfly larvae and the ant species may have been affected by temperature, which may have led to indirect impacts on adult butterfly size. *Plebejus argus* has the closest relationship with ants of the three species and density of *P. argus* populations has been shown to positively correlate with ant nest density (Ravenscroft, 1990). An additional factor to consider is the life cycle of the species. *Polyommatus bellargus* for example, overwinters as a larvae whereas *P. argus* and *P. coridon* overwinter as eggs (Thomas and Lewington, 2014). Yet, they are all direct developers (i.e. there is no pupal diapause) and therefore may be subject to more seasonal time constraints and variation than species which diapause in the pupal phase, such as *A. cardamines* (Davies, 2019). This may explain why all three lycaenid species I have studied, and *H. comma*, respond to temperature in the larval stage (Table 2.5; Fenberg *et al.*, 2016) but the pierid butterfly *A. cardamines*, which overwinters in the pupal stage, instead responds to temperature in the pupal stage (Davies, 2019).

Finally, I note that, while climate change is resulting in warmer mean day-time air temperatures and the earlier emergence of many butterfly species, my study does not take into account the effect of night-time temperatures or temperatures at ground level on size. Advances in phenology may result in certain life stages (particularly early larval stages) being exposed to shorter days, so that, although day-time temperature is higher, these stages will be exposed to cooler night-time temperatures for longer. This is particularly relevant for *P. coridon* and *P. argus*, which are nocturnally active (Thomas and Lewington, 2014). Yet, it is likely that ground temperatures, especially around the larval host plants, will increase even during the night if day-time temperatures are higher, which is probably why these two species show the same general temperature-size trends as *P. bellargus*, which has diurnal larvae.

2.5.1 Conclusions

Temperature-size responses can vary according to life cycle factors (e.g. voltinism and life history stage) and sex. If these factors are not included in analyses, adult size of a species may not appear responsive, especially when compared to annual or seasonal temperatures. However, a temperature-size response can emerge if studies separately analyse sex, generation, and life history stage with monthly temperatures during growth. While the species studied here all responded in some way to temperatures during immature stage development, the responses varied in strength and direction. Generation one male and female *P. bellargus* and males of *P. coridon* and *P. argus* responded in a similar way to previously studied univoltine species (Horne, Hirst and Atkinson, 2015; Fenberg *et al.*, 2016), and became larger in years with warmer temperatures during late larval stages and, therefore, showed the reverse of the TSR. Conversely, adult body size decreased in size with increasing temperature during early larval instar development and, in *P. coridon*, during pupal development, in line with the TSR. This suggests that, within a species, the temperature-size response can change direction as the immature stages progress (Table 2.5). Furthermore, these results suggest that for species which follow this trend, there will likely be an increase in SSD due to climate warming, if increases in size during late larval instars are greater than decreases in size during early larval instars. This not only highlights the importance of integrating life history factors into temperature-size response analyses, but also emphasises that size declines in response to climate warming are not "universal" and that the TSR is too simplistic, especially for species with complex life cycles.

While not all aspects of ecology can be included directly in a study using museum specimens, these collections can provide useful insights into the responses of organisms to temperature change in the recent historical past. These data, when used in combination with monthly temperature records, can unravel the complex interactions between individuals and some of the factors that control body size. Furthermore, museum specimens provided the best opportunity to study the size response of these species to a wide range of temperatures as field studies would require continuous monitoring over many years and laboratory experiments would be hard to conduct due to the strong relationship between the juvenile stages of these species and ants.

Future studies examining temperature-size responses for species with complex life histories should be aware that the strength and direction of temperature effects may vary according to growth stage, sex, and generation. Where possible, studies using natural history collections can be used in combination with field studies and laboratory experiments to give a more holistic

approach to studying temperature-size responses and predicting how these responses may be affected by climate change.

Chapter 3 Temperature-size responses in British butterflies using computer vision applied to natural history collections

This chapter is currently under revision in the Journal of Animal Ecology: Wilson, R. J., Brooks, S. J., Van der Walt S.J., Feng D., Price B.W. and Fenberg, P. B. (2020) 'Temperature-size responses in British Lepidoptera using computer vision applied to natural history collections', *Journal of Animal Ecology. [in review]*

3.1 Abstract

Reduction in body size was thought to be a common response to climate warming among ectotherms, but recent studies suggest that the strength and direction of response is partly a function of individual species traits and phylogenetic relationships. In fact, some butterfly species have been shown to increase in size with increasing temperature, and in some cases, show varying responses to temperature between developmental stages, sex, voltinism, habitat type and family. Most of this research uses manual measurements of museum specimens, but the number of specimens and species that can be analysed is time limited. Here, I use a newly developed computer vision pipeline to automatically measure wing lengths, and to determine what factors affect the adult body size response of species to temperature variation during the immature stages. The pipeline automatically measured wing lengths of 39,111 digitised museum specimens of 21 British butterfly species. The resulting measurements were then used to analyse temperature-size responses within the early larval, late larval and pupal stages of each species (for 14,381 specimens with a corresponding date and location). I also used multi-species analyses to compare temperature-size responses between species from different families, habitats, overwintering stages, and average sizes. The results show that, for most species, adult size increases during years with warm temperatures during the late larval stage. For some species, there were also changes in adult size with increasing temperatures during the early larval and pupal stages, but these varied in strength and direction, with many showing no change at all. Additionally, where males and females were analysed separately, the sexes often showed different responses to temperature. Multi-species analyses revealed that taxonomic family

explained the highest proportion of variance in species responses to temperature, and there were also some differences between species from different habitat types. These results highlight the importance of considering the temperature-size responses of species separately for each life stage, and for each sex. The use of automated technology has allowed us to analyse a large number of species relatively quickly.

3.2 Introduction

Three major ecological responses to climate warming have been identified: changes in phenology, geographic range and body size (Gardner *et al.*, 2011). For body size, it is thought that most species follow the temperature-size rule (TSR) by decreasing in size with increasing temperature (Gardner *et al.*, 2011; Sheridan and Bickford, 2011; Ohlberger, 2013). In reality, this is not always the case; in fact, species can show varying responses to temperature by decreasing or increasing in size, or showing no response (Horne, Hirst and Atkinson, 2015; Tseng *et al.*, 2018; Wilson, Brooks and Fenberg, 2019; Wonglersak *et al.*, 2020). This is especially true for insects (Horne, Hirst and Atkinson, 2015; Tseng *et al.*, 2018; Wonglersak *et al.*, 2020) which, due to their complex and diverse life cycles, can lead to a variety of temperature-size responses. For example, each life stage of holometabolous insects can experience different environmental conditions, which may cause each stage to respond in a different way to temperature (Forster, Hirst and Atkinson, 2011; Kingsolver *et al.*, 2011; Fenberg *et al.*, 2016; Wilson, Brooks and Fenberg, 2019). Thus, it is important that life stages, and the environmental conditions experienced by them, are considered separately where possible when investigating a species' response to temperature.

Lepidoptera from temperate climates are a useful study taxon for this type of research as their life stages are clearly defined and experience a range of conditions across seasons. Species generally have relatively short generation times, with most species having at least one generation per year. If adult body size measurements are paired with historic temperature records across multiple generations and years, then it is possible to determine the direction and strength of adult body size responses to temperature. For example, two Arctic butterfly species (*Boloria chariclea* and *Colias hecla*) decreased in size with increasing summer temperatures between 1996 and 2013 (Bowden *et al.*, 2015). In contrast, *Anthocharis cardamines* increased in adult size with increasing temperatures at the start and end of the pupal stage between 2004 and 2017 (Davies, 2019). While these studies focused on species in their natural environment over multiple generations, the temporal scope is limited to less than two decades.

In the absence of data from long-term field studies, natural history collections (NHCs) paired with temperature records can provide a useful resource for studying temperature-size responses. This is because NHCs often span many decades, over which a large range of inter- and intra-annual (e.g. seasonal) temperature records may be available. In recent years, the use of NHCs to study temperature-size responses in insects has become increasingly common, but responses may vary among taxa (Baar *et al.*, 2018; Tseng *et al.*, 2018; Polidori *et al.*, 2020). For example, the body sizes of British Zygoptera (damselflies) are more sensitive to temperature than Anisoptera (dragonflies) (Upton *et al.*, 2016; Christou, Brooks and Price, 2017; Wonglersak *et al.*, 2020). This suggests that, at least in some insect groups, the phylogenetic relationships among species are also an important predictor of the direction and magnitude of temperature-size responses. Butterflies, for example, often increase in adult size with increasing temperatures. *Colias meadii* had an 11% increase in size over a 60 year period, with temperatures during the developmental period being the most important predictor of wing length (MacLean, Kingsolver and Buckley, 2016). In addition, analysis of four UK butterfly species found that response to temperature varies according to life stage, but adult size in all four species increased when temperature increased during the late larval stage. However, some species showed decreases in adult size in relation to increasing temperature during early larval and pupal stages (Fenberg *et al.*, 2016; Wilson, Brooks and Fenberg, 2019). But in order to determine if these are general responses, more species and specimens need to be analysed.

Digitised collections of NHCs are becoming widespread and will be particularly useful for research into biotic response to climate change, such as temperature-size responses (Johnson *et al.*, 2011). Insect specimens are often fragile and can be damaged if manual measurements of physical specimens are required. Measuring specimens from images is more practical, and has often been done using software such as ImageJ to measure morphological features manually (Fenberg *et al.*, 2016; Christou, Brooks and Price, 2017; Baar *et al.*, 2018; Wilson, Brooks and Fenberg, 2019). NHCs, which have already been digitised, such as the NHM iCollections (Paterson *et al.*, 2016), can provide valuable resources for this type of research. However, there are time-constraints to the number of species and specimens that can be analysed manually. In order to make better use of the huge numbers of specimens available, ecologists need to capitalise on recent advances in computer vision, which enable specimens to be measured automatically.

Here, I use a newly developed software pipeline to automatically measure the wing lengths of multiple British butterfly species. First, I test the software, which was specifically developed for measuring forewing lengths of butterflies, by comparing the results to manual measurements of seven species. Once validated, I use the software to measure wing lengths of 21 species of butterflies from the NHM iCollections digitisation project. The results are paired with monthly

temperature records experienced by the immature stages to determine which months are most important for predicting adult size, and the percentage changes in size for each species. Data are also combined with the same measurements from previously published results (Wilson, Brooks and Fenberg, 2019). I hypothesise that univoltine species (and first generations of bivoltine and multivoltine species) will increase in size as adults with increasing temperatures during the late larval stages, and that males and females will respond differently. This was the case for the four British butterflies studied in Fenberg *et al.* (2016) and Wilson, Brooks and Fenberg (2019), where an increase in adult size with increasing temperature in the late larval stage occurred in all four species, but responses varied between sexes and generations. Previous research has also shown that increasing temperatures during the early larval stage causes some species to become smaller as adults (Fenberg *et al.*, 2016; Wilson, Brooks and Fenberg, 2019). Response to temperature during the pupal stages varies (Davies, 2019; Wilson, Brooks and Fenberg, 2019). I therefore hypothesise that there will be a temperature-size response during the early larval and pupal stages for some species, but the direction of these responses may vary. Using a multi-species meta-analysis, I hypothesise that temperatures during the late larval stage will be significant, and sex will be an important factor in determining adult wing length. I also predict that family will be an important factor given recent studies in other insect taxa (Wonglersak *et al.*, 2020).

3.3 Material and Methods

3.3.1 Pipeline building and testing

The “butterfly wings” software pipeline (Feng *et al*., 2020) is written in python with the following dependencies: Matplotlib (Hunter, 2007), scikit-image (van der Walt *et al*., 2014), opencv-python (PyPI, 2020a), numpy (van der Walt, Colbert and Varoquaux, 2011), scipy (Virtanen *et al*., 2020), pandas (PyData, 2020), pytest-timeout (PyPI, 2020b), and joblib (Joblib, 2020).

The pipeline is divided into three stages: (1) scale detection, (2) butterfly location, and (3) tracing & measurement, and relies on similar positioning of elements across images to identify three parts: a ruler, a butterfly, and specimen labels (an example is given in Appendix A).

Scale detection: to measure butterfly attributes, a link needed to be established between image scale (in pixels) and world scale (millimeters). The bottom third of the image was searched for a ruler and, after removing numbers, use the Fourier transform to determine the frequency of tick marks. Butterfly location: an accurate binary mask is calculated to provide the butterfly outline. Then the foreground was separated from the background using thresholding in HSV space

or graph cuts (depending on species). Tracing and measurement: the butterfly mask is split in to two halves, and each half is analyzed separately. The antenna is then digitally removed. Then, the wing tip is defined as the pixel closest to the corner of the bounding box. The background pixel furthest from that same corner is the shoulder. Measurements are made by calculating pixel distance, and then applying the scale found in the first step. An overview of the pipeline is provided on the software repository (https://github.com/machine-shop/butterfly-wings).

3.3.2 Comparison to manual measurements

The absolute differences between manual and automated measurements were calculated for specimens previously measured manually: *Hesperia comma* (463 specimens, Fenberg *et al*., 2016), *Polyommatus bellargus*, *Polyommatus coridon* and *Plebejus argus* (790, 445 and 490 specimens respectively; Wilson, Brooks and Fenberg, 2019). In addition 100 specimens each of *Apatura iris*, *Favonius quercus* and *Papilio machaon* were measured both automatically and manually (Wilson *et al*., 2020) following the methods outlined in Wilson, Brooks and Fenberg (2019). Correlations between the automated and manual measurements were conducted both before and after outliers were removed.

3.3.3 Species selection

In order to determine which species worked best using the current pipeline, a subset of 10 or more specimens from each of the 66 butterfly species digitized in the NHM iCollections project were measured automatically. I then compared the absolute differences between left and right forewings for each specimen and took the average difference for each species. If this average was higher than 2 mm, I removed the species from my study because previous work using manual measurements showed that 2 mm was the maximum difference found between left and right forewing lengths (Fenberg *et al*., 2016; Wilson, Brooks and Fenberg, 2019). This, however, would not necessarily account for cases where the pipeline had measured both wings incorrectly, and therefore, the output images (showing the points where the measurements were taken from) of retained species were inspected to check if the pipeline had measured the specimens in the correct place (see Figure A.1 in Appendix A). Following this test, 21 species were selected as suitable to be measured using the current pipeline. Where a species had multiple sub-species, these were grouped together for all subsequent analyses. The selected species were distributed between four families and a range of habitats and overwintering stages (see Table A.1 in Appendix A).

3.3.4 Removing outliers

The output images for all specimens were checked manually for error in measurements (e.g. the points of measurement were not on the wing tip or point of attachment to the thorax). It was also noted which specimens were pinned ventrally rather than dorsally and, for species where there was a clear difference in wing pattern or colour between the sexes, it was noted which were male or female. Before the data were analysed, specimens were removed where measurements were clearly incorrect (i.e. outliers in terms of the expected size for the species), or where there was a large discrepancy (more than 2 mm in species with forewing lengths of 10-20 mm, and scaled-up for larger species) following Wilson, Brooks and Fenberg (2019; chapter 2).

3.3.5 Individual species analyses

For each species, only specimens with a location and year of collection were analysed. Where applicable, specimens were separated into generations. If a species had a partial second generation, or a variable number of generations from year to year, some specimens were removed to keep the number of generations per year consistent within a species. For example, *Aglais urticae* has one generation in Scotland and two generations a year in other parts of the UK, so Scottish specimens were removed. Additionally, when there were specimens with dates of collection outside the expected range of adult flight season for that species (based on Thomas and Lewington (2014)), these specimens were also removed. For *Pararge aegeria*, one sub-species (*P. aegeria oblita*) is known to be larger than the other sub-species (Thomas and Lewington, 2014), and therefore, this sub-species was removed. Generally, specimens of a species were not analysed if there were fewer than three specimens available per year (and sex where applicable). Information about the life cycles of each species given in Thomas and Lewington (2014) was used to determine which monthly temperatures were appropriate for analyses. Temperatures from months when species were in the early larval, late larval and pupal stages were added to the data set; winter months were not used as growth would be limited during this time. Mean monthly temperature data from the Central England Temperature Record (CET; obtained from https://www.metoffice.gov.uk/hadobs/hadcet/) were used in all analyses.

Following Fenberg *et al.* (2016) and Wilson, Brooks and Fenberg (2019; chapter 2), wing length was compared to average monthly temperatures using multiple linear regression analyses. R statistical packages MASS and MuMMIn were used to run stepwise regression in both directions to select variables for the final model and information theoretic (IT) model selection with model averaging based on Akaike Information Criterion (AIC). This was not possible for *Nymphalis polychloros* as there were insufficient specimens with a year and location to analyse. For the

remaining 20 species, multiple linear regressions were run using average forewing lengths for specimens which were pinned dorsally. In each case, adult size was compared to the average monthly temperatures covering the early larval, late larval and pupal stages for that species to determine if the temperatures experienced during the immature stages affect the adult size.

Where applicable, males and females, and generations one and two were run in separate models. For *P. aegeria*, it was only possible to run models for generations one and two (of three). Furthermore, this species can overwinter in the larval or pupal stage so it was not possible to tell if temperature-size responses were related to temperatures during the larval or pupal stage. However, the models were not significant for this species and therefore it was excluded from further analyses. Including the three Lycaenidae (*P. bellargus, P. coridon* and *P. argus*) analysed in Wilson, Brooks and Fenberg (2019), a total of 22 species were included in the final analysis. In 12 species, males and females could be identified by eye from the images, and two species had two generations that could be analysed separately, giving a total of 37 models. For each species with a significant model, the percentage change in adult size per °C was calculated for the most significant month in early larval, late larval and pupal stages. Where there was not a significant variable for a particular life stage, the most important non-significant variable was used. Percentage changes were calculated from slopes of the natural log of average forewing length versus temperature: $((\exp^{(slope)}-1) \times 100)$.

3.3.6 Multi-species analysis

The data were compiled for all species in two ways. Firstly, the percentage change in adult size per °C of the three immature stages were compiled for each species and, where applicable, each sex and generation. Secondly, the natural log of average forewing lengths for all specimens used in the individual analyses were compiled. Natural logs were used to allow for species of different sizes to be compared without the effects of scaling. Temperature data from the most important month for predicting adult size during each immature stage were used for multi-species analyses. Four other variables (family, habitat, size category, overwintering stage) were included in the multi-species analyses in the form of multi-level factors (Table 3.1) to determine which may affect the strength and direction of temperature-size responses. These four variables were selected *a priori* as likely having an influence on temperature-size response based on previous research (Fenberg *et al.*, 2016; Tseng *et al.*, 2018; Davies, 2019; Wilson, Brooks and Fenberg, 2019; Wonglersak *et al.*, 2020).

The percentage change in adult size per °C in temperature increase during each of the three immature stages were compared between the four variables (Table 3.1). Linear mixed effects

models were run using the natural log of average forewing lengths of specimens from all 22 species, with temperature during the early larval, late larval and pupal stages as fixed effects. A mixture of random effects (family, overwintering stage, habitat and size category) were also used in each model. The models were used to determine if there were relationships between the average forewing lengths and the fixed and random effects. ANOVAs and AIC values were used to determine which model gave the best fit. This was repeated using only species where sex of the specimens could be determined, with sex included as a random factor.

Table 3.1 Factors used in multi-species analysis and their levels

Factor	Number of levels	Name of levels	Notes
Family	4	Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae	There was only one species of Papilionidae, which did not have a significant model
Overwinter stage	4	Egg, larva, pupa, adult	
Habitat type	4	Grass, wood, any, other	Grass=Grassland, Wood=Woodland, Any=species that live anywhere, Other=species that don't fit into the other categories. No species in 'any' had significant models
Size category	3	Small, Medium, Large	Based on average forewing lengths: Small=≥10mm-20mm, Medium=>20-30mm, Large=>30-40mm

3.4 Results

3.4.1 Pipeline testing

For *Hesperia comma*, *Favonius quercus, Apatura iris* and *Papilio machaon* the majority of automated measurements were within 1 or 2 mm of the manual measurements (Table 3.2, Fig 3.1). The majority of manual measurements were larger than the automated measurements for *H. comma*, *A. iris* and *P. machaon* (96%, 96% and 86% respectively), and the reverse was true for *F. quercus* (90%). For all four species, the average difference in measurements was less than 1 mm when outliers were removed (Table 3.2). There were significant positive correlations between

automated and manual forewing measurements for all species, and strength of correlations increased when outliers were removed (Table 3.2, Fig 3.1).

For *Polyommatus coridon*, *Plebejus argus* and *Polyommatus bellargus*, the percentages of automated measurements within 0.5 and 1.0 mm of the manual measurements was low (Table 3.2), and there were a large number of specimens with more than 2 mm difference in the measurements; 167 (38%), 249 (51%) and 503 (64%) specimens respectively. In all three species, the automated measurements were on average larger than the manual measurements in at least 75% of specimens, with average differences of 2.25 mm for *P. coridon*, 2.22 mm for *P. argus* and 3.33 mm for *P. bellargus* (see Figure A.3 in Appendix A). The current pipeline was therefore not accurate in measuring these species, so the manual measurements from Wilson, Brooks and Fenberg (2019) were used in further analyses.

Table 3.2 Comparisons between automated and manual measurements of seven species. Correlations before and after outliers were removed, the percentage of automated measurements that were +/- 0.5 mm and +/- 1 mm* different from manual measurements, and the average absolute difference in automated and manual measurements once outliers were removed. *For *A. iris* and *P. machaon*, this was +/- 1mm and +/- 2mm as these species have a larger average size. Outliers were not removed for *P. coridon*, *P. bellargus* and *P. argus*; NS=Not significant.

Species	With outliers					Without outliers			
	Correlation			Percentage of specimens within 0.5 or 1 mm of manual measurements		Correlation			Average absolute difference (mm)
	All	Male	Female	0.5 mm	1 mm	All	Male	Female	
H. comma	0.76	0.76	0.57	72	98	0.97	0.93	0.96	0.39
F. quercus	0.86	0.76	0.57	66	93	0.92	0.91	0.94	0.47
A. iris	0.39	NS	NS	53 *	86 *	0.97	0.94	0.95	0.96
P. machaon	0.54	NA	NA	61 *	78 *	0.95	NA	NA	0.92
P. coridon	0.61	0.72	0.64	46	50	NA	NA	NA	NA
P. argus	0.45	0.31	0.41	24	33	NA	NA	NA	NA
P. bellargus	0.29	0.50	0.19	22	27	NA	NA	NA	NA

3.4.2 Individual species analyses

The total number of specimens analysed per species is given in Table 3.3, along with the number of specimens measured by the pipeline, and the percentage of specimens left after the outliers were removed. On a standard desktop computer (an iMac with a 3.5 GHz Intel Core i7 processor) images took approximately 14 seconds to go through the pipeline. When average forewing lengths were compared to monthly temperatures using multiple linear regression models, 21 of the 37 models were significant. This accounted for 17 of the 22 species analysed; the five species with no significant models were *Melitaea cinxia, F. quercus, Aglais io, Thecla betulae* and *P. machaon*. In all but three of the significant models, an increase in adult size with increasing temperature during the late larval stage was significant; the three exceptions were *Argynnis paphia* females, *Thymelicus lineola* males and *P. coridon* females. The responses of adult size to temperatures experienced during the early larval and pupal stages were less consistent. Only nine of the 21 models had a significant change in adult size in relation to changes in temperature during the early larval stage and 10 models had significant changes in adult size in relation to changes in temperature in the pupal stage, with both having a mix of increases and decreases in size with increasing temperatures. The percentage changes in adult size per °C increase during each immature stage are given in Table 3.4, and detailed individual model results can be found in the supplementary information.

3.4.3 Multi-species analyses

The influence of temperature during the immature stages on percentage changes in adult size for each species were compared in two ways: using percentage change in size from all species, and using only those with significant individual models (see Table 3.4). There was little difference in the results between the two methods and, therefore, the results presented here are for species with significant models only unless stated otherwise. The correlation between average size of a species and percentage change in size with temperature during early larval ($t=-0.206$, $df=19$, $p=0.839$; Fig 3.2i) and late larval ($t=0.135$, $df=19$, $p=0.894$; Fig 3.2ii) stages were not significant. There was, however, a marginally significant correlation between average size and percentage change in size for temperatures during the pupal stage ($t=0.765$, $df=17$, $p=0.0455$, correlation=0.182; Fig 3.2iii). There was no significant difference in the mean percentage change in adult size between species in the four different size categories or between species that overwintered in different life history stages ($p>0.05$). There were no significant differences in mean percentage change in adult size between species occurring in different habitat types when using significant models only. However, there were marginally significant differences in the mean percentage change in adult size in response to temperatures during the pupal stage between

species occurring in a woodland habitat and those in grass habitats (p=0.0455) when all species were included (F=3.027, df=3 and 31, p=0.0442; Fig 3.3).

Table 3.3 The number of specimens measured and analysed for 21 species of British butterflies in the iCollections at the NHM

Species	Total number of specimens	Number of outliers or errors	Percentage remaining after outliers removed	Number of specimens in ventral view	Number of specimens with a location and year	Number of specimens used in individual species models
Aglais io	1326	242	81.7	90	574	399
Apatura iris	569	49	91.4	110	180	130
Argynnis paphia	2217	359	83.8	336	1692	1158
Aglais urticae	3729	271	92.7	251	1484	774
Euphydryas aurinia	6459	885	86.3	730	2874	2212
Erynnis tages	1233	183	85.2	112	993	664
Fabriciana adippe	1526	170	88.9	423	1151	668
Favonius quercus	1212	158	87.0	272	523	276
Hesperia comma	875	31	96.5	158	497	484
Hipparchia semele	2926	264	91.0	817	2007	1267
Melitaea athalia	2271	286	87.4	571	1473	902
Melitaea cinxia	1878	116	93.8	574	688	384
Nymphalis polychloros	819	115	81.1	86	95	N/A
Ochlodes sylvanus	1283	70	94.5	115	1068	859
Pararge aegeria	3026	444	85.3	604	1698	664
Papilio machaon	833	299	64.1	108	238	94
Pyrgus malvae	1701	330	80.6	154	1297	814
Speyeria aglaja	1899	133	93.0	227	1499	1192
Thecla betulae	1220	133	89.1	240	271	138
Thymelicus lineola	839	25	97.0	88	734	548
Thymelicus sylvestris	1270	131	89.7	113	1008	754
Totals	**39,111**	**4694**	**88.0 (on average)**	**6179**	**22,044**	**14,381**

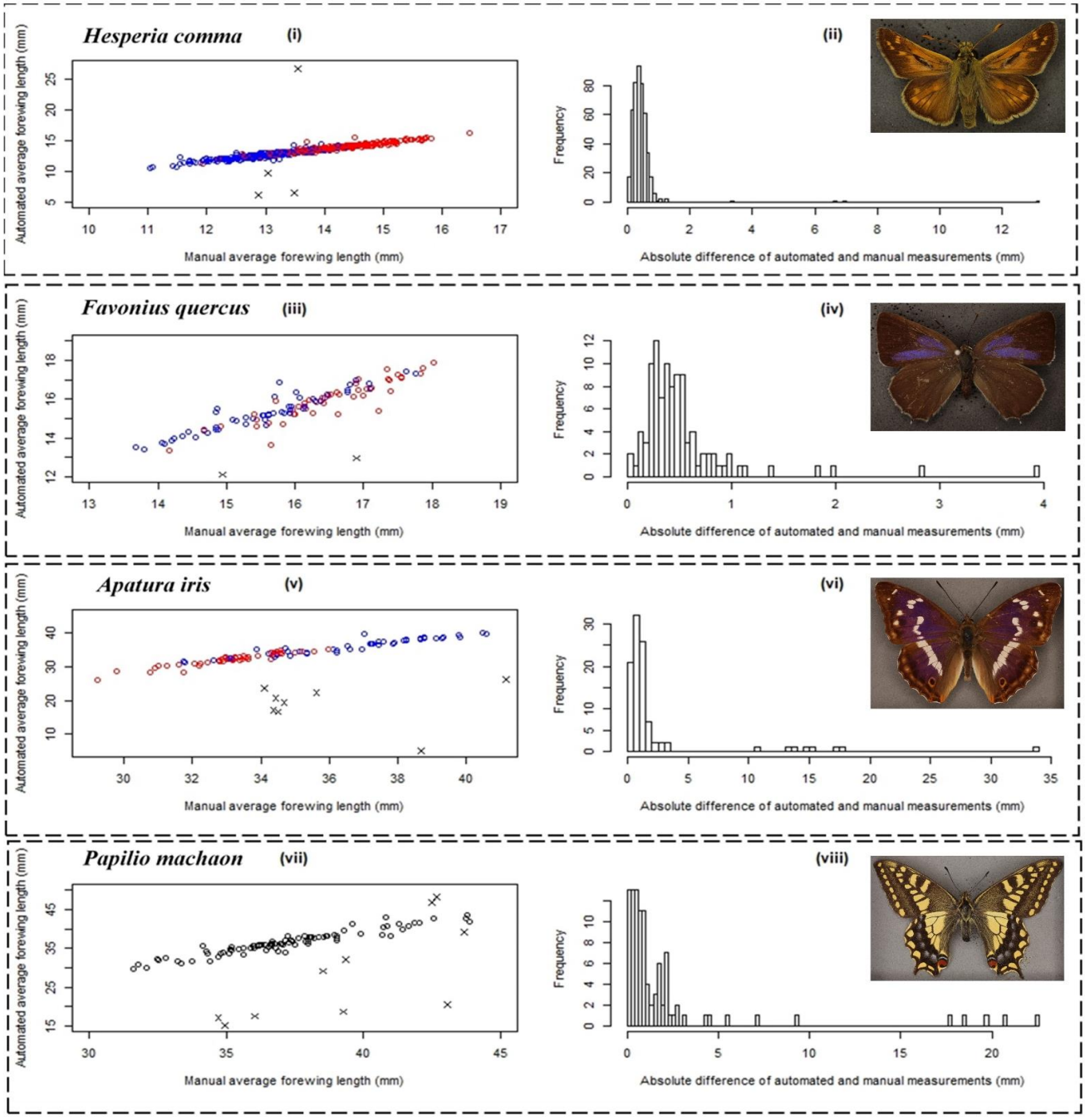

Figure 3.1 Scatter plots of manual versus automated measurements for each individual, and frequency plots of the absolute difference in values between the manual and automated measurements for specimens of i and ii) *Hesperia comma*, iii and iv) *Favonius quercus*, v and vi) *Apatura iris*, and vii and viii) *Papilio machaon*. For the scatter plots, males are indicated by the blue points, and females are indicated by the red points (for *P. machaon* males and females could not be identified). Each plot includes outliers, which are indicated by black crosses.

There was a significant difference in percentage change in adult size according to the three developmental stages ($F=13.12$, $df=2$ and 58, $p<0.001$), with differences between percentage changes per °C in the late larval stage and both the early larval and pupal stages ($p<0.001$; Fig 3.4i). On average, forewing length increased by 0.86% per °C increase in temperature during the late larval stage (SE=0.16), decreased by 0.22% per °C in the early larval stage (SE=0.15), and increased by 0.05% per °C in the pupal stage (SE=0.16). Additionally, there were significant differences in percentage change in adult size per °C temperature increase in the early larval stage between species from different families ($F=18.27$, $df=2$ and 18, $p<0.001$), with differences between the Lycaenidae and both the Hesperiidae and the Nymphalidae (both $p<0.001$). On average, there was a 0.25% increase in average forewing length per °C temperature increase in the early larval stage for the Hesperiidae, a 1.15% decrease in size per °C for the Lycaenidae, and a 0.11% decrease in size per °C in the Nymphalidae (Fig 3.4ii). A two-way ANOVA to test for differences in percentage changes in adult size between stages and families also found a significant interaction between life stage and family ($F=4.627$, $df=4$ and 52, $p=0.00285$, Fig 3.4ii). There is a large difference in responses to temperatures between larval stages for the Lycaenidae; there is on average a large increase in adult size per °C temperature increase in the late larval stage (1.34%), but a large decrease in adult size per °C in the early larval stage (-1.15%; Fig 3.4ii).

For the linear mixed effects models, various models were tested using combinations of factors for the fixed effects, starting with a simple model and becoming more complicated. Family was given priority in selection of factors to include as the analyses above showed there were significant differences in responses between families. The model with the lowest AIC value was Average forewing length (Ln) ~ Early larval temperature + Late larval temperature + Pupal temperature + (1|Family) (AIC=-17250; Table 3.5i). For the species where sex could be determined, the model which was most significant was Average forewing length (Ln) ~ Early larval temperature + Late larval temperature + Pupal temperature + (1|Sex) + (1|Family) (AIC=-11884; Table 3.5ii). In both models (for all species and those which can be sexed), family explained the highest proportion of variance in the results (Tables 3.5i and 3.4ii).

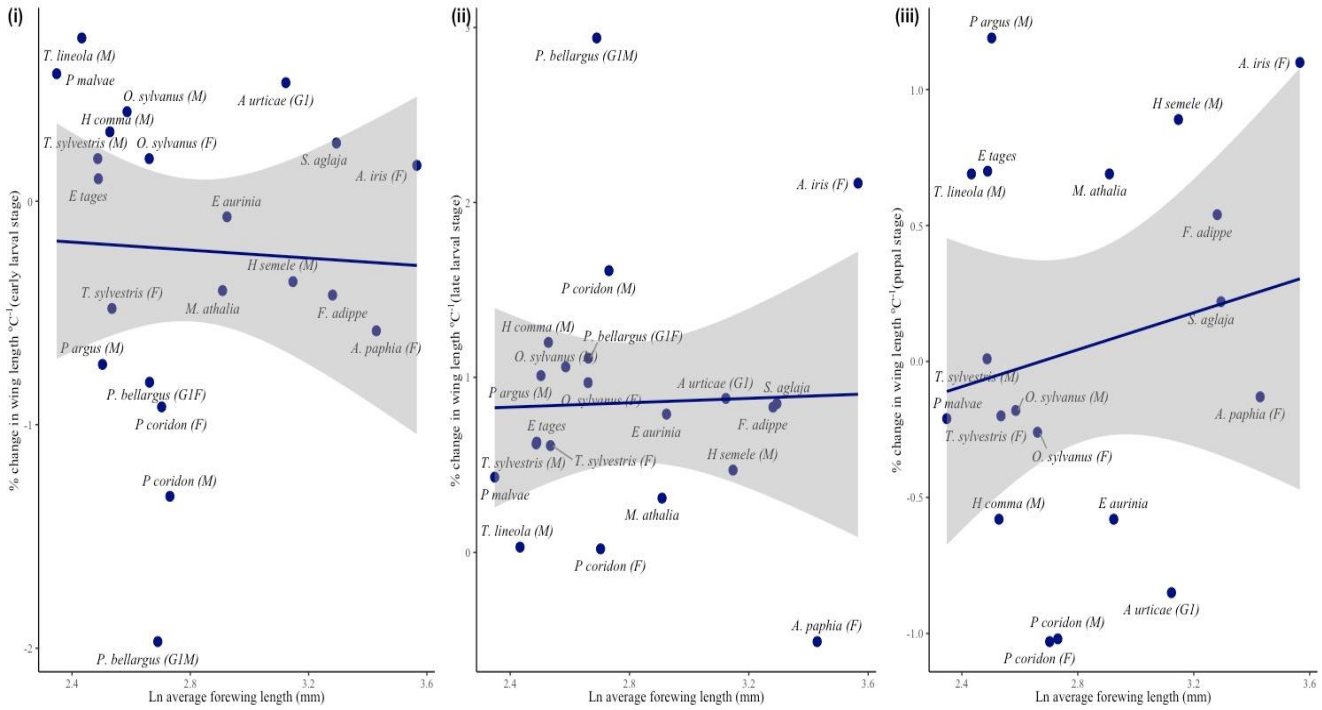

Figure 3.2 Percentage change in adult size per °C versus Ln average size for each species (and sex or generation within species) for temperatures during i) early larval stage, ii) late larval stage, and iii) the pupal stage. Data are from species with significant individual models only.

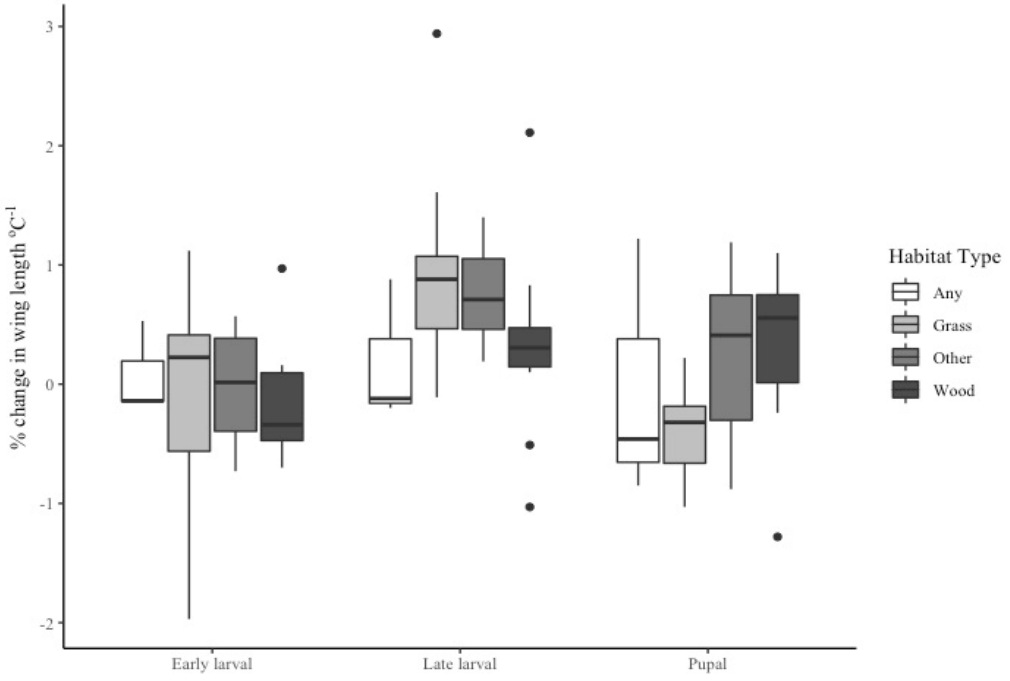

Figure 3.3 Boxplot of percentage change in adult size per °C change during the early larval, late larval and pupal stages for all species grouped by general habitat type. The habitat type "Any" is for species which occur in both habitat types, and "Other" is for species which do not occur in either woodland or grassland.

Table 3.4 Percentage changes in size per °C change in temperature for each developmental stage (early larval, late larval and pupal) for each species (or sex/generation) when the model of average forewing length versus monthly temperatures was significant. Values in bold indicate results which were in line with the predicted results (increase in size in relation to temperature in the late larval stage and decreases in size in relation to the early larval and pupal stages). NS indicates the monthly temperatures during that stage were not significant variables, and NA indicates that the stage was excluded from analysis. The percentage change is based on the slope given by the model for the most significant month in that stage if there was more than one month used per stage in analysis.

Family	**Species**	**Sex**	**Change in size with temperature in Late larval stage (%)**	**Change in size with temperature in Early larval stage (%)**	**Change in size with temperature in Pupal stage (%)**
Hesperiidae	*H. comma*	Male	**1.20**	NS	**-0.58**
	O. sylvanus	Male	**1.06**	NS	NS
		Female	**0.97**	NS	NS
	T. sylvestris	Male	**0.62**	NS	NS
		Female	**0.61**	NS	NS
	T. lineola	Male	NS	0.73	NS
	E. tages	Both	**0.63**	0.10	0.70
	P. malvae	Both	**0.43**	0.57	NS
Nymphalidae	*F. adippe*	Both	**0.83**	**-0.42**	0.54
	A. iris	Female	**2.11**	NS	1.1
	A. paphia	Female	NS	**-0.58**	**-0.13**
	M. athalia	Both	**0.31**	NS	0.69
	S. aglaja	Both	**0.85**	NS	NS
	H. semele	Male	**0.47**	**-0.52**	0.89
	E. aurinia	Both	**0.79**	NS	**-0.58**
	A. urticae (G1)	Both	**0.88**	NS	**-0.85**
Lycaenidae	*P. argus*	Male	**1.01**	**-0.73**	NS
	P. coridon	Male	**1.61**	NS	NS
		Female	NS	**-0.92**	**-1.03**
	P. bellargus (G1)	Male	**2.94**	**-1.97**	NA
		Female	**1.11**	NS	NA

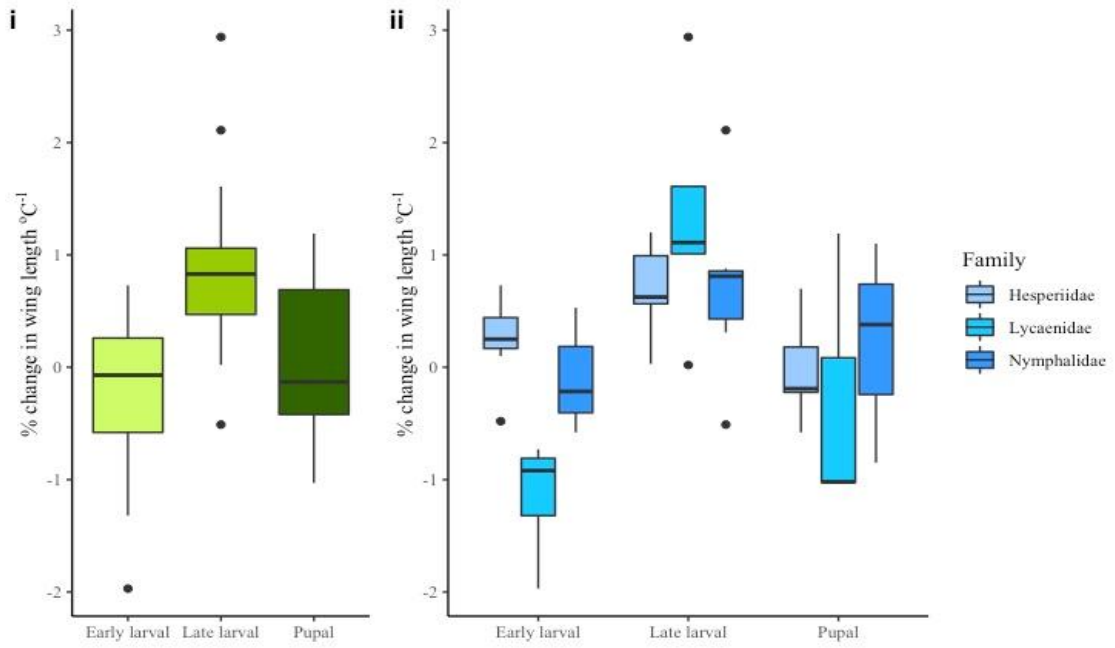

Figure 3.4 Boxplot of percentage change in adult size per °C change during (i) the early larval, late larval and pupal stages and (ii) for species grouped by family within each stage.

Table 3.5 Coefficients (with standard errors) of the linear mixed effects model for examining the significant effects of monthly temperatures on butterfly forewing length in the early larval, late larval and pupal stages. A negative symbol shows a negative relationship between size and the variable. Significant levels are indicated as *, **, *** for p<0.05, 0.01 and 0.001, respectively for fixed effects (monthly temperatures). For random effects, coefficients represent the variance explained by that factor.

i) Model for all data: Average forewing length (Ln) ~ Late T + Early T + Pupal T + (1|Family)

Variables	**Monthly temperatures per stage**			**Intercept**	**Random effects**
	Early larval	**Late larval**	**Pupal**		**Family**
Coefficients	-0.0164*** ± 0.000290	0.0198*** ± 0.000417	0.0209*** ± 0.000421	2.624*** ± 0.270	0.291 ± 0.539

ii) Model species which can be sexed: Average forewing length (Ln) ~ Late T + Early T + Pupal T + (1|Sex) + (1|Family)

Variables	**Monthly temperatures per stage**			**Intercept**	**Random effects**	
	Early larval	**Late larval**	**Pupal**		**Family**	**Sex**
Coefficients	-0.00106*** ±0.000314	0.0138*** ± 0.000594	0.0186 ***± 0.000941	2.390 ***± 0.245	0.179 ± 0.423	0.000844 ± 0.0291

3.5 Discussion

Temperature size responses can be highly variable, with individual species often showing different responses between developmental stages, habitat, sex, and taxonomic category (Forster, Hirst and Atkinson, 2011; Fenberg *et al.*, 2016; Wilson, Brooks and Fenberg, 2019; Wonglersak *et al.*, 2020). Digitised museum collections can be very useful for determining if there are general patterns, yet the number of specimens/species analysed will be limited with time intensive manual measurements. Here, I overcame this problem by using a newly developed computer vision pipeline to automatically measure the sizes of 39,111 specimens of 21 British butterfly species (14,381 with a date and location were used in analyses). I confirmed previous findings that an increase in adult size in relation to increasing temperature during the late larval stage is a consistent response across most species. The responses of adult size to temperature during the early larval and pupal stages varied between species and, as expected, responses often differed between males and females of the same species. Finally, I have also shown that family is an important factor in affecting direction and magnitude of temperature-size responses in each key developmental stage.

3.5.1 Temperature-size responses in British butterflies

For the majority (15/21) of species in my study, adult size increases with increasing temperature during the late larval stage. This is consistent with previous research, which was based on only a few species (Fenberg *et al.*, 2016; Wilson, Brooks and Fenberg, 2019). While there were some species that did not show this response, there were no species, sexes or generations that showed the reverse response. This suggests that warm temperatures during the late larval stages result in larger adults. I suggest that this is because a higher volume and/or quality of food is available during years with warmer temperatures during the late larval stages of species. Therefore, late larval stage individuals can reach optimum growth rates when food quality and quantity are plentiful, resulting in larger adults (Suhling, Suhling and Richter, 2015). However, the optimum temperature for growth and the highest rate of growth possible vary between species (Suhling, Suhling and Richter, 2015), and, therefore, how size is affected by temperature will also vary. Another possible explanation for the consistent response to temperatures during late larval stages, is that the final larval stage may be less oxygen-stressed than other developmental stages. Previous research on *Pieris napi* found that in the final larval instar, both respiration and metabolic rate increased with growth, but in the previous instar it was the reverse (Kivelä, Lehmann and Gotthard, 2016). This suggests that in the final instar, oxygen is not a limiting factor and, therefore, if food supply is plentiful and temperatures are optimal, growth rate increases and results in a larger adult.

While my results are largely consistent for the effect of temperature during the late larval stage on adult size, there was much more variation in adult size responses to temperature during the early larval and pupal stages. A previous study found that increasing temperatures at the beginning and end of the pupal stage for *A. cardamines* led to an increase in adult size (Davies, 2019). Yet, I found this to be true in only five species (Table 3.5), and these species have a variety of ecological and life history traits not shared by *A. cardamines*. For other species, adult size decreased with increasing temperature during the pupal stage (e.g. *P. coridon*; Wilson, Brooks and Fenberg, 2019). There were only three species (all Hesperiidae) which showed an increase in adult size with increasing temperature during the early larval stage, while four species followed the TSR with regards to early larval temperatures.

Of the species that did not respond to temperature, two were Lycaenidae, two were Nymphalidae (plus *P. aegeria* which was excluded from further analyses) and one was Papilionidae. Given the strong response of the three Lycaenidae species analysed in Wilson, Brooks and Fenberg (2019), also included here, it seems surprising that the two additional Lycaenidae species analysed here showed no response to temperature in males or females. This difference may be due to habitat type as *F. quercus* and *T. betulae* are both found in woodland habitats, whereas *P. bellargus* and *P. coridon* live on chalk grasslands, and *P. argus* is found primarily on heathland. Additionally, unlike the other Lycaenidae I have studied, *F. quercus* and *T. betulae* are only tended and protected by ants during the pupal stage (Thomas and Lewington, 2014). Two other species which did not show a temperature-size response were *M. cinxia* and *A.io.* In these species, larvae may regulate their temperature by huddling together, basking in groups or living and feeding in communal silk webs (Thomas and Lewington, 2014). This suggests they may regulate temperature to their thermal optimum and explains why size is not influenced by monthly air temperatures. However, not all species which thermoregulate show no response to temperature (e.g. *E. aurinia*).

As expected, the sexes and different generations did not respond in the same way to temperature (Wilson, Brooks and Fenberg, 2019). For the three bivoltine species, two had a response in the first generation and not in the second (*P. bellargus* and *A. urticae*). The third species, *P. aegeria,* showed no response in either generation and was excluded from the analysis because it overwinters in both the pupal and larval stage. The different responses between generations were expected as the larvae of each generation experience different environmental conditions, which can affect adult body size (Horne, Hirst and Atkinson, 2017). Some studies have shown differences in temperature-size responses between the sexes, which may lead to changes in sexual size dimorphism in some cases (Høye *et al.*, 2009; Fenberg *et al.*, 2016; Wilson, Brooks and Fenberg, 2019). Other research, however, has shown that temperature-size responses can be

similar between sexes in butterflies (Bowden *et al.*, 2015; Davies, 2019) and other insect species (e.g.McCauley *et al.*, 2015; Wonglersak *et al.*, 2020). Out of the 12 species in which the sexes were analysed separately, males had a significant temperature-size response in eight species and females responded in six species (four species had a significant response in both males and females), and in two species there was no response from either sex (*F. quercus* and *T. betulae*). In all but two of the species with significant results, the responses to temperature differed between males and females.

In the multispecies analyses, family explained the highest proportion of variance in temperature-size responses. Although significant responses to temperature in the late larval stage were always positive, the magnitude was greater for Lycaenidae compared to Nymphalidae or Hesperiidae (Fig. 3.4ii). The response to early larval stage temperatures shows the clearest differences between families: most Hesperiidae species showed an increase in adult size with increasing temperature (with the exception of *H. comma*) and most Lycaenidae species showing a decrease in adult size with increasing temperature (with the exception of *P. argus* males). Overall, the Lycaenidae show the largest variation in responses to temperature between the immature stages, with a large increase in adult size (1.34% per °C on average) with increasing temperatures in the late larval stage and a large decrease in adult size (1.15% per °C on average) with increasing temperature in the early larval stage. In the pupal stage, there was a range of positive and negative responses within each family.

There are also some differences in response between species from different habitat types, particularly to temperature during the pupal stage. For example, species associated with grassland generally decreased in adult size with increasing temperature in the pupal stage and showed varied responses to temperature in the early larval stage. In contrast, woodland species generally increased in adult size with increasing temperature in the pupal stage but decreased in adult size with increasing temperature in the early larval stage. This may be due to differences in the microclimates within the habitats experienced by each stage. Grassland habitats will likely be more exposed, but temperatures may not be as high within a woodland setting (especially if it is a dense area of wood), which could particularly influence growth rate in early larval stages.

I found little evidence for the relative size of the species having an effect on the temperature-size responses, although there may be a small impact during the pupal stage (Fig. 3.2iii). Generally, larger species take longer to develop and size has been previously found to increase with mean temperature (Garcia-Barros, 2000). Therefore, an increase in adult size with increasing temperatures in these larger species may have been expected. This was seen to a

certain extent in the response to temperature during the pupal stage, but not the other immature stages.

This research confirms the importance of considering the development stages separately when studying effects of climate change on species with complex life cycles. Different stages respond in different ways to temperature within a species. This may be largely due to the fact that each stage lives within a different micro-climate/habitat (Kingsolver *et al.*, 2011). In general, I found that years with warm monthly temperatures that coincide with the late larval stages of species result in larger adults (Fig. 3.4i). I suggest this is because late larval stage individuals will take advantage of the higher food quality/quantity during warm years, ultimately resulting in more growth and larger adults. However, family is also shown to be an important predictor, especially among the Lycaenidae which are more temperature sensitive than the other families (Fig. 3.4ii). As I do not know the exact micro-climatic conditions the specimens grew in, it is impossible to include more specific factors in this type of research. In the future, it would be useful to conduct laboratory or field experiments with *in situ* measurements of environmental variables over the growth period of each species.

3.5.2 Advantages and limitations of automated measurements

On the whole, the image analysis pipeline was successful in automating the measurement of butterfly wing length. In four of the seven species tested (*H. comma, A. iris, P. machaon,* and *F. quercus*), there was a strong positive correlation between the manual and automated measurements when outliers had been removed, and the average differences between the two sets of measurements were also relatively low (i.e. less than 1mm, Fig. 3.1, Table 3.3). This was not the case, however, for *P. bellargus, P. coridon* and *P. argus*, which had lower correlations between automated and manual measurements, and a high percentage of specimens with a large difference between the two measurements. The failure of the current pipeline on the species with blue wings is likely due to the current binarization stage not separating the butterfly from their grey background and more development is required in order to make segmentation of the butterfly more robust. Additionally, for the 21 species measured using the pipeline, less than 20% of specimens were removed as outliers or measurement errors (except for *P. machaon*). Visually checking output images for errors is timing consuming, but it ensures that the data are more reliable, and it is still overall less time consuming than doing the same amount of manual measurements. Most importantly however, I can be confident that temperature size responses measured using automated measurements versus manual measurements produce the same results. For example, warm temperatures during the late larval stage for *H. comma* (i.e. June)

result in larger adult males using both manual (Fenberg *et al.*, 2016) and automated measurements.

3.5.3 Conclusions

Our results show that automated measurements can be used reliably instead of laborious manual measurements to analyse temperature-size responses in butterflies. Consistent with previous findings, in most British butterfly species studied, an increase in adult size follows an increase in temperature during late larval stages. Adult size responses in relation to temperature in the early larval and pupal stages are more variable and may be more dependent upon family, and habitat type (for the pupal stage). There are also differences in how the sexes respond within and between species, and in some species, responses are different between generations. Therefore, it is important to consider multiple life history factors when predicting how a species will respond to temperature. In the future, improvements to the pipeline need to be made so that more species can be measured, and potentially other insect groups. Additionally, more collections need to be digitized to make use of this and similar technology, and so that more species and data can be included in multi-species analyses to help unravel complex ecological responses to climate warming.

Chapter 4 Temporal and spatial trends in the size and shape of rocky shore gastropods across the UK

4.1 Abstract

Many factors can affect the morphology of gastropods on rocky shores, from natural abiotic and biotic factors to anthropogenic impacts such as climate warming and harvesting. Marine ectotherms are predicted to follow the temperature-size rule, and thus are expected to decrease in size over time due to climate warming. Previous research has found this to be the case for some species, and have also found differences in morphology between different regions. Here, I study temporal and spatial variation in morphology using museum specimens covering a period of over 100-years in combination with field data from across the UK coastline for seven species of intertidal rocky shore gastropod (*Patella vulgata*, *Patella depressa*, *Patella ulyssiponensis*, *Littorina littorea*, *Steromphala umbilicalis*, *Steromphala cineraria* and *Phorcus lineatus*). Size and shape of the individuals were compared between year groups and regions for each species. In contrast to previous studies, none of the species studied here decreased in size over time; yet, there was variation in size and shape between regions and variations in shape over time. This suggests that these species likely do not follow the temperature-size rule, and that other factors may be more important in determining morphology. These species are all grazers, which may be less vulnerable to size changes than predators and may also benefit from the reduction in size or abundance of predators. Future changes in morphology of rocky shore species should be monitored as temperatures continue to rise as changes to one species may have knock-on effects on other species.

4.2 Introduction

Rocky shores are constantly changing environments, which provide a unique set of challenges for the organisms living there. Unlike in marine or terrestrial environments, the individuals living in the intertidal zone are exposed to a range of environmental conditions from the sea and the land. These conditions can also vastly differ between shores, due to factors such as wave exposure, which have a large impact on the structure of the communities present (McQuaid and Branch, 1985; Queiroga *et al.*, 2011). In addition to these natural abiotic factors, the individuals and communities are also being affected by anthropogenic impacts. This can be directly through

harvesting or trampling, or indirectly via the effects of climate change or pollution, which have been shown to affect the size and geographic ranges of species, and alter community compositions (Southward, Hawkins and Burrows, 1995; Branch and Odendaal, 2003; Roy *et al.*, 2003; Helmuth *et al.*, 2006; Fenberg and Roy, 2008; Hawkins *et al.*, 2008; Wilson-Brodie, MacLean and Fenberg, 2017).

Geographically separated populations of species can respond in different ways to local abiotic conditions. In particular, growth of individuals varies between locations due to factors such as wave exposure and temperature. This can result in populations from different locations having differences in maximum size and shell shape. For example, *Patella spp.* in Portugal were found to have a smaller maximum size than those in North West Europe (Guerra and Gaudencio, 1986). Growth rates for *Patella vulgata* have also been shown to differ between wave exposed and sheltered sites when populations were in had natural conditions (Jenkins and Hartnoll, 2001). Shell shape may also respond to wave exposure, and to risks from predation and desiccation (Cabral, 2007; Queiroga *et al.*, 2011). For example, a study of the gastropod *Buccinanops globulosus* found that different populations had different shapes, with those on sheltered shores tended to have large, thick shells with small apertures, compared to small, thin shells with wide apertures on exposed shores (Avaca *et al.*, 2013). On sheltered shores, shape is a response to predation pressure and risk of desiccation, while dislodgement is a bigger threat on exposed shores (Cotton, Rundle and Smith, 2004; Queiroga *et al.*, 2011). A study of patellid limpets in Portugal found that shells were often flat and more eccentric (having the apex off-centre) than the optimum, which is likely to aid with resistance to heat stress and avoid desiccation (Cabral, 2007). Additionally, individuals may have different morphologies across the same shore. For example, *Patella* spp. increase shell height higher up the shore or have smoother shells in shaded areas (Vermeij, 1973). This is also true for *L. littorea,* which increases shell height from low to high shore, although factors such as wave exposure can alter this pattern (Vermeij, 1973).

In addition to these natural differences in size and shape, previous studies have found that some species have decreased in size due to increasing temperatures or harvesting pressure (Roy *et al.*, 2003; Fenberg and Roy, 2008; Munroe *et al.*, 2016; Ortega *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017), and become thinner due to ocean acidification (Pfister *et al.*, 2016). Many studies and experiments have found that marine molluscs decrease in size in response to climate warming, and thereby follow the temperature-size rule (TSR) (Irie, Morimoto and Fischer, 2013; Munroe *et al.*, 2016; Ortega *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017). This is despite the fact that calcification rates (needed to produce calcium carbonate shells) are slower at lower temperatures and thereby should result in smaller shells (Irie and Fischer, 2009; Irie, Morimoto and Fischer, 2013). Some of these species have also been impacted by fishing or

harvesting (Roy *et al.*, 2003; Munroe *et al.*, 2016), while others may have also been impacted by other human impacts such as pollution (Wilson-Brodie, MacLean and Fenberg, 2017). In some species the maximum size has been found to decrease dramatically since the onset of climate warming; for example, the maximum size of *N. lapillus* in the southern UK decreased by at 18 mm in the last century, and the maximum size of *Spisula solidissima* decreased 20 mm since 1982 due to anthropogenic impacts (Munroe *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017). Climate warming is likely to negatively affect species which have a narrow thermal range or cold-water affinities as they may not be able to adapt to warming temperatures (Munroe *et al.*, 2016; Ortega *et al.*, 2016), and therefore, it can be predicted that species living in cooler conditions may be more impacted by rising temperatures.

Ocean acidification, as a result of climate warming, may adversely affect marine species, particularly ones with calcite shells (Pfister *et al.*, 2016). A study of the mussel *Mytilus californianus* found that the largest modern mussels were thinner per unit length than those from shell middens (over 1000 years ago), but smaller shells were not thinner in 2011-12 than in the 1970s (Pfister *et al.*, 2016). An experiment to determine how well two gastropod species responded to low pH levels (caused by increased CO_2) found that individuals adapted to low pH were smaller than those under normal conditions and adapted to have lower calcification rates (Garilli *et al.*, 2015). Conversely, a study of specimens of the brachiopod *Calloria inconspicua* found that most shell characteristics have remained unchanged between 1900 and 2014. There was, however, an increase in shell density over time and shell perforations became narrower, likely to reinforce the shell (Cross, Harper and Peck, 2018).

Another factor which could affect a species' response to climate warming is trophic level. Predators such as *Carcinus meanus* are thought to regulate herbivore abundance; for example, *L. littorea* was found to grow slower when predator cues were present, which in turn increases algal density due to lack of grazing (Trussell, Ewanchuk and Bertness, 2002). Previous research of *N. lapillus* has shown that in southern UK, there was a decrease in size over the past 100 years, which correlated with an increase in temperature (Wilson-Brodie, MacLean and Fenberg, 2017). This species is predatory, so it is unclear whether the many grazing gastropods on UK shore will respond in the same way. If predators become smaller or less common, then grazers may become more successful in response. *Littorina littorea* was found to have a higher behavioural response to predators compared to species which were morphologically adapted (i.e. with flatter shells) such as *Phorcus lineatus, Steromphala umbilicalis and S. cineraria.* This means that *L. littorea* may be at a competitive disadvantage for foraging food against these species because it moves in response to predator cues and, therefore, this limits foraging (Cotton, Rundle and Smith, 2004).

Given that it is predicted that aquatic ectotherms are likely to shrink in size in response to rising temperatures, and species can vary in size and shape across their range, studies into morphological changes across time and space are required to predict future responses and gain a better understanding of how communities may be affected as a whole. In order to do this, data from past decades (or centuries) and several locations are required to compare to modern field data. Museum collections provide a useful source of historical data for spatial and temporal comparisons. For species that have been well collected, specimens are often present in large quantities covering a wide range of locations and dating back over at least the last century (Johnson *et al.*, 2011; Lister *et al.*, 2011). While there may be issues and inconsistencies over sampling methods and collector bias, using only specimens with a date and location of collection can provide a reliable long term data-set (Johnson *et al.*, 2011; Lister *et al.*, 2011; Kharouba *et al.*, 2018). Previous studies have successfully used museum specimens to show that individuals in the larger size classes of intertidal gastropods have reduced in size over time (Roy *et al.*, 2003; Wilson-Brodie, MacLean and Fenberg, 2017).

Here, I aim to use museum specimens and field data to determine whether there is temporal or spatial variation in the size and shape of seven intertidal gastropod species in the UK. Previous research of *N. lapillus* has shown that in southern UK, there was a decrease in size over the past 100 years, which correlated with an increase in temperature (Wilson-Brodie, MacLean and Fenberg, 2017). Intertidal gastropods, however, have a wide thermal tolerance given the nature of their environment (Wang, Surge and Mithen, 2012), and a study of gastropods and bivalves in the Cenozoic era found that there was no general trend of decreasing size (Chattopadhyay and Chattopadhyay, 2019). Therefore, not all species may respond in this way, and some intertidal gastropods may be able to adapt to temperature changes (Sorte, Jones and Miller, 2011). Furthermore, the species used here are all grazers rather than predators and so may respond differently due to the lower trophic level. I do, however, expect there to be differences in size and shape between different regions. As previously mentioned, if predators reduce in size or become less abundant then grazers may thrive in this environment and not show a size decline over time.

In the study of *N. lapillus*, there was a significant difference in size between individuals in the South East UK and those from the South West UK (Wilson-Brodie, MacLean and Fenberg, 2017). Intertidal crustacean species have also previously been found to vary in size over latitude, generally increasing with latitude (Jaramillo *et al.*, 2017), and therefore, I also predict that there will be a difference in size of intertidal gastropods between northern and southern UK. Shape of intertidal gastropods is known to vary between shores due to environmental conditions and interactions between species present (Kemp and Bertness, 1984; Cotton, Rundle and Smith, 2004;

Cabral, 2007; Avaca *et al.*, 2013), and so I predict that shape will also vary between regions for all species.

4.3 Material and Methods

Seven gastropod species, which can be found commonly on UK shores and in museum collections were selected for this study: *Littorina littorea, Patella vulgata, Patella ulyssiponensis, Patella depressa, Steromphala umbilicalis, Steromphala cineraria* and *Phorcus lineatus*. These species cover three different families (Littorinidae, Patellidae and Trochidae), but all are grazers. Details of data collection and analyses are given below.

4.3.1 Data collection – museum data

Data were collected from specimens at four UK museums; Natural History Museum (London), National Museum Wales (Cardiff), Oxford Museum of Natural History and National Museum Scotland. Each species was measured in two dimensions, a primary size measurement and a secondary measurement so that shape could be calculated (see below). For *L. littorea,* height was the primary measurement and width was the secondary measurement. For *S. umbilicalis, S. cineraria* and *P. lineatus*, the diameter (primary measurement) and height of the shells were measured, and for *P. vulgata, P. ulyssiponensis* and *P. depressa*, length (primary measurement) and width of the shells were measured. For some species, a threshold size was used for the primary measurement to ensure that only the largest individuals were sampled as I am only interested in comparisons between adult sizes in analyses of those species. For *L. littorea*, only individuals with a height of 20 mm or more were included, while *P. vulgata, P. ulyssiponensis* and *P. depressa* needed to measure at least 40 mm, 30 mm and 20 mm in length respectively. Measurements to the nearest 0.01 mm were taken of each size parameter of each individual using Mitutoyo digital callipers.

Specimens were only included if they had good quality metadata, such as a specific location (i.e. name of a town or beach rather than just a county) and a year of collection or enough information to estimate the latest possible date of collection (based on biographic information of the collector). The location data was georeferenced to give latitude and longitude co-ordinates for each specimen. The number of specimens measured per species at each museum are given in Table 4.1, details of the spatial and temporal spread of data are given in Table 4.2, and locations where specimens were collected for each species are shown in Figure 4.1 (i-vii).

Table 4.1 The number of specimens measured per species at each museum; NHM – Natural History Museum, NMW – National Museum Wales, NMS – National Museum Scotland, OMNH – Oxford University Museum of Natural History

Species	NHM	NMW	NMS	OMNH	Total
Patella vulgata	217	171	193	122	703
Patella depressa	125	153	33	69	380
Patella ulyssiponensis	137	154	317	20	628
Littorina littorea	484	242	654	111	1491
Phorcus lineatus	111	150	44	70	375
Steromphala umbilicalis	299	190	553	123	1165
Steromphala cineraria	205	243	1369	175	1992

4.3.2 Data collection – field data

The georeferenced locations of the museum specimens were used to create a map of data points for each species (Fig 4.1i-vii), which were used to determine possible field sites for modern surveys to take place (Fig 4.1viii). As a large proportion of the data came from Scotland or southern England, I selected eight sites from Scotland, South West England and South East England to survey. The two southern regions were also selected to allow for comparison with previous results of *N. lapillus* in these regions, which found there was a difference in size between the two areas both in modern and historical data (Wilson-Brodie, MacLean and Fenberg, 2017). The boundary between South West and South East England lies just to the west of the Isle of Wight. As species can also vary in size with latitude, I selected an area of Scotland (the Outer Hebrides) that was relatively far north to provide a stark contrast with the data collected in southern England. Field surveys took place between summer 2018 and autumn 2019, and details of the sites selected are given in the supplementary information (Appendix B, Table B.1).

At each field site, metadata were taken (e.g. date, co-ordinates, weather condition; Table 4.2 and the supplementary information). For *L. littorea* and the three limpet species (*Patella spp.*), a timed search and measure was carried out for 45 minutes across the site, measuring only those individuals over the threshold size. For the *Steromphala spp.* and *P. lineatus*, a three-minute timed search and collection was carried out at four different locations on the shore to ensure a range of shore heights in the littoral zone was included. Diameter and height of the individuals collected were measured, and the individuals were returned to the shore. Measurements to the nearest 0.1 mm were taken using plastic DialMax callipers.

Table 4.2 The spatial (latitude and longitude) and temporal (years) ranges of museum specimens for each species.

Species	Latitudinal range (museum)	Longitudinal range (museum)	Years covered (museum)	Latitudinal range (field)	Longitudinal range (field)
Patella vulgata	49.164 °N to 60.306 °N	007.410 °W to 001.424 °E	1852 to 2004	50.114 °N to 57.664 °N	007.524 °W to 001.444 °E
Patella depressa	49.470 °N to 51.725 °N	007.099 °W to 001.551 °W	1894 to 1998	50.114 °N to 50.833 °N	005.531 °W to 001.551 °W
Patella ulyssiponensis	49.172 °N to 60.306 °N	007.543 °W to 000.393 °W	1902 to 2004	50.114 °N to 57.664 °N	007.414 °W to 000.650 °W
Littorina littorea	49.165 °N to 60.758 °N	007.310 °W to 001.423 °E	1883 to 2004	50.114 °N to 57.664 °N	007.524 °W to 001.444 °E
Phorcus lineatus	49.172 °N to 52.826 °N	006.309 °W to 002.179 °W	1885 to 1990	50.114 °N to 51.210 °N	005.531 °W to 000.650 °W
Steromphala umbilicalis	48.629 °N to 60.479 °N	007.486 °W to 001.297 °E	1872 to 2009	50.114 °N to 57.664 °N	007.524 °W to 001.444 °E
Steromphala cineraria	49.164 °N to 58.483 °N	007.485 °W to 001.379 °E	1825 to 2004	50.114 °N to 57.664 °N	007.524 °W to 001.444 °E

4.3.3 Statistical analyses

In both the museum and field data, the locations were split into areas; North East UK (museum data only), North West UK, South East UK and South West UK. The North-South divide ran parallel from the northern most point of Wales. In the South, the East-West divide was to the west of the Isle of Wight (at a longitude of around 001.600°W), while in the North, the divide was between East and West Scotland with the divide in the northern most parts at around 004°W. This meant that Wales and the Chanel Islands were included in the South West, the Outer and Inner Hebrides were included in the North West and the Shetland Islands were included in the North East. Specimens from Ireland were excluded. The museum data was also split into year groups; earliest date of collection-1922, 1923-1969, 1970-1989 and 1990-latest date of collection. If the exact year was not given for museum specimens, obituaries and other biographic information held at the museums were useful in finding a latest possible date of collection, these dates were then used to create groupings so that these estimated dates would easily fit into a group. For example, many specimens in the NHM and the NMW were collected by JT Marshall, who died in 1922 according to his obituary held at the NHM, and therefore, these specimens were labelled pre-1922 and this was selected as a sensible break point for the earliest group. In some species, the

date range in this group is larger due to specimens dating back to 1825 (Table 4.2), but in most species this group does not have more specimens than other year groups (Table 4.3) due to specimens with good meta data being less common from 100 years ago. The number of data points per species in each group are given in Table 4.3.

Table 4.3 The number of specimens of each species used in analyses given by region and year group.

Species	**Region**				**Year Group**					**Total number**
	North East	**North West**	**South East**	**South West**	**1825-1922**	**1923-1969**	**1970-1989**	**1990-2004**	**modern**	
P. vulgata	69	931	620	714	99	103	133	43	1956	2334
P. depressa	N/A	N/A	2	386	165	48	36	53	86	388
P. ulyssiponensis	64	114	8	433	89	152	144	68	166	619
L. littorea	264	1000	900	1147	467	303	426	154	1961	3311
P. lineatus	N/A	N/A	11	745	138	99	67	1	451	756
S. umbilicalis	N/A	502	1468	1455	199	197	365	230	2434	3425
S. cineraria	359	1004	180	667	162	204	720	692	432	2210
Totals	**756**	**3551**	**3189**	**5547**	**1319**	**1106**	**1891**	**1241**	**7486**	**13043**

Shape was calculated as a ratio between the primary and secondary measurements (aspect ratio) for each specimen or individual; for *L. littorea*, height was divided by width, for *Steromphala* spp. and *P. lineatus*, diameter was divided by height, and for *Patella spp.,* length was divided by width. For each species, I tested for correlations between the two size parameters, and the primary size parameter with shape. Both size and shape parameters were then compared between areas and year groups, as well as between individuals from museum collections and measured in the field, for all species to determine if there were any spatial or temporal patterns. As the data were not normal non-parametric tests were used in the form of Mann-Whitney, Kruskal-Wallis and Spearman's correlation tests. I also tested for correlations between size or shape and latitude, longitude or year of collection (where there was an exact year of collection). For significant results, this was followed by a quantile regression analysis to determine the significance and direction of slopes at each of the following quantiles: 0.05, 0.10, 0.25, 0.50, 0.75, 0.90, 0.95.

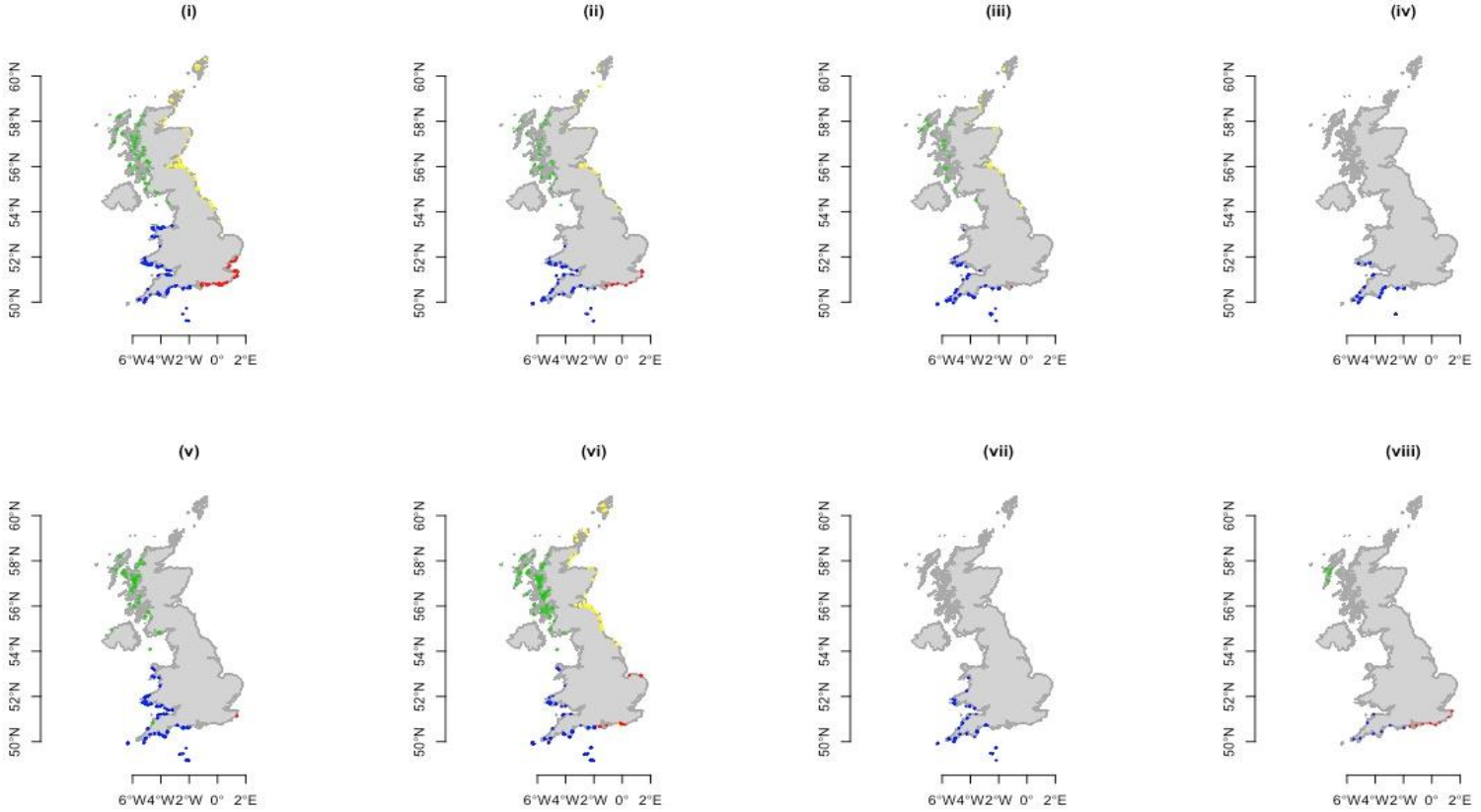

Figure 4.1 Maps showing locations of museums specimens (i-vii) and field sites (viii) for i) *L. littorea*, ii) *P. vulgata*, iii) *P. ulyssiponensis*, iv) *P. depressa*, v) *S. umbilicalis*, vi) *S. cineraria*, vii) *P. lineatus* and viii) all species.

4.4 Results

The results for each species are given below. All regional comparisons are for combined museum and field data; i.e. the North West, South East and South West data include museum and field data, whereas the North East group is only museum data as there are no field sites in this region. The comparisons between size or shape and latitude or longitude are also a combination of museum and field data, so they are spatial patterns irrespective of year of collection. Conversely, the year group comparisons are for data across all regions; i.e. all past groups (museum data) is for specimens collected in any region, and the modern group (field data) is for individuals in the North West, South East and South West. The comparisons between size or shape and year are a combination of individuals from all regions, so they are temporal patterns irrespective of location.

4.4.1 Littorina littorea

There was a significant positive correlation between height and width of individuals (rho=0.915, p<0.001, Fig. 4.2i). This species had the steepest slope (aspect ratio) when comparing the two size parameters. The aspect ratio for this species is height:width so a low aspect ratio indicates that an individual is relatively short and round (i.e. globose), whereas a high aspect ratio indicates that an individual is tall and thin (elongated).

There were significant differences in height (chi^2=328.11, df=3, p<0.001; Fig. 4.3iii) and shape (chi^2=110.49, df=3, <0.001) between regions, with differences between all regions (p<0.05) except the North East and North West (p>0.05). On average, the South West had the lowest average height over 20 mm, and the North West had the largest average height, with the South East also having lower averages than the northern areas. The North West had the largest individual (45.0 mm; Cheesebay, Outer Hebrides in the field data), but the maximum size of an individual measured from the South East was considerably smaller (35.9 mm; Beachy Head, Sussex in the field data). This is similar to results for *N.* lapillus, which had a higher maximum size in the South West UK than the South East, but unlike *L. littorea,* the South West had a higher average size than in the South East (Fig. 4.4iii; Wilson-Brodie, MacLean and Fenberg, 2017). There was a significant positive correlation between height and latitude (rho=0.331, p<0.001); quantile regression analysis showed that all quantiles tested had positive slopes, which were significantly different from 0 (p<0.001). There was a significant correlation between height and longitude (rho=-0.123, p<0.001), with slopes of all quantiles between 0.05 and 0.95 being negative and significantly different from 0 (p<0.05). The North East had the narrowest range of aspect ratios (1.161 to 1.550), whereas the South West has the largest range in aspect ratios (0.849 to 2.548).

There was a significant positive correlation between shape and latitude (rho=0.205, p<0.001; Fig. 4.2ii), all slopes at quantiles between 0.05 and 0.95 were positive and significant (p<0.001). There was a significant negative correlation between shape and longitude (rho=-0.155, p<0.001; Fig. 4.2iii), and all slopes at quantiles between 0.05 and 0.95 were negative and significantly different from 0 (p<0.01).

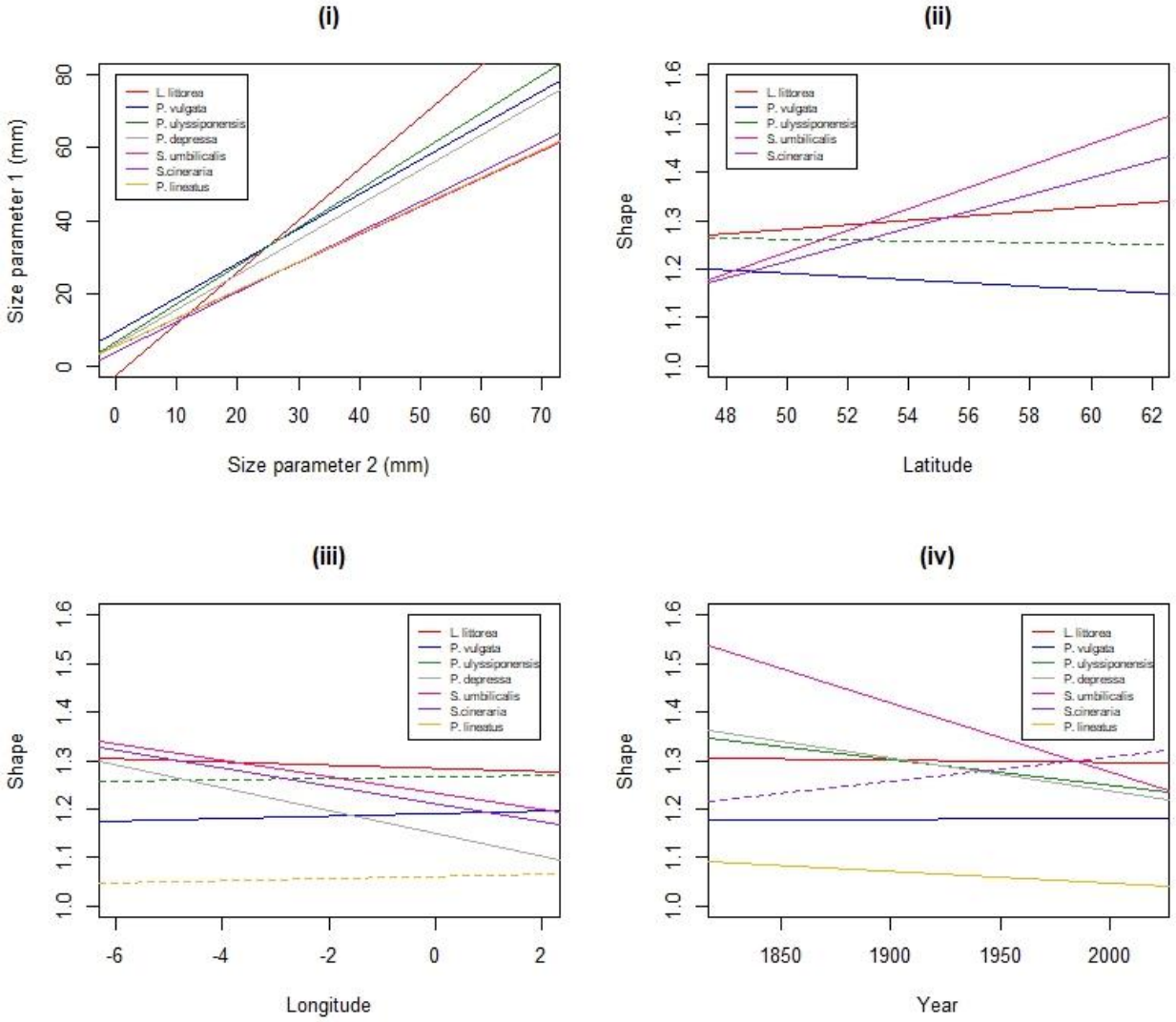

Figure 4.2 i) Plot of the main size parameter for each species versus the secondary size parameter, and plots of shape versus ii) latitude, iii) longitude, and iv) year. The lines represent linear models of shape (e.g. for plot i, a linear model of shape versus size), and each species is represented by a different colour line. Non-significant models are represented by dashed lines. N.B. Size parameter 1 is height for *L. littorea*, length for *Patella* spp., and diameter for the trochids. These plots include both significant and not significant correlations (see text for more details).

There was no significant difference in height between specimens from museum collections and those measured in the field (W=1356982, p=0.218), and as Figures 4.3i and ii show, in both the museum and field collections the majority of specimens were under 30 mm in height and very few were over 40 mm. This is in contrast to *N. lapillus*, which had considerably fewer individuals over 30 mm in the field compared to the museum collections as well as having a much lower maximum size (Fig. 4.4i-ii; Wilson-Brodie, MacLean and Fenberg, 2017). There were, however, significant differences in height between year groups (chi^2=90.07, df=4, p<0.001; Fig. 4.3iv). The 1970-1989 group had the largest average size over 20 mm, and 1883-1922 had the lowest average size and the lowest maximum size (37.74 mm; Balta Sound, Shetland collected before 1911), yet the modern group had the tallest individual (45.0 mm; Cheesebay, Outer Hebrides). There was a significant positive correlation between height and year (rho=-0.0804, p<0.001), and quantile regression analysis revealed that quantiles between 0.05 and 0.50 had a significant positive slope, and at the 0.90 and 0.95 quantiles there were significant negative slopes (p<0.05). There was no significant difference in shape between year groups (chi^2=8.144, df=4, p=0.0865) or between museum specimens and those measured in the field (W=1319095, p=0.866), but the aspect ratios for northern individuals were consistently higher (or equal to) on average than the southern individuals across year groups (i.e. the shells were more elongated in the North). There was a significant negative correlation between shape and year (rho=-0.115, p<0.001; Fig. 4.2iv), but in the quantile regression analysis, only negative slopes at the 0.75 and 0.95 quantiles were significantly different from 0 (p<0.05).

4.4.2 *Patella* spp.

For *P. vulgata*, there was a significant positive correlation between length and width (rho=0.904, p<0.001; Fig. 4.2i). For *P. ulyssiponensis*, there was a significant positive correlation between length and width (rho=0.949, p<0.001; Fig. 4.2i). For *P. depressa*, there was a significant correlation between length and width (rho=0.951, p<0.001; Fig. 4.2i). When length and width were compared, the three species had a similar slope (aspect ratio), which is shallower than the slope for *L. littorea* but steeper than the slope for the trochids (Fig. 4.2i). For these species, aspect ratio is length:width and therefore, an individual with a low aspect ratio would be more circular in shape at the base and those with a high aspect ratio would have a more oval or elongated in shape at the base.

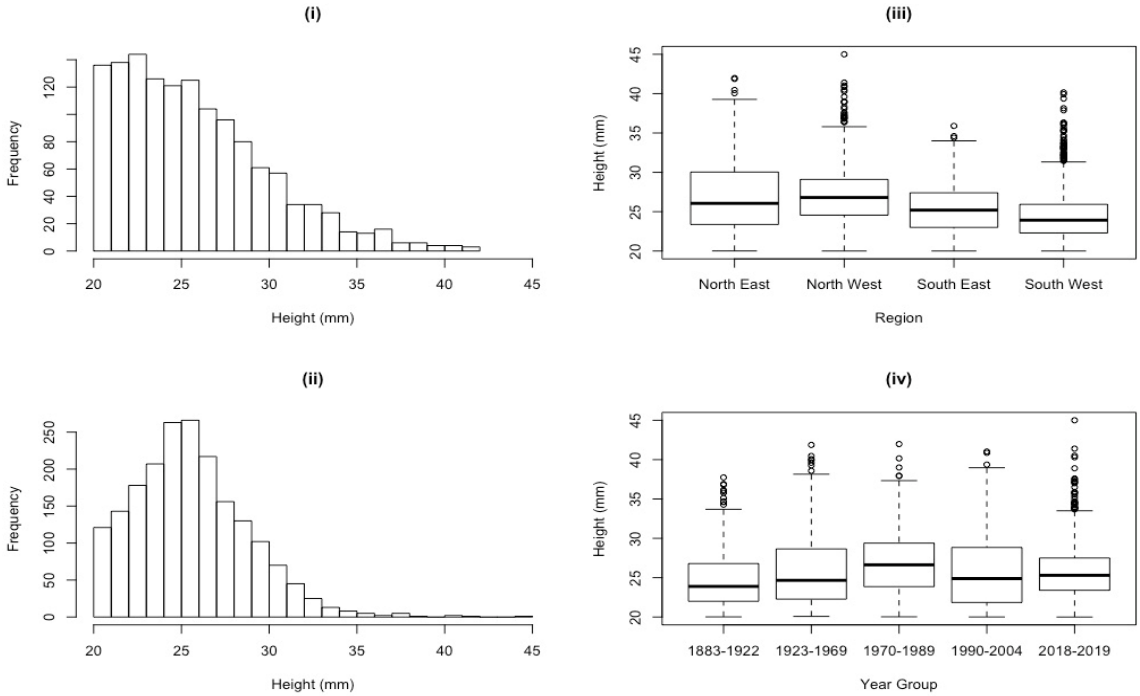

Figure 4.3 Plots of height for *L. littorea*. Frequency distribution of height of individuals (over 20 mm) for those measured in i) the museums, and ii) the field. Boxplots of height of individuals (over 20 mm) in iii) each region (irrespective of year), and iv) each year group (irrespective of location). The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers.

For *P. vulgata,* there were differences in length (chi^2=138.996, df=3, p<0.001; Fig. 4.5iii) and shape (chi^2=93.783, df=3, p<0.001) between regions. The North East had the lowest average length (over 40 mm) and the South West had the highest average length. The South East had the smallest maximum size (67.8 mm; Seaview, Isle of Wight in the field data), while the South West and North West had larger maximum sizes (74.01 and 74.21 mm respectively; Lyme Regis, Dorset collected pre-1965 and Loch Sween, West Scotland collected in 1982). The North East had the narrowest range in aspect ratios (1.043 to 1.280) and the North West had the largest range in aspect ratios (0.916 to 2.257). There was a significant difference in length between specimens in the museum collections and those measured in the field (W=410576, p<0.001), with the museum specimens having a larger maximum size but a lower median size (46.5 mm) than those in the field (48.45 mm; Fig. 4.5i-ii). There were significant differences in length (chi^2=87.418, df=4, p<0.001; Fig. 4.5iv) and shape (chi^2=18.319, df=4, p=0.00107) between year groups. The year group 1970-1989 had the lowest average length over 40 mm, yet the largest individual (74.21 mm; collected in 1982 at Loch Sween, West Scotland), whereas 1923-1969 had the highest average length, and 1990-2004 had the lowest maximum size (62.51 mm; collected in 1990 in Ayrshire, West Scotland). The modern group had the largest range in aspect ratios (0.916 to

2.257) and the 1923 to 1969 group had the narrowest range in aspect ratios (1.071 to 1.306). There was a significant positive correlation between length and year (rho=0.201, p<0.001). Quantile regression analysis showed that quantiles between 0.05 and 0.50 had positive slopes, that were significantly different from 0, and the 0.90 and 0.95 quantiles had negative slopes that were significantly different from 0 (p<0.05). The was a significant negative correlation between length and latitude (rho=-0.190, p<0.001); quantile regression analysis showed that quantiles between 0.50 and 0.95 had negative slopes, which were significantly different from 0 (p<0.05). There was a positive correlation between longitude and length (rho=0.0455, p=0.0318), with quantiles between 0.05 and 0.25 having a significant negative slope, and quantiles between 0.75 and 0.95 having significant positive slopes (p<0.05). There was a significant negative correlation between shape and latitude (rho=-0.166, p<0.001; Fig. 4.2ii), but a significant positive correlation between shape and longitude (rho=0.158, p<0.001; Fig. 4.2iii). There were significant positive correlations between shape and year (rho=0.121, p<0.001, Fig. 4.2iv), and quantile regression analysis found that there was a significant negative slope at the 0.10 quantile and a significant positive slope at the 0.95 quantile (p<0.05).

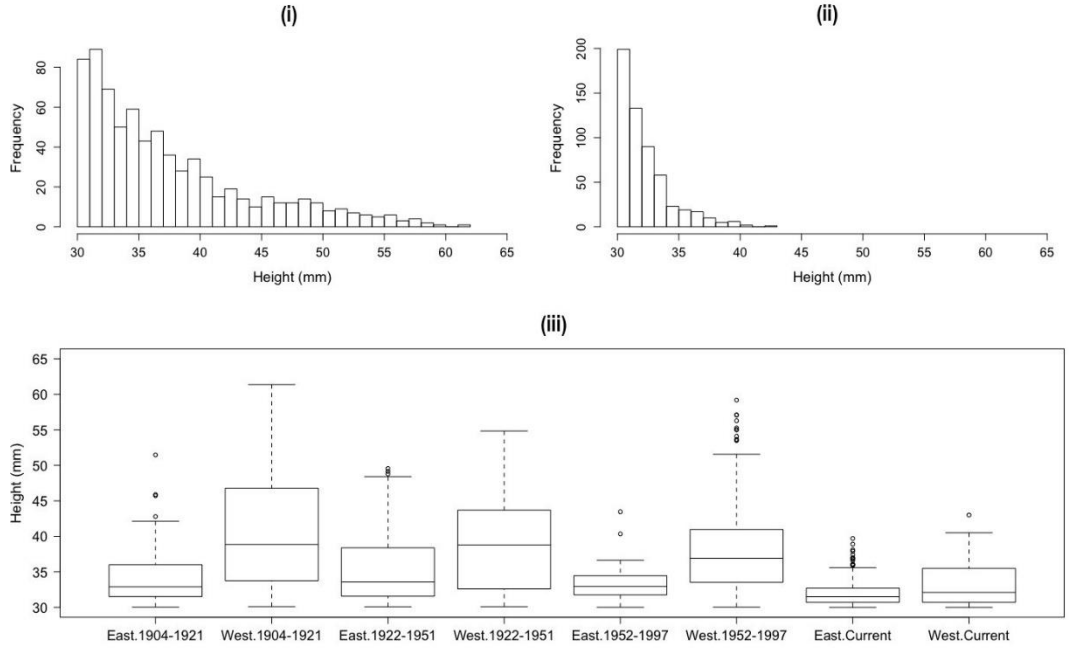

Figure 4.4 Plots of height for *N. lapillus* from a previous study on the southern UK coastline. Frequency distribution of height of individuals (over 20 mm) for those measured in i) the museums, and ii) the field. iii) Boxplots of height of individuals (over 20 mm) by region and year group. The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers. Only the South East and South West are included in this data set, field data (current) were collected between 2014 and 2016, and museum year groups are different to those used in the current study (Wilson-Brodie, MacLean and Fenberg, 2017).

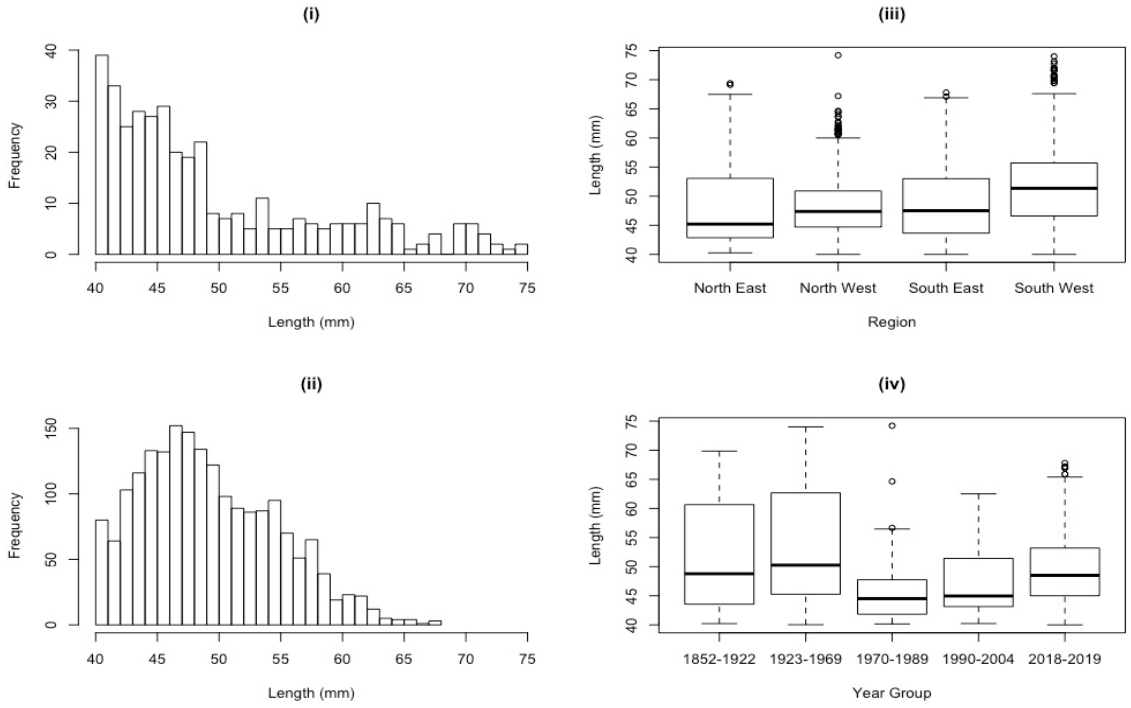

Figure 4.5 Plots of length for *P. vulgata*. Frequency distribution of length of individuals (over 40 mm) for those measured in i) the museums, and ii) the field. Boxplots of length of individuals (over 40 mm) in iii) each region (irrespective of year), and iv) each year group (irrespective of location). The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers.

For *P. ulyssiponensis,* there were significant differences in length (chi^2=21.336, df=3, p<0.001; Fig. 4.6iii) and shape (chi^2=12.056, df=3, p=0.00720) between regions, with differences in length between the South West and the northern regions (p<0.05) and differences in shape between the South East and both the South West and North East (p<0.05; there are only 8 data points in the South East). The South East had the largest average size over 30 mm, but the lowest maximum size (61.79 mm; Whitecliff Bay, Isle of Wight collected in 1991), whereas the North East had the smallest average size, and the South West had the largest maximum size (70.54 mm; Penzance, Cornwall collected in 1971). The South East had the narrowest range in aspect ratios (1.164 to 1.227) and the South West had the widest range in aspect ratios (1.053 to 2.089). There was a significant difference in length between museum specimens and those measured in the field (W=46183, p<0.001), with the field individuals having a higher average size, but a lower maximum size than the museum specimens (Fig. 4.6i-ii). There was a significant difference in length (chi^2=21.851, df=4, p<0.001; Fig. 4.6iv) and shape (chi^2=54.817, df=4, p<0.001) between year groups, with differences between the modern group and all other groups (p<0.05). The modern data had the largest average size over 30 mm, but the averages of the other groups were

fairly similar; 1902-1922 had the smallest maximum size (58.88 mm; Ilfracombe, Devon collected before 1913), and 1970-1989 had the largest individual (70.54 mm; collected in 1971 in Penzance, Cornwall). The 1970-1989 group had the largest range in aspect ratios (1.106 to 2.089) and the 1990-2004 group had the narrowest range in aspect ratios (1.142 to 1.398). There was a significant positive correlation between length and year (rho=0.168, p<0.001); quantile regression analysis revealed that quantiles between 0.10 and 0.50 had positive slopes, which were significantly different from 0 (p<0.05). There was also a significant negative correlation between length and latitude (rho=-0.174, p<0.001). Quantile regression analysis found that quantiles between 0.75 and 0.95 had negative slopes, which were significantly different from 0 (p<0.001). There was not a significant correlation between length and longitude (p=0.514). There was a significant negative correlation between shape and year (rho=-0.282, p<0.001; Fig. 4.2iv), but quantile regression showed all slopes were not significantly different from 0 (p>0.05). There were no significant correlations between shape and latitude (p=0.335; Fig. 4.2ii) or shape and longitude (p=0.291; Fig. 4.2iii).

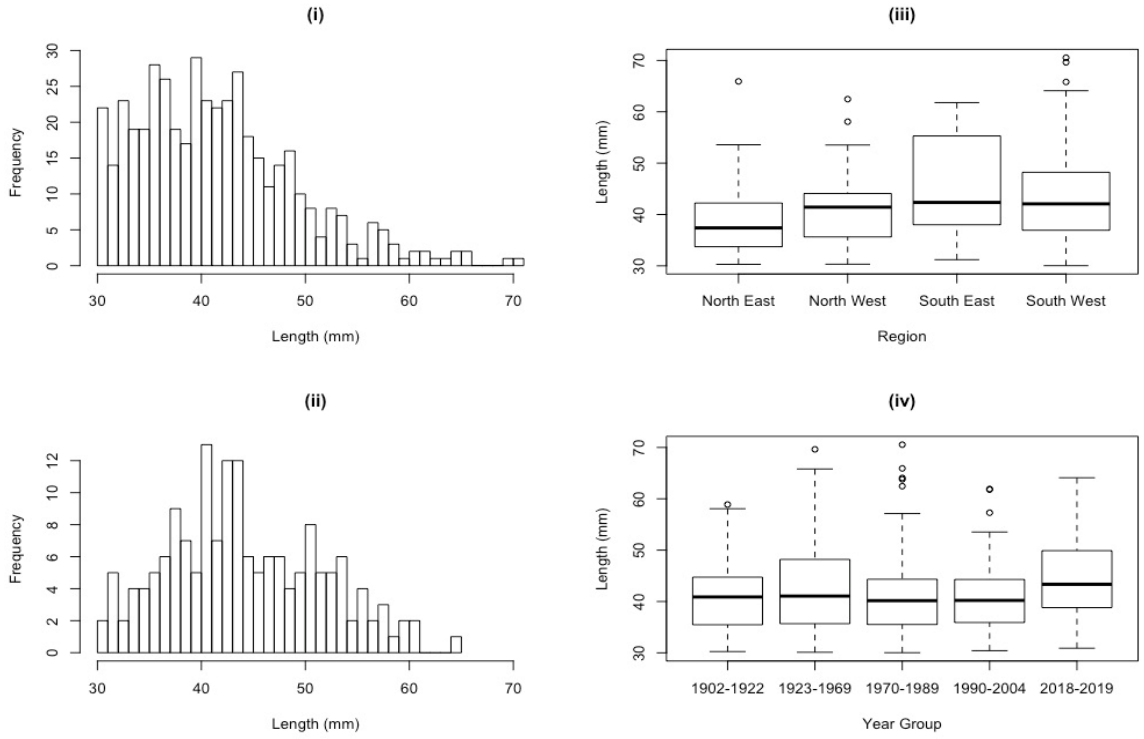

Figure 4.6 Plots of length for *P. ulyssiponensis*. Frequency distribution of length of individuals (over 40 mm) for those measured in i) the museums, and ii) the field. Boxplots of length of individuals (over 40 mm) in iii) each region (irrespective of year), and iv) each year group (irrespective of location). The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers.

For *P. depressa,* almost all of the specimens were in the South West, with two in the South East, and therefore, there was no comparison between regions for this species. This species is only found as far north as Wales (Table 4.2). There was a significant difference in length between museum specimens and those measured in the field (W=15256, p=0.0134), with field individuals having a higher average size and larger maximum size than museum specimens (Fig. 4.7i-ii). There was a significant difference in length between year groups (chi^2=15.559, df=4, p=0.00367; Fig. 4.7iii), but there was no significant difference in shape between year groups (chi^2=37.005, df=4, p<0.001). The year group 1923-1969 had the largest average length over 20 mm, 1970-1989 had the lowest average length and the lowest maximum size (37.69 mm; Swanage, Dorset collected in 1974), while the modern group had the largest maximum size (43.6 mm; Swanage, Dorset in the field data). The modern group had the narrowest range of aspect ratios (1.000 to 1.432) and 1990-1998 had the largest range in aspect ratios (1.049 to 3.278). There was a significant positive correlation between length and year (rho=0.317, p<0.001); quantile regression analysis found that quantiles between 0.05 and 0.95 had positive slopes, which are significantly different from 0 (p<0.005). There was a significant positive correlation between length and longitude (rho=0.185, p=0.00395), and slopes in quantile regression analysis between 0.5 and 0.95 were positive and significantly different from 0 (p<0.05). There was a significant negative correlation between shape and year (rho=-0.348, p<0.001; Fig. 4.2iv) and between shape and longitude (rho=-0.201, p=0.00307; Fig. 4.2iii).

4.4.3 Trochids

As there was no size threshold for the diameter of the three throchid species, I was more interested in shape (aspect ratios) rather than changes in maximum size.

For *S. umbilicalis*, there was a significant positive correlation between diameter and height (rho=0.869, p<0.001; Fig. 4.2i). For *S. cineraria*, there was a strong positive correlation between diameter and height (rho=0.931, p<0.001; Fig. 4.2i). For *P. lineatus*, there was a significant positive correlation between diameter and height (rho=0.932, p<0.001; Fig. 4.2i). These species all had a similar slope (aspect ratio) when comparing diameter to height, and they were the shallowest slopes of the species studied here. This is likely because the shell shape for these species is relatively spherical compared to the other species (i.e. height and diameter are very similar). For these species, aspect ratio is diameter:height and therefore, individuals with a lower aspect ratio as relatively tall and those with a high aspect ratio are relatively flat.

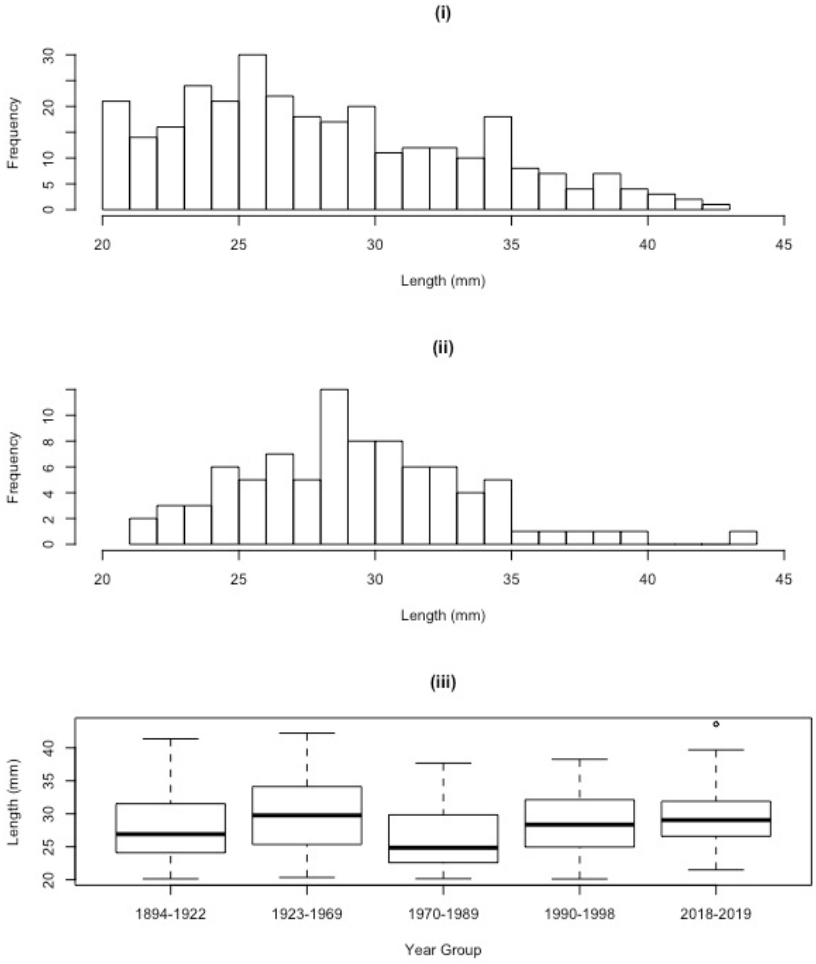

Figure 4.7 Plots of length for *P. depressa*. Frequency distribution of length of individuals (over 40 mm) for those measured in i) the museums, and ii) the field. iii) Boxplot of length of individuals (over 40 mm) in each year group. The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers.

For *S. umbilicalis*, there were no specimens in the North East, but specimens were compared between the other three regions. There were significant differences in diameter (chi^2=55.312, df=2, p<0.001) and shape (chi^2=348.820, df=2, p<0.001; Fig. 4.8iii) between regions, with differences between all three regions (p<0.05). The largest individual was in the South West (21.32 mm in diameter; Isles of Scilly collected in 1983) and the South East had the lowest maximum size (20.0 mm; Ramsgate measured in the field). The South East had the lowest average aspect ratio and the South West had the highest average ratio, with the North West having the smallest range in aspect ratios (1.005 to 1.938) and the South West having the largest range (0.546 to 1.925). There was also a significant positive correlation between shape and latitude (rho=0.233, p<0.001; Fig. 4.2ii), and quantile regression found that at quantiles between 0.05 and

0.95 had positive slope, which were significantly different from 0 ($p<0.005$). There was a significant negative correlation between shape and longitude (rho=-0.232, $p<0.001$; Fig. 4.2iii), with all quantiles between 0.05 and 0.95 being significantly negative ($p<0.001$). There was no significant difference in diameter between museum specimens and individuals measured in the field ($W=1192731$, $p=0.612$; Fig. 4.8i-ii). There were also significant differences in diameter ($chi^2=33.112$, $df=4$, $p<0.001$) and shape ($chi^2=390.946$, $df=4$, $p<0.001$; Fig. 4.7iv) between year groups. The year group 1970-1989 had the largest individual (21.32 mm; Isles of Scilly collected in 1983) and 1825-1922 had the smallest maximum size (19.7 mm; Isles of Scilly collected 1919). The modern group had the lowest average aspect ratio and the 1825-1922 group had the highest average aspect ratio as well as the lowest range in ratios (1.084 to 1.719), whereas the 1990-2009 group had the widest range of aspect ratios (0.546 to 1.938). There was a significant negative correlation between shape and year (rho=-0.380, $p<0.001$; Fig. 4.2iv), with slopes at all quantiles between 0.05 and 0.95 being significantly negative ($p<0.001$).

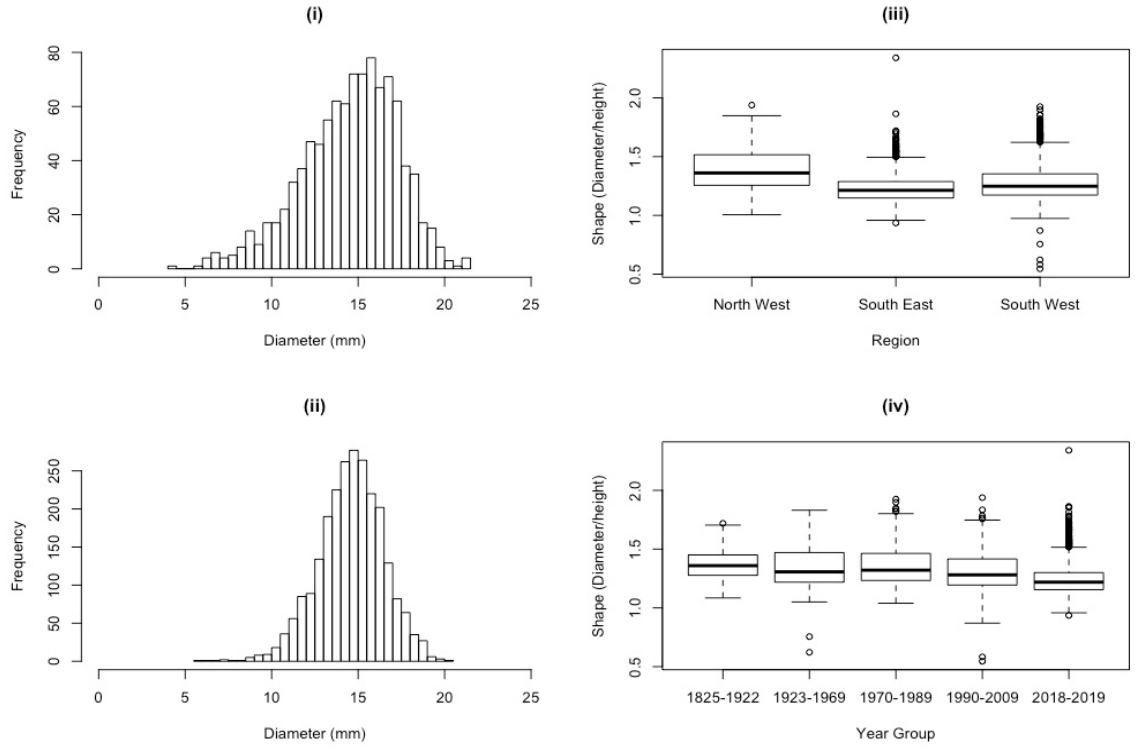

Figure 4.8 Plots of diameter and shape (aspect ratio) for *S. umbilicalis*. Frequency distribution of diameter of individuals for those measured in i) the museums, and ii) the field. Boxplots of shape of individuals in iii) each region (irrespective of year), and iv) each year group (irrespective of location). The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers. N.B. Shape is calculated as diameter divided by height.

For *S. cineraria,* there were significant differences in diameter (chi^2=53.917, df=3, p<0.001) and shape (chi^2=271.083, df=3, p<0.001; Fig. 4.9iii) between regions, with differences in size between the North and South (p<0.001), and differences in shape between all regions (p<0.05). The largest individual was in the North East (diameter of 22.77 mm; Moray Firth, East Scotland collected in 1976), with the South East having the lowest maximum size (17.05 mm; Brighton, Sussex collected before 1959). The South East had the lowest average aspect ratio and the narrowest range in values (0.804 to 1.612), with The North East having the highest average aspect ratio and the North West having the largest range (0.821 to 3.143). There was a significant correlation between shape and latitude (rho=0.152, p<0.001; Fig. 4.2ii), and all slopes at quantiles between 0.05 and 0.95 were significantly positive (p<0.001). There was a significant negative correlation between shape and longitude (rho=-0.176, p<0.001; Fig. 4.2iii), with slopes at quantiles between 0.05 and 0.90 being significantly negative (p<0.001). There was a significant difference in diameter between museum specimens and those measured in the field (W=451017, p<0.001), with the field individuals having a higher average size, but a lower maximum size than the museum specimens (Fig. 4.9i-ii). There were significant differences in diameter (chi^2=128.014, df=4, p<0.001) and shape (chi^2=223.4, df=4, p<0.001; Fig. 4.8iv) between year groups. The year group 1970-1989 had the largest individual (22.77 mm in diameter; Moray Firth, East Scotland collected in 1976), with the 1923-1969 group having the lowest maximum size (17.8 mm; Gosford Bay, East Scotland collected in 1968). The 1990-2004 year group had the highest average aspect ratio, with the 1872-1922 group having the lowest average; the modern group had the largest range in aspect ratios (0.819 to 3.143) and the 1923-1969 group had the lowest range (0.804 to 1.674). There was not a significant correlation between shape and year (rho=0.0138, p=0.151; Fig. 4.2iv).

For *P. lineatus*, the majority of the specimens were in the South West, with 10 in the South East but none in northern regions, and therefore size was not compared between regions. There was a significant difference in diameter between the museum specimens and the individuals in the field (W=44517, p<0.001), with the museum specimens having a higher average size and a larger maximum size than in the field (Fig. 4.10i-ii). There was a significant difference in diameter (chi^2=63.484, df=4, p<0.001) and shape (chi^2=23.716, df=4, p<0.05; Fig. 4.10iii) between year groups. Excluding the individual collected in 1990, the modern group had the lowest maximum diameter (26.1 mm; Bognor Regis, Sussex from the field data), while the 1923-1969 group had the largest individual (diameter of 31.25 mm; Helford, Cornwall collected before 1965). The year groups all had similar average aspect ratios, and all had relatively small range of shapes as the entire dataset only has a range from 0.846 to 1.388. There was a significant negative correlation between shape and year (rho=-0.150, p<0.001; Fig. 4.2iv), and quantile regression showed that

slopes of quantiles between 0.5 and 0.95 were negative and significantly different from 0 ($p<0.05$). There was not a significant correlation between shape and longitude ($p=0.772$; Fig. 4.2iii).

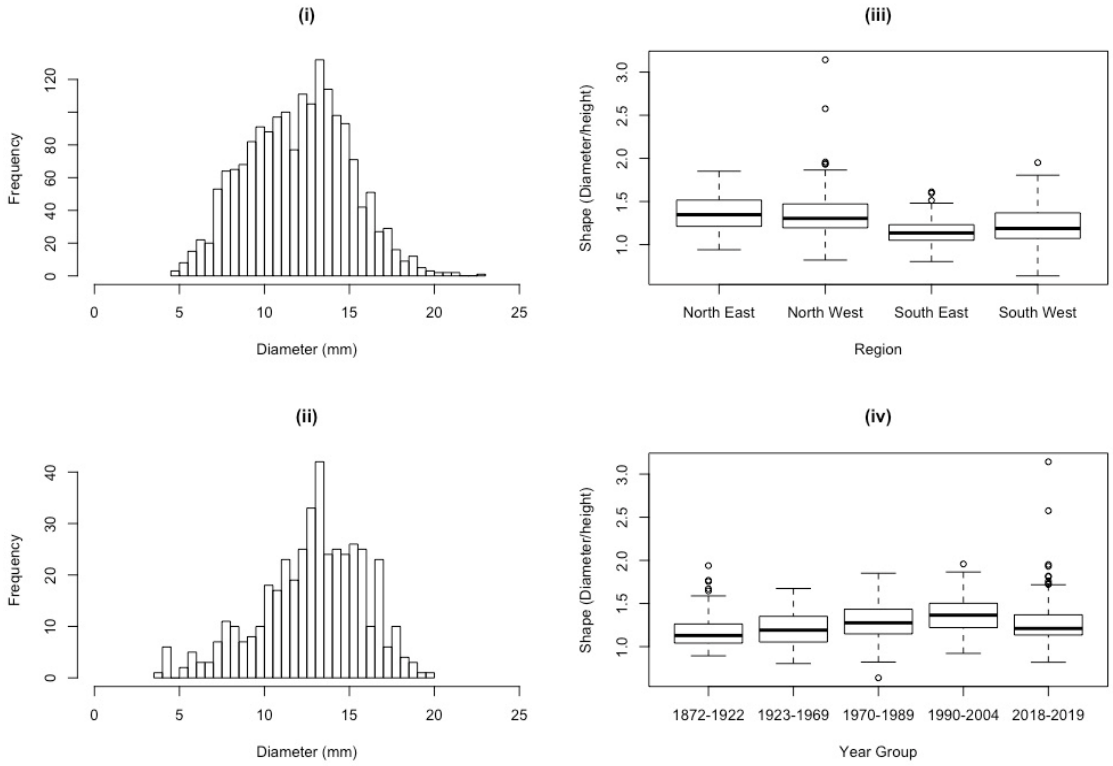

Figure 4.9 Plots of diameter and shape (aspect ratio) for *S. cineraria*. Frequency distribution of diameter of individuals for those measured in i) the museums, and ii) the field. Boxplots of shape of individuals in iii) each region (irrespective of year), and iv) each year group (irrespective of location). The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers. N.B. Shape is calculated as diameter divided by height.

4.5 Discussion

Previous research has shown that some species of marine gastropod have decreased in size over time, thereby following the temperature-size rule (Roy *et al.*, 2003; Wilson-Brodie, MacLean and Fenberg, 2017). It is thought that aquatic species will be more susceptible to decreases in size as a result of climate warming due to oxygen limitation in aquatic environments (Horne, Hirst and Atkinson, 2015). Here, I use a combination of historical museum specimens (from 100+ years ago) and modern field data to show that not all marine species have decreased in size over time. In

contrast to previous research on the predatory gastropod *N. lapillus* (Wilson-Brodie, MacLean and Fenberg, 2017), the maximum size and mean size of the larger size classes of the grazers studied here did not decrease over time. There were however, differences between regions, as was previously seen with *N. lapillus* (Wilson-Brodie, MacLean and Fenberg, 2017), and variations between temporal groupings. There were also temporal and spatial variations in shape of all species; these variations in size and shape are discussed below.

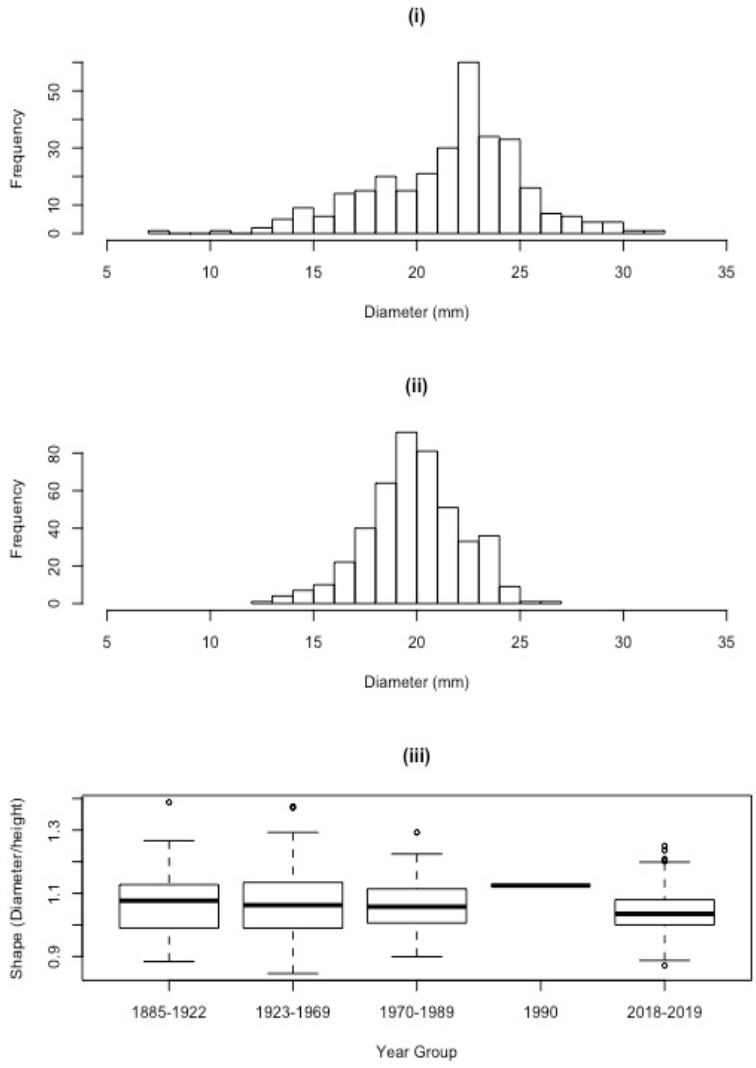

Figure 4.10 Plots of diameter and shape (aspect ratio) for *P. lineatus*. Frequency distribution of diameter of individuals for those measured in i) the museums, and ii) the field. iii) Boxplot of shape of individuals in each year group. The box represents the interquartile range, the line in the box represents the median value in each group, the whiskers are the 5th and 95th percentiles, and the circles represent the outliers. N.B. Shape is calculated as diameter divided by height.

4.5.1 Temporal and spatial variations in size

For all seven species, there were temporal and spatial (where applicable) differences in size. Within the temporal groups, the 1970-1989 group had the maximum size for four of the seven species (*P. vulgata, P. ulyssiponensis, S. umbilicalis* and *S. cineraria*); although, this group had the lowest maximum size for *P. depressa.* The modern group (field data) had the highest maximum size for *L. littorea* and *P. depressa*, but had the lowest maximum size for *P. lineatus*. Additionally, most species had a positive relationship between size and year (for specimens with a known year of collection), and while I cannot conclude that there has been an increase in size over time, these species have not decreased in size. This is in contrast to several other studies of marine molluscs, which have decreased in size over time (Roy *et al.*, 2003; Munroe *et al.*, 2016; Ortega *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017). As shown in Figure 4.4, these results differ from those found for *Nucella lapillus*, which had the largest maximum size (62 mm) in the oldest year group and the lowest maximum size in the field data (43 mm). *N. lapillus* also had a negative correlation between height and year (Wilson-Brodie, MacLean and Fenberg, 2017). A similar pattern was seen in four gastropods in California, which all had a lower average size in modern field data compared to museum specimens collected before 1960 (Roy *et al.*, 2003).

As expected, there were differences in size between regions, but these patterns varied between species. In two of the species (*L. littorea* and *S. cineraria*), individuals in the North were larger than those in the South both in terms of maximum size and average size (over 20 mm in height for *L. littorea*). The reverse was true for *P. ulyssiponensis* (for individuals over 30 mm length), while for *P. vulgata* there were similar maximum sizes but the South had a larger average size (for shells of length over 40 mm). For *S. umbilicalis*, however, the North had a larger average size but the South had a larger maximum. A previous study of crustaceans on the Pacific coastline found that intertidal species generally increase in size with increasing latitude (Jaramillo *et al.*, 2017), and this was also the case for the gastropod *Tegula funebralis* on the western US coast (Frank, 1975), suggesting this is a pattern which is common in multiple taxa. There was less variation in size between individuals from the East and West; only two species had a significant difference between regions (*P. vulgata* and *P. ulyssiponensis*), with those in the West being larger and having a larger maximum size than those in the East. This difference was also found previously in a study of *N. lapillus* in the southern UK (Fig. 4.4; Wilson-Brodie, MacLean and Fenberg, 2017). These differences between regions are also reflected in the correlations between size and latitude or longitude (Fig. 4.1). For the four species where there was a significant correlation for both size with longitude and latitude, a positive correlation for latitude accompanied a negative correlation with longitude and vice versa. This resulted in there being a general increase in size from the South East to the North West for *L. littorea, S. umbilicalis* and *S.*

cineraria, and the reverse for *P. vulgata*. Although regions were not tested for *P. lineatus* and *P. depressa,* there was a general increase in size from West to East within sites where those species were present.

For five of the seven species, there was a significant difference in size between museum specimens and those collected in the field (all but *L. littorea* and *S. umbilicalis*), and in most cases the field average size was higher than the museum average size. As I do not know the methods used for collecting the museum specimens, I can't determine if there were larger individuals present in each location at that time; I only know that they were at least as large as the largest individual collected. Furthermore, size of a species can change across the height of each shore; for example, *L. littorea* generally increases in height from low to high shore (Vermeij, 1973) and so if specimens were preferentially collected from one area of the shore this could skew results in a certain location or time period to have a smaller or larger average size than the actual value.

4.5.2 Temporal and spatial variation in shape

For all five of the species where regions were compared, there was a significant difference in shape between regions, and shape differed between year groups in all but two species (all except *L. littorea* and *P. depressa*). For species where regions were compared, eastern regions generally had narrower ranges of aspect ratios than in the West suggesting there is less variation in shape on those shores. Where there was a significant correlation, shape often had a positive correlation with latitude (in 3 out of 4 cases) and a negative correlation with longitude (in 5 out of 6 cases). Interestingly, if there is a positive correlation between shape and latitude, there is a negative correlation between shape and longitude and vice versa. This suggests that generally aspect ratios increase from the South East to the North West for *L. littorea, S. umbilicalis* and *S. cineraria*, and the reverse is true for *P. vulgata*. For *L. littorea,* this in turn means there is likely a higher proportion of globose individuals in the South East and a higher proportion of elongated individuals in the North West. Similarly, for *Steromphala* spp., there is likely a higher proportion of tall individuals in the South East and a higher proportion of flatter individuals in the North West. Whereas for *P. vulgata*, the individuals in the South East are more likely to have an oval shape and those in the North West more likely to be more circular. There was no significant correlation between latitude or longitude and shape for *P. ulyssiponensis*, or between longitude and shape for *P. lineatus*. Previous research on *P. ulyssiponensis* found that shape remained stable throughout growth and not related to length (Cabral, 2007), and therefore, shape may be more consistent between shores and conditions in this species.

The range in aspect ratios for each species varied between year, but later year groups (from 1970 onwards) generally had wider ranges of aspect ratios. The variation in shape between groups may be due to the specific locations where specimens were collected, and the wider range in later groups may be due to a wider range of sites collected from. Six of the seven species had a significant correlation between shape and year (all except *S. cineraria*); this was a negative correlation for all species apart from *P. vulgata*, which was also the only species to have a positive correlation between shape and longitude and a negative correlation between shape and latitude.

For the *Patella* spp., as length increases, the aspect ratio decreases suggesting longer shells are relatively wider than shorter shells (i.e. more circular). This is consistent with previous research for *P. vulgata*, which showed positive allometric growth (more circular shape as gets larger) (Cabral, 2007). Similarly, in the three throchid species, the aspect ratio decreases as diameter increases. Yet, in *L. littorea,* the aspect ratio increased as height increased suggesting that taller shells are relatively thinner compared to shorter shells. This was as expected given that previous studies have found that in intertidal gastropods (including littorinids) that shape of individuals differs with wave exposure. This was found in *Buccinanops globulosus* and littorinids *Littorina saxatilis* and *Littorina obtusata*, where wave exposed shores have small, thin shells with large apertures and sheltered shores have large, thick shells, with small apertures (Trussell *et al.*, 1993; Queiroga *et al.*, 2011; Avaca *et al.*, 2013).

Given the above, I would expect that in species where there was a positive relationship between size and year, there would be a negative correlation between shape and year (except for *L. littorea* where I would expect a positive correlation due to the positive correlation between shape and height). Yet, this was not the case for most species, in fact, only *P. ulyssiponensis* and *P. depressa* followed this. For most of the other species, the correlations between shape and year were quite low ($r<0.2$). As there was generally a large variation in aspect ratios, even within regions and year groups, these correlations may have been influenced by outliers (individuals with very high or low aspect ratios). Additionally, as previously mentioned, I cannot determine whether a species has increased in size over time, and therefore, for species which become increasingly wider as they get taller, I cannot determine if shape has decreased over time as there may be a bias of larger (and so likely wider) individuals missing from past samples. The only species which goes against this is *L. littorea*, but as the correlations are low and the ranges in aspect ratios are high I do not think that shape has changed much over time, and thereby is not likely being affected by temperature.

4.5.3 Biological implications

The growth of species can be impacted by the other species that are present on the shore due to competition between the species. *Patella vulgata* is one of the most ubiquitous species on UK rocky shores, which may be because this species is tolerant to a range of wave exposures (Blackmore, 1969). This species is also found at most heights on the shore, and at low levels is found alongside *P. ulyssiponensis* in some regions (Blackmore, 1969). *P. vulgata* is a northern cold-adapted species, but its range extends into southern Europe, whereas other pattelids found on UK shores (*P. ulyssiponensis* and *P. depressa*) are southern warm-adapted species (Guerra and Gaudencio, 1986; Hawkins *et al.*, 2008). Therefore, it would not have been surprising if *P. vulgata* had been affected by the recent climate warming, but this was not the case. An experiment found that when *P. depressa* and *P. ulyssiponensis* were also present in pools, the growth of *P. vulgata* decreased but growth was not affected when found alongside *P. depressa* on open rock (Firth *et al.*, 2009). I found that *P. vulgata* was largest in the South West, where it was found alongside *P. ulyssiponensis* and *P. depressa*, and although I cannot be certain if they were living on open rock or in rock pools it does not appear as though the presence of the other limpet species has affected the growth of *P. vulgata* in the South West UK.

Wave exposure can affect the growth, size, and shape of a rocky shore species; I did not measure that here as it cannot be accounted for in museum data, but it may have had some influence on the morphology of individuals, particularly the shape. For example, individuals of *P. vulgata* on wave sheltered shores have a slower growth rate than those on exposed shores, despite there being higher food availability on sheltered shores (Jenkins and Hartnoll, 2001). Grazing rate also differs between these shores, with sheltered shores having stable rates compared to seasonal patterns in grazing on exposed shores, leading to higher overall grazing on exposed shores (Jenkins and Hartnoll, 2001). Shape (and height) of some intertidal gastropods is often related to stress factors such as predation, desiccation and dislodgement (Queiroga *et al.*, 2011; Avaca *et al.*, 2013). Shells tend to be taller and thicker on sheltered shores to give better protection against mobile predators, which are abundant on these shores, and against heat stress and desiccation. Conversely, on wave exposed shores shells tend to be shorter with wide apertures to prevent dislodgement (Trussell *et al.*, 1993; Queiroga *et al.*, 2011; Avaca *et al.*, 2013). Varying wave exposure between shores may have led to a wide range in maximum sizes and aspect ratios within regions or year groups, but as there is a large amount of data in each group this should not have affected the overall results.

Gastropods with taller shells, such as *L. littorea*, are less well morphologically adapted to avoid predation than species with lower aspect ratios, such as *S. umbilicalis, S. cineraria* and *P.*

lineatus. A study of the four species found that *L. littorea* instead relied more on behavioural response to predators, but this could lead to a competitive disadvantage to the species with a lower aspect ratio (Cotton, Rundle and Smith, 2004). Furthermore, *L. littorea* do not have stronger shells on sheltered shores (Currey and Hughes, 1982), this suggests they are solely relying on behavioural adaptations on sheltered shores. Changes in predator abundance on these shores, could therefore have knock on effects on *L. littorea* populations. If predators were to decrease in abundance or in size, as was seen previously with *N. lapillus* (Wilson-Brodie, MacLean and Fenberg, 2017), then this may benefit *L. littorea* as this species is regulated by predator abundance. This is in line with results found in this study, where grazers, including *L. littorea,* did not decrease in size over time. Predators are likely to be most vulnerable as they are food limited and would require more food at higher temperatures to counteract increasing metabolic rates. If individuals cannot compensate for this, then adult body sizes are predicted to get smaller over time as temperatures rise (Sheridan and Bickford, 2011; Wilson-Brodie, MacLean and Fenberg, 2017). Furthermore, size is thought to affect communities through its effects on predation, competition and energy use (Nelson *et al.*, 2017), and therefore, changes in size of one species could have an impact on other species on the shore. At lower trophic levels, local biotic and abiotic conditions may be as important than climate warming for determining size and shape of grazing gastropods on UK rocky shores, and while they are not currently decreasing in size, impacts on other species could have a knock-on effect in the future.

4.5.4 Conclusions

I have shown that the size and shape of seven gastropod species vary both spatially and temporally on UK rocky shores. Unlike patterns seen previously in other gastropod species, there has not been a decrease in size over time. There was however, clear spatial trends with size along latitudinal and longitudinal gradients, and the direction of the gradients depended upon the species. These gradients were also found in some species in relation to their shape. Complex interactions between species and their biotic and abiotic environment can affect both size and shape, as well as abundance. Therefore, any changes to a species morphology, distribution or abundance due to climate warming (or other anthropogenic impacts) will likely have knock on effects on other species on the shore. This means that in the future there could be more species that show changes in morphology, and although the grazers measured here seem relatively robust compared to other species, this may change in the future.

Chapter 5 Natural history collections as proxies for spatial and seasonal abundance patterns: a test using butterflies and snails

5.1 Abstract

Museum collections are useful resources for ecological, biogeographical, and conservation research. However, museum collections are rarely used to assess changes in patterns of spatial or seasonal abundances of species over historical time-scales. To assess whether NHC data do reflect actual abundance patterns, I compare the spatial abundance of four species of rocky shore gastropods along the North Pacific coastline, and temporal abundance of five species of butterflies in the UK. The results show that for most species, there was no significant difference in the abundance curves of the museum and field data. In two species, however, differences could be explained by changes in the modern phenology or distribution in response to climate change. These results suggest that museum collections can be a useful proxy for spatial and seasonal abundance patterns of both marine and terrestrial invertebrates.

5.2 Introduction

The spatial and temporal distribution of the abundance of individual species are necessary datasets for many ecological, biogeographic, and conservation biology studies. At a minimum, the acquisition of such data requires a field record of the abundance of individuals of a species at geo-referenced locations and times (e.g. year/season). Monitoring programmes can provide such datasets, and have been instrumental in providing the means to test a range of questions, from large scale biogeographic analyses testing the 'abundant centre hypothesis' (Sagarin and Gaines, 2002) and biogeographic structure and diversity patterns (Fenberg *et al.*, 2015), to documenting inter-annual variation in biotic responses to climate change, including phenological and range shifts (e.g. Roy and Sparks, 2000; Diamond *et al.*, 2011). While these datasets are valuable for their utility in asking basic and applied research questions, they are usually limited in scope as

they are either restricted to a single or small handful of species, or may be restricted to a narrow spatial and/or temporal range or habitat type (Magurran *et al.*, 2010; Fenberg and Rivadeneira, 2011; Johnson *et al.*, 2011; Robbirt *et al.*, 2011; Meineke *et al.*, 2018). For example, there is often spatial bias towards the northern hemisphere, and developed countries (Magurran *et al.*, 2010), and some taxa are more well suited to long-term monitoring programmes (or Citizen Science projects), such as birds or butterflies (Roy and Sparks, 2000; Bartomeus *et al.*, 2011, 2018; Polgar *et al.*, 2013). While these studies provide scientists with useful information on how the abundances of species may respond to anthropogenic stressors, such as recent climate warming, they are often restricted to more recent decades (e.g. post 1970's). Therefore, they do not include a comparison against a historical baseline prior to the recent onset of climate warming (pre-1970's) (Meineke and Davies, 2018). An alternative source of spatial and temporal abundance data may be acquired from the vast collections housed at the Natural History Collections (NHC's) of the world – but they are rarely used for that purpose.

If well-documented, a natural history specimen is an unambiguous data point confirming the presence of a species at a specific time and location (an occurrence) (Pyke and Ehrlich, 2010; Johnson *et al.*, 2011), and therefore, collections may also inform us about past distributions (Pyke and Ehrlich, 2010). Often, a single occurrence contains many specimens, and the number of specimens collected within a spatial bin (e.g. per 1º latitude) or time period (e.g. per season, month or week) could be related to its actual spatial and/or seasonal abundance in the field. This is because collectors would encounter common individuals in the field more often than rare ones – just as field ecologists do when estimating individual species abundances (spatially or seasonally). Thus, it may be possible to approximate species' spatial and/or seasonal abundance patterns by quantifying the relative abundance of individuals collected across space and/or time. For such data to be as un-biased as possible, multiple museums containing specimens from multiple collectors and spread across the geographic range of the species in question should be prioritised. Likewise, if interest is in seasonal abundance patterns, data should be gathered from museums with collections spread across season(s) when the species in question are active.

Museum collections can provide scientists with long term datasets, for example from the 19th century, for which field data would not usually be available (Lister *et al.*, 2011). This data can be useful in studying changes in range and phenological timings by providing a baseline to compare against recent changes (Pyke and Ehrlich, 2010; Johnson *et al.*, 2011; Kharouba *et al.*, 2018; Meineke *et al.*, 2018). These collections, especially in large national museums with world collections, often cover a broad range of taxa and geographical locations (Johnson *et al.*, 2011). This allows for broad-scale investigations into spatiotemporal abundances that would not be possible from field surveys. Meta-data of museum specimens can be particularly useful in helping

to establish changes in the historical range of a species, and date of collections may help establish changes in the phenology of some short-lived species, such as butterflies, through time (Bartomeus *et al.*, 2011; Fenberg and Rivadeneira, 2011; Robbirt *et al.*, 2011; Polgar *et al.*, 2013; Fenberg, Posbic and Hellberg, 2014; Brooks *et al.*, 2017; Meineke and Davies, 2018; Meineke *et al.*, 2018). For example, a study of the owl limpet (*Lottia gigantea*) using museum specimens and field observations from the last 140 years in California (USA) and Baja California (Mexico) found that the northern range had contracted since 1963, and that the highest abundances were in the centre of the range in both the museum specimens and field observations (Fenberg and Rivadeneira, 2011). Another study using museum specimens found that there had been a northern range expansion and southern range contraction for the gastropod *Mexacanthina lugubris* (Fenberg, Posbic and Hellberg, 2014). Some studies have used NHCs in combination with long-term monitoring data, field data or citizen science data to show that the phenology of insects such as bees and butterflies are advancing over time and in relation to increasing temperatures (Bartomeus *et al.*, 2011; Polgar *et al.*, 2013; Brooks *et al.*, 2017). Yet, until now NHCs have not been used to show changes in peak abundance of a species.

There are, however, some drawbacks to using museum collections. For example, in studies of distribution, museum collections can only reveal where a species was present in the past, but not where they were absent as they are unlikely to have been collected at every location across their range (Bartomeus *et al.*, 2018; Kharouba *et al.*, 2018). This, however, may also be true of modern data depending on the collection methods. For example, monitoring surveys would reveal if a species was absent from a specific location, but distributional data recording would only record where a species was present. Some specimens are not accompanied by good quality meta-data, and therefore vital information such as date and location of collection may be unknown (Polgar *et al.*, 2013), and ecological context is rarely provided (Pyke and Ehrlich, 2010; MacLean *et al.*, 2018). Furthermore, sampling methods are usually unknown; it is unlikely that systematic sampling, which would be part of a well-designed field study, would have been used to collect museum specimens (Kharouba *et al.*, 2018; Meineke and Davies, 2018; Meineke *et al.*, 2018). Opportunistic collectors may be biased in their choice of specimens to collect, taking for example, the largest, brightest coloured or rarest individuals and avoiding damaged individuals (Pyke and Ehrlich, 2010; Bartomeus *et al.*, 2018; Kharouba *et al.*, 2018). There may also be a bias in timing, locations and effort of sampling, which may lead to collecting more specimens at the start or end of a specific event e.g. the flowering of a plant species or certain areas (likely those which are easily accessible) being more prevalent in collections (Pyke and Ehrlich, 2010; Kharouba *et al.*, 2018; MacLean *et al.*, 2018; Meineke and Davies, 2018). Due to the lack of standardized sampling methods, there will be some form of collection bias, and therefore specimens may not always be

representative of the natural environment. Having said this, some opportunistic collectors kept good notes of their collections, which were passed onto museums along with the collections, and collected a variety of species from a range of locations (Pyke and Ehrlich, 2010; Johnson *et al.*, 2011). For example, some collections include information on other species present at the location or about the abiotic environment (e.g. wave exposure or type of rocks present), or give a specific point within the location where the specimen was collected from. It would be useful to have records of sampling methods so that researchers could determine whether the specimens are appropriate for use in their studies.

In order to test whether NHC datasets are representative of field abundances (e.g. from monitoring programmes), then individual species from both types of datasets should be statistically compared to each other. If, for example, a researcher is interested in the abundance pattern of a species across its range, then a geo-referenced dataset of NHC occurrences across its geographic range could be compared to a corresponding georeferenced dataset of field abundances. For seasonal abundances, the abundances per time unit across the year (e.g. per week) in the field could be compared to the distribution of NHC specimens across the same time units. While the absolute number of individuals cannot be compared between datasets because sampling strategies are different, the relative shape of abundance distributions can be compared by examining density curves derived from frequency data (i.e. from field abundances or number individuals per occurrence). Yet, until now this has not been previously studied.

Such a study would be based on several assumptions. For example, it would assume that the species that are most common at a particular point in time or location in the natural environment are the species that have been most commonly collected and are present in the highest numbers in museum collections. It would also assume that the metadata given for museum collections (used for georeferencing) are correct. By using specimens from multiple collectors and multiple museums (where possible), I can reasonably assume that the most common organisms in the field will be the most commonly collected. If museum collections can be used as a proxy of the shapes of seasonal/spatial abundance patterns, then this will provide even further evidence of the importance of NHCs for ecological research, and may even pave the way for their use in tracking shifts in abundance over time as a result of climate warming or making predictions of future effects.

So, with all of the above considered, are the spatial and temporal abundances of specimens in NHCs representative of real life abundances? In order to answer this question, I use museum specimens and field data collected after the mid-1970s to determine if patterns of spatial and temporal (i.e. seasonal) abundance of species in the field is similar to the relative abundance of

NHC specimens collected across space and time. Two taxa which are useful for this type of research are Mollusca and Lepidoptera as these groups are well-represented both in monitoring programmes and in museum collections (Wilson-Brodie, MacLean and Fenberg, 2017; Kharouba *et al.*, 2018; MacLean *et al.*, 2018). For spatial abundance, I use gastropod data from the western North American coast, and for temporal abundance, I use butterfly data from the UK. I hypothesize that the spatial abundance distribution of gastropods from museum collections will be representative of the distribution in the field data, and that the temporal (seasonal) abundance of butterflies from museum collections will be representative of their abundance distribution in the field data. I anticipate, however, that there may be some differences in the timing or location of peak abundances due to shifts in phenology and distribution that have occurred in the recent past due to climate change. This is particularly likely for the butterflies as UK butterflies have been shown to have generally emerged earlier in recent decades (Roy and Sparks, 2000), and therefore I expect a mismatch in timings of first and peak abundance.

5.3 Material and Methods

5.3.1 Gastropod spatial abundance data

Spatial abundances of four gastropod species (*Lottia gigantea, Tegula funebralis, Fissurella volcano* and *Mexacanthina lugubris*) were measured from the west North American coastline (from Washington, USA, to Baja California Sur, Mexico; 48°N to 26°N) using field data collected between 2000 and 2010. Field data were obtained from the Multi-Agency Rocky Intertidal Network (MARINe; https://marine.ucsc.edu/index.html), using quadrat data from biodiversity surveys. The quadrats (0.5m x 0.5m) were placed on eleven transects at 3m intervals in each of the high, mid and low shore zones, giving 33 quadrats at each site. Within each quadrat, the numbers of each mobile invertebrate species present were recorded. Some sites had more or less than 33 quadrats, but I excluded these data to ensure consistency in collection methods between sites. Furthermore, some sites were sampled in multiple years and therefore I averaged the count numbers of each species across years at these sites. The full sampling methods used by MARINe can be found at https://marine.ucsc.edu/ methods/biodiversity-methods.html.

The museum data were obtained from meta-data taken from searches of collections on museum data portals and for *L. gigantea*, from Fenberg and Rivadeneira (2011). The museums used were: Los Angeles County Museum, San Diego Museum of Natural History, Santa Barbara Museum of Natural History, California Academy, Hertz private collection (San Diego), Scripps Institute of

Oceanography (Benthic Invertebrate Collection), Burke Museum (Seattle), University of California Museum of Palaeontology, and the Smithsonian National Museum of Natural History. Specimen data were obtained from data portals for California Academy (http://researcharchive.calacademy.org/research/izg/iz_coll_db/index.asp), Santa Barbara Museum of Natural History (http://researcharchive.calacademy.org/research/izg/ iz_coll_db/ index.asp), the Smithsonian (https://collections.nmnh.si.edu/search/iz/), and GBIF (gbif.org; Groves and Mertz, 2020). Specimens were only included where a latitude or an exact location (which could be converted into latitude) was given, and islands were excluded. A summary of the data used is given in Table 5.1.

Table 5.1 Latitudinal range and number of specimens used in the gastropod spatial abundance analysis from both museum and field data. Note: for number of individuals in the field, the value in brackets is the number used in analyses after the data was averaged across years at each site.

Species	Number of specimens (museums)	Latitudinal range (museums)	Number of individuals (field)	Latitudinal range (field)
Lottia gigantea	1896	26.983-41.750°N	998 (430)	26.705-38.881°N
Tegula funebralis	3442	28.089-43.488°N	38737 (13172)	32.665-48.171°N
Fissurella volcano	4011	26.723-38.417°N	1791 (914)	26.705-36.638°N
Mexacanthina lugubris	920	26.723-34.276°N	438 (401)	26.705-32.871°N

5.3.2 Butterfly phenology data

For the temporal abundance datasets, weekly abundance data was obtained for five bivoltine (or multivoltine) British butterfly species (*Polyommatus bellargus, Polyommatus icarus, Lycaena phlaeas, Aricia agestis* and *Pieris napi*). For the field collected data, weekly count data throughout the flight season (recorded from 1st April to 30th September) were obtained from the UK Butterfly Monitoring Scheme (UKBMS) for years between 1976 and 2018. For the museum data, collection dates (converted into number of specimens collected each week) were obtained from meta-data of specimens held in the Natural History Museum (NHM) Lepidoptera collections. UKBMS data were restricted to sites for the counties which were most common in the museum collections to limit the effects of locality on the results. For *P. bellargus*, which was common in both the

museum and UKBMS data, only data from Folkestone was used. *Polyommatus coridon* was also common in the collections, and in this case only data from Kent (the most common county) were used. For all other species, all of the NHM data (from across the UK) were compared to county level data from the UKBMS. The counties selected for the UKBMS data for these species were based on which counties were most common in the NHM data to minimise the effect of locality on the results. A summary of the data used for each species is given in Table 5.2.

Table 5.2 Year range, localities and number of specimens for the data used in the butterfly phenology analysis for both museum (NHM) and field (UKBMS) data.

Species	NHM year range	NHM localities	NHM number of specimens	UKBMS year range	UKBMS localities	UKBMS number of specimens
P. bellargus	1884-1969	Folkestone	1321	1987-2017	Folkestone	5033
P. icarus	1847-1994	Kent	557	1976-2018	Kent	75495
A. agestis	1878-1981	All UK	751	1976-2018	Dorset, Hertfordshire	20582
L. phlaeas	1877-1992	All UK	1012	1990-2018	Hertfordshire	3328
P. napi	1884-1996	All UK	710	1976-2018	Devon, Hertfordshire	43116

5.3.3 Data analysis

For the gastropod data, only the latitudes of the sites were used for analysis to give a count of the number of individuals at each latitude. This was done for the field data and the museum data separately. The count data for museum specimens were not averaged across years (unlike the field data) as many sites only had data from one year. For the butterfly data, the weekly count data were combined across years to give a total count per week for both the field and museum data.

For both gastropod and butterfly species, density curves were produced for both museum and field data, and the area under the curve was calculated, per week for temporal data (butterflies) and per 1° latitude for the spatial data (gastropods). The proportion of data in each bin for the museum and field density curves were then compared to determine if the distributions of the data were significantly different using a two-sample K-S test. However, K-S tests require a continuous distribution, but as the data included ties due to some latitude and weekly bins having the same proportion of data, the distributions were not continuous. Therefore, I bootstrapped

(n=1000) the data to overcome this problem. Data were analysed using the function ks.boot in the R statistical package Matching (Sekhon, 2011). The graphs for the density curves were created using bandwidths of 0.6 for the gastropods and 0.75 for the butterflies to smooth out the curves. A higher bandwidth equates to more smoothing of the curve; this, however, did not affect the area under the curves calculated to be used in the K-S tests, as this was done without smoothing. Smoothing of the curves allows for a more general trend of the data to be seen as it 'smooths' out the natural oscillation of the data, but too much smoothing may result in some peaks being lost. A lower bandwidth was used for the gastropod data as there are more peaks and troughs in those curves compared to the butterflies (which generally have two clear peaks), and when a higher value was used some of the peaks merged together. Additionally, a lower bandwidth of 0.4 was used for *T. funebralis* as this species has some peaks which were lost when using a bandwidth of 0.6.

For *P. icarus,* there was a large amount of data points in the UKBMS data from Kent; there were 75,789 individuals counted between week 0 (week starting 25th April) and week 30 (week starting 21st October) in the period from 1976 to 2018. The data was separated into year groups covering 5 year intervals starting at 1976, apart from the final year group, which covered the years 2016 to 2018. The peak abundance was then determined for both generations one and two within each year group; the peaks were deemed to be at the weeks in which the total count was highest within that year group. The peak abundances were compared between year groups for both generations.

5.4 Results

5.4.1 Gastropod spatial abundance

There were no significant differences between the density curves of spatial abundance of museum and field specimens for *Lottia gigantea* (D=0.3125, $p_{k.sboot}$=0.379; Fig. 5.1)*, Fissurella volcano* (D=0.3846, $p_{k.sboot}$=0.248; Fig. 5.2) and *Mexacanthina lugubris* (D=0.3333, $p_{k.sboot}$=0.588; Fig. 5.3). However, there was a marginally significant difference in spatial abundance between museum and field specimens for *Tegula funebralis* (D=0.3913, $p_{k.sboot}$=0.045; Fig. 5.4).

For *L. gigantea*, both the museum and field abundance peaked between 33 and 34°N; the field data stops at 38°N, but there is still a small proportion of museum data between 38 and 42°N (Fig. 5.1), which represents a confirmed range contraction since the 1960's (Fenberg and Rivadeneira,

2011). For *M. lugubris,* both museum and field data show two peaks; an initial small peak at 27-28°N and a second larger peak at 31-32°N (Fig. 5.2).

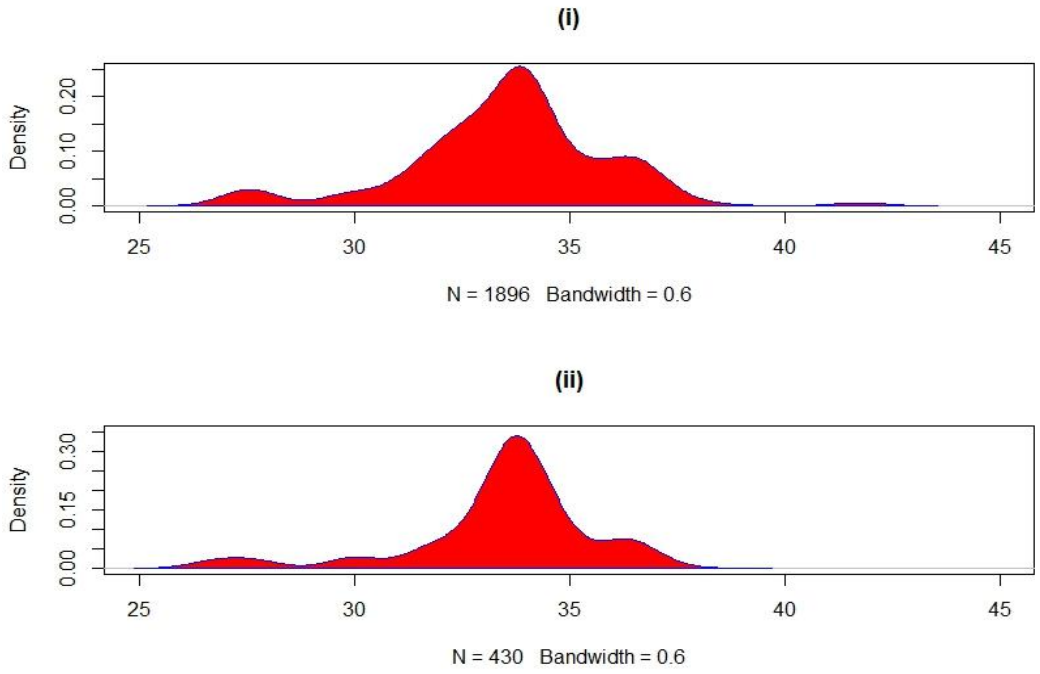

Figure 5.1 Density curves of abundance of *Lottia gigantea* i) in museum collections, and ii) at field sites between 26 and 42°N on the western North American coastline. Bandwidth refers to the amount of smoothing applied to create the curves.

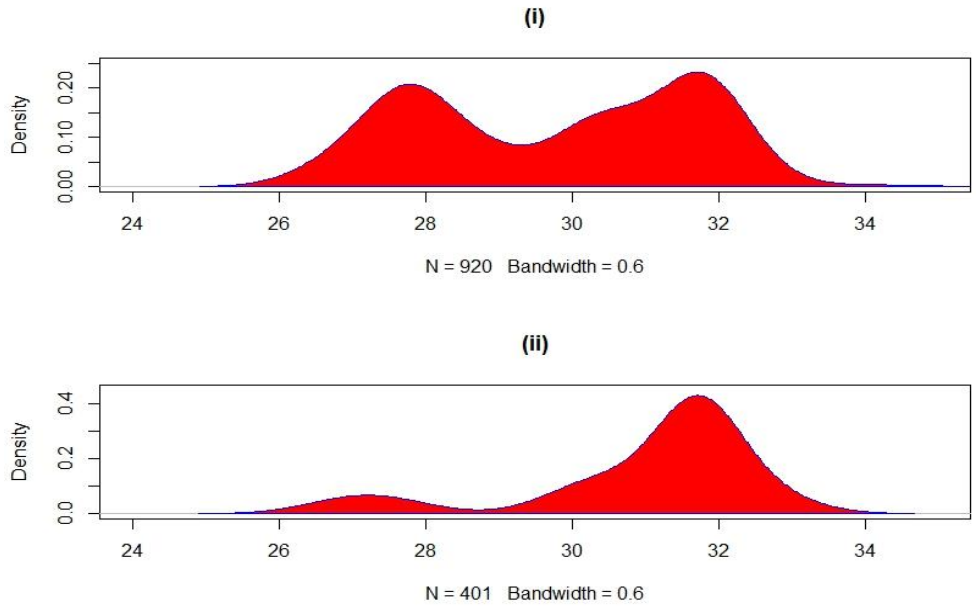

Figure 5.2 Density curves of abundance of *Mexacanthina lugubris* i) in museum collections, and ii) at field sites between 26 and 35°N on the western North American coastline.

There are some differences in the spatial abundance of museum and field data for *F. volcano*, the largest peaks in both museum and field data, however, are at 33-34°N (Fig. 5.3). For the field data, there is another large peak at 27-28°N; there is a peak at the same latitude for museum data but it is relatively small. The field data has two additional (but smaller) peaks at 30-31°N and 36-37°N. Additionally, the field data stops at 37°N but the museum data continues to 39°N (Fig. 5.3).

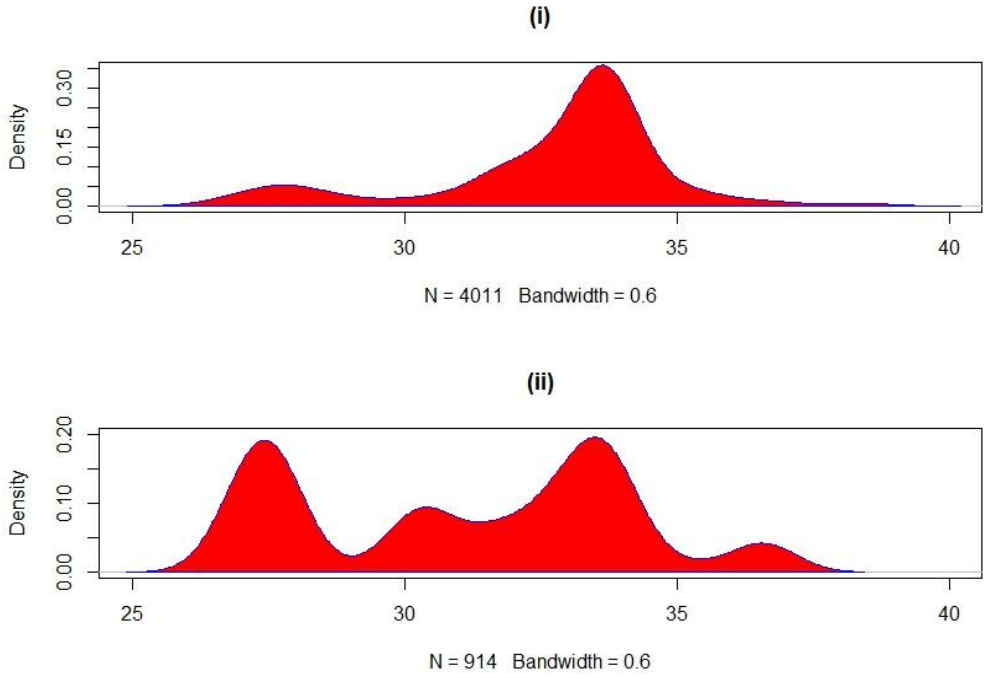

Figure 5.3 Density curves of abundance of *Fisurella volcano* i) in museum collections, and ii) at field sites between 26 and 39°N on the western North American coastline.

For *T. funebralis*, the field data has a main peak at 37-38°N, yet at this latitude there is a dip in abundance in the museum data (Fig. 5.4), and a smaller peak at 35-36°N. The museum data has two large peaks at 35-36°N and 38-39°N, and two other small peaks at 43-44°N and 48-49°N. The field data starts at 32°N whereas the museum data starts at 28°N (Fig. 5.4); this is because in the field data, below 32°N *T. funebralis* co-exists with *T. gallina* and the two species cannot be easily distinguished.

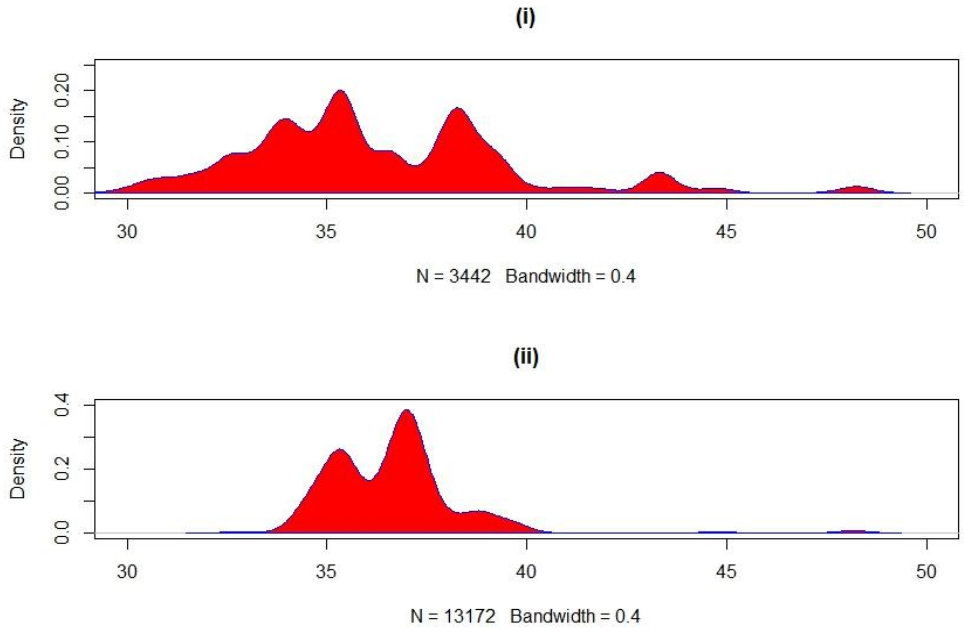

Figure 5.4 Density curves of abundance of *Tegula funebralis* i) in museum collections, and ii) at field sites between 28 and 49°N on the western North American coastline.

5.4.2 Butterfly temporal abundance

There was no significant difference between the museums and UKBMS density curves in temporal abundance for *P. icarus* (D=0.2581, $p_{k.sboot}$=0.222), *A. agestis* (D=0.333, $p_{k.sboot}$=0.139), *P. napi* (D=0.2759, $p_{k.sboot}$=0.189) and *L. phlaeas* (D=0.1724, $p_{k.sboot}$=0.739). There was, however, a significant difference between the museum and UKBMS density curves of abundance of *P. bellargus* at Folkestone (D=0.4783, $p_{k.sboot}$=0.01). Although the density curves were generally similar, there were differences in the timings of peak abundances between the NHM and UKBMS data for most species.

For *P. bellargus*, the peak abundance was earlier for the UKBMS data for both generations (Fig. 5.5). In generation one, the NHM peak abundance is week 10 (week starting 3rd June) compared to week 9 (week starting 27th May) in the UKBMS data, whereas in generation 2, the NHM and UKBMS peak abundances were in week 23 (week starting 2nd September) and week 22 (week starting 26th August) respectively. Additionally, UKBMS data showed emergence as early as week 4 (week starting 22nd April), whereas the earliest museum specimen was collected in week 6 (week starting 5th June).

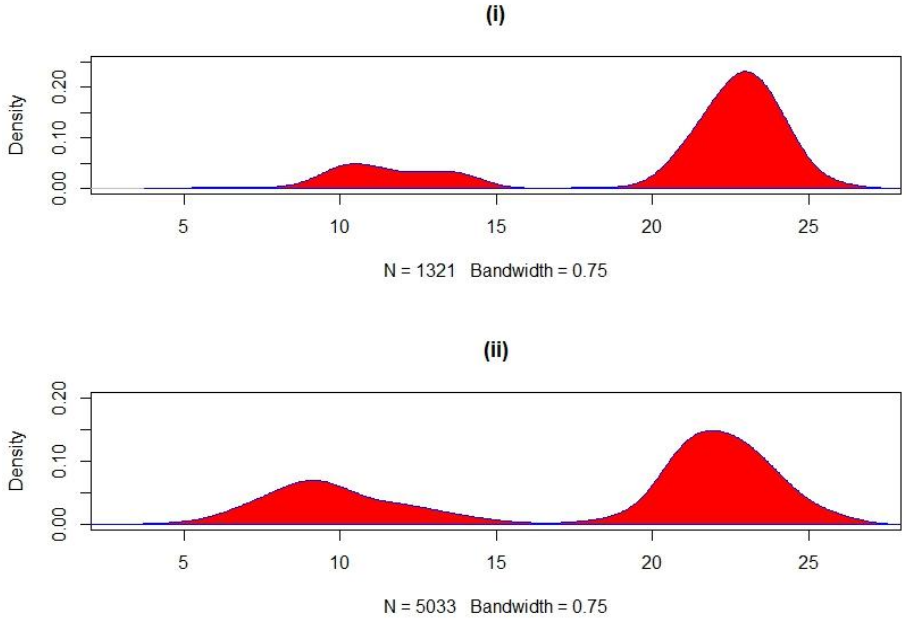

Figure 5.5 Density curves of the abundance of adult *Polyommatus bellargus* at Folkestone based on i) collection dates on specimens held in the NHM collections, and ii) data collected by UKBMS.

For *P. icarus,* there was a difference in the timing of peak abundance for both generations between the museum and field data (Fig. 5.6). For the NHM specimens, the peak abundance of generation one was in week 10 (starting 3rd June) and the peak abundance of generation two was in week 20 (starting 12th August). The UKBMS data peaked a week earlier in both cases; in week 9 (starting 27th May) and week 19 (5th August) for generations one and two respectively.

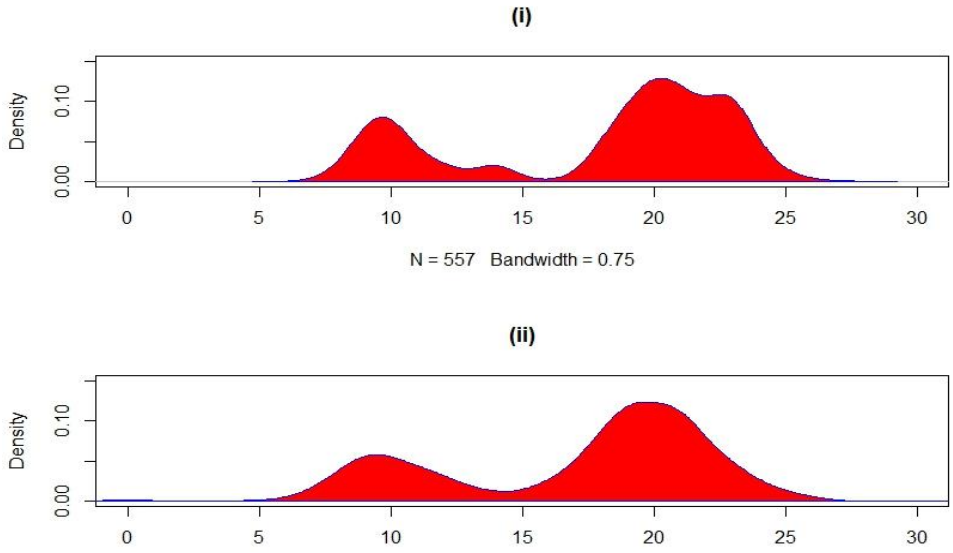

Figure 5.6 Density curves of the abundance of adult *Polyommatus icarus* in Kent based on i) collection dates on specimens held in the NHM collections, and ii) data collected by UKBMS.

Due to the large quantity of UKBMS data available for *P. icarus* in Kent, I was able to split the data into 5-year bins (apart from the last bin which was 2016-2018). The peak abundance for both generations vary over time (Fig. 5.7); generally, the timing of the peak abundance advances over time, with the earliest peak abundances occurring in the most recent years.

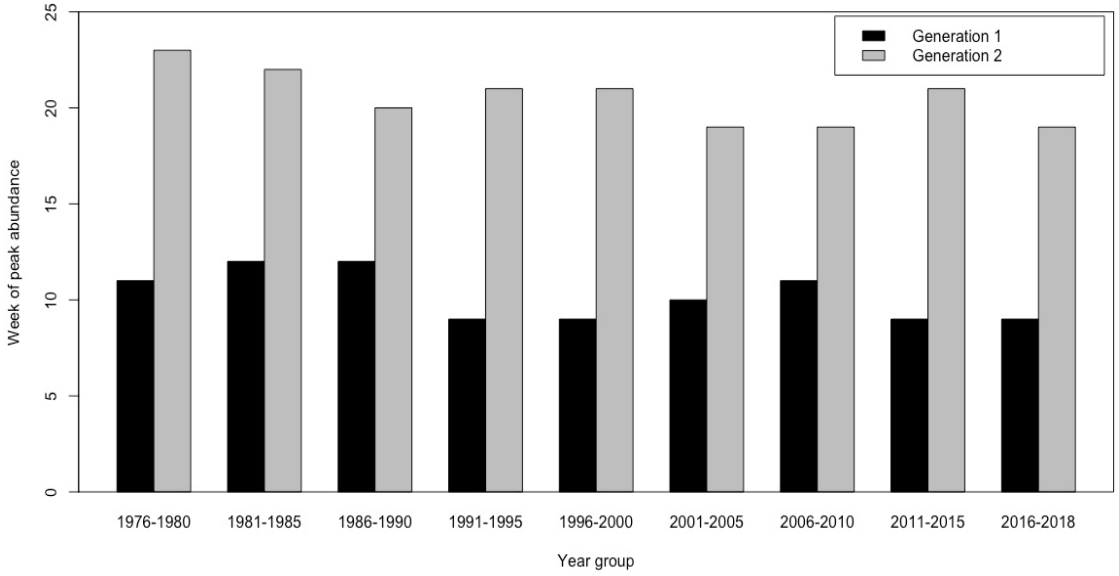

Figure 5.7 The timing of peak abundance of generation one and two adult *P. icarus* in Kent between 1976 and 2018 based on count data provided by the UKBMS.

For *A. agestis*, there was a difference in the timing of peak abundance between museum and field data for both generations (Fig. 5.8). In generation one, the peak abundances were in weeks 9 and 10 (weeks starting 27th May and 3rd June) for the UKBMS and NHM data respectively. For generation two, the peak abundance for UKBMS data was in week 21 (starting 19th August) and the peak abundance of the NHM data was in week 20 (starting 12th August).

For *P. napi,* both the museum and field data had the peak abundances in generations one and two occurred in the same weeks (Fig. 5.9). For generation one, the peak abundance was in week 7 (starting 13th May) and for generation two, peak abundance was in week 18 (starting 29th July).

For *L. phlaeas* generation one, there was no difference in the timing of peak abundance between UKBMS and NHM data; peak abundance occurred in week 9 (starting 27th May; Fig. 5.10). In generation two, however, there was a difference in peak abundance timing, with peaks occurring in weeks 19 and 21 (weeks starting 5th and 19th August) for the UKBMS and NHM data respectively (Fig. 5.10). In the third generation (less well defined peak, Fig. 5.10), the peak

abundance for UKBMS data was in week 25 (starting 16th September), and the peak abundance for NHM data was in week 26 (starting 23rd September).

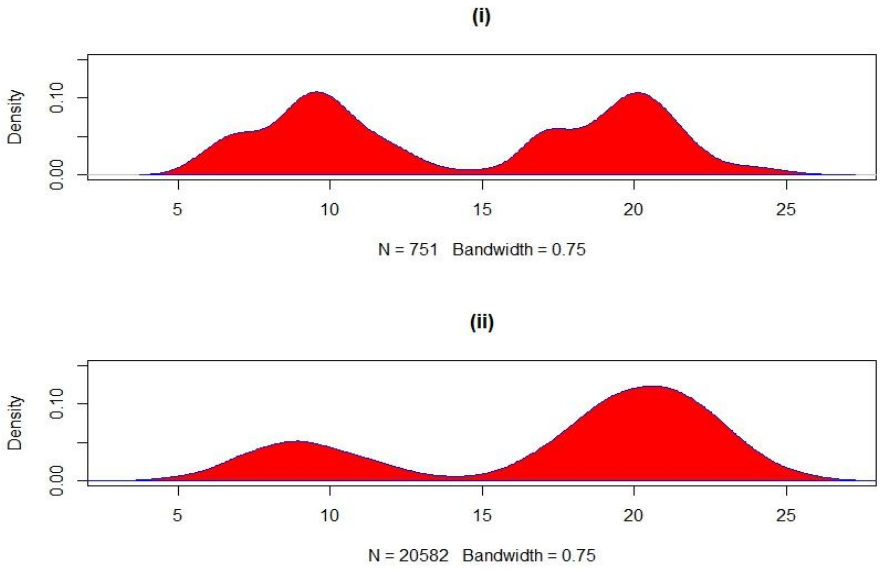

Figure 5.8 Density curves of the abundance of adult *Aricia agestis* in the UK based on i) collection dates on specimens held in the NHM collections, and ii) data collected by UKBMS.

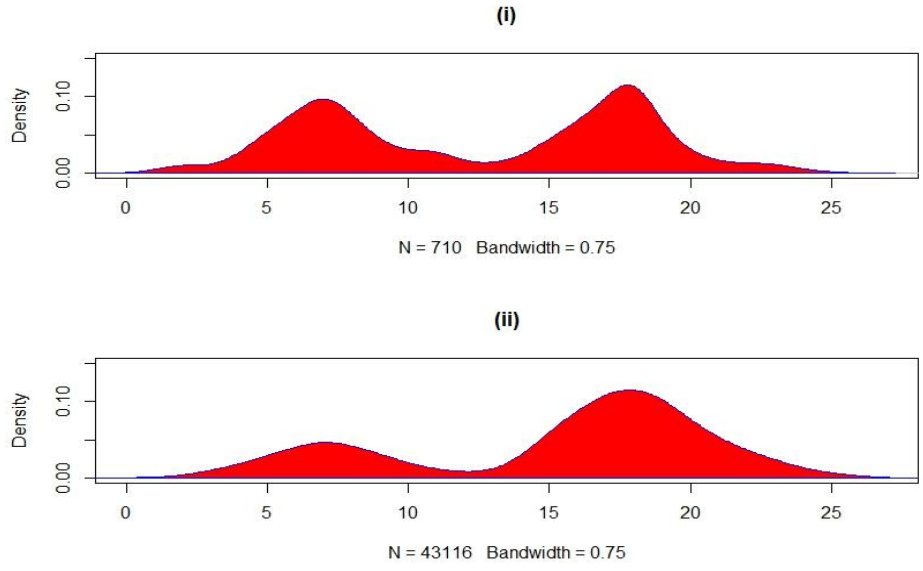

Figure 5.9 Density curves of the abundance of adult *P. napi* in the UK based on i) collection dates on specimens held in the NHM collections, and ii) data collected by UKBMS.

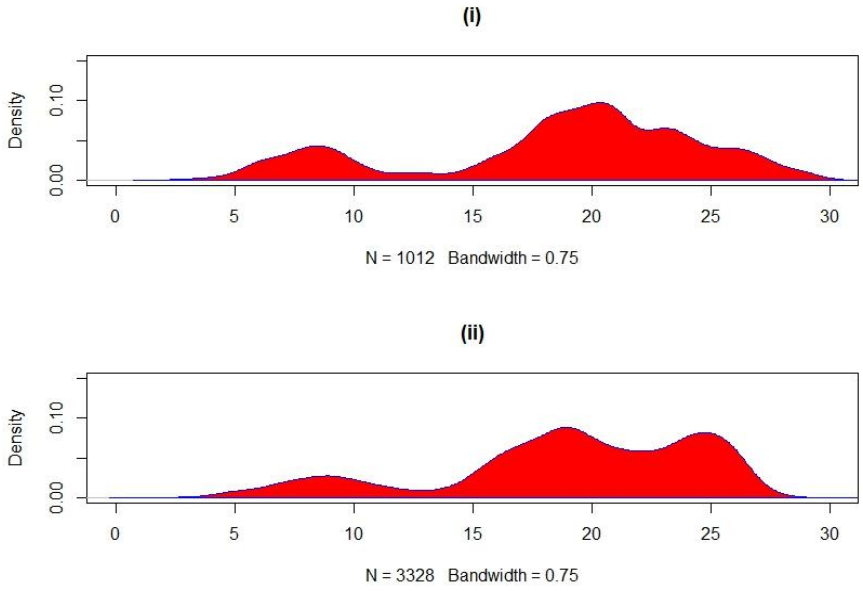

Figure 5.10 Density curves of the abundance of adult *Lycaena phlaeas* in the UK based on i) collection dates on specimens held in the NHM collections, and ii) data collected by UKBMS.

5.5 Discussion

Spatial and temporal patterns of species abundances are needed for many research questions in biogeography, ecology, and conservation biology. These data are often obtained in the field from large scale and/or long term monitoring programmes. But the collection of such field data is time and resource intensive, generally restricted to certain taxonomic groups, and often spatially restricted to a few regions (usually in the northern temperate hemisphere; Magurran *et al.*, 2010). Here, I show that museum collections may be used as an alternative resource for broad scale patterns of spatial and temporal species abundances. To do this, I compared museum specimens to field data to determine whether natural history collections are broadly representative of patterns of spatial and temporal abundance occurring in the natural environment.

For seven out of the nine species studied here, there was no statistical difference between the density abundance curves for museum and field data. The two species where there were differences between the abundance curves were the butterfly *P. bellargus* and the gastropod *T. funebralis*. However, the difference for *P. bellargus* may be largely due to the shift in timing of peak abundance, as the curves appear to have a similar shape but the peaks are displaced and

therefore, when the weekly data bins were compared they differed significantly. Although the patterns of abundance are generally similar between the museum collections and the field, the percentage of abundance present in each bin is not the same in the museums and in the field. For example, *Pieris napi* and *Aricia agestis* have similar abundance in generations one and two in the museum data, but in the field generation two has a much higher abundance than generation one for both species. Therefore, although I can conclude that the museum collections are generally representative of broad scale spatial and temporal patterns in abundance, they should not be used to estimate absolute population sizes per location or time. This is in agreement with a previous review of the use of museum collection data, which suggested that due to biases and methods, museum collections are usually not useful for studying population sizes (Pyke and Ehrlich, 2010).

Museum specimens can be useful for tracking changes in seasonal abundance of species, and comparing museum data to modern data to determine how seasonal abundance has changed over time. This is particularly useful for species with a short generation time (e.g. when there are one or more generations per year) as abundance can vary annually or between generations. For four of the five butterfly species studied here (all except *P. napi*), the peak abundance was earlier in the UKBMS data than in the museum data for at least one generation, which is consistent with previous research into emergence times of British butterflies (Brooks *et al.*, 2017). This is clearest in *P. bellargus* and *P. icarus*, where I used a more localised data set. In the other species, there may be some variation in timings due to a wider range of localities (especially in the museum data) as latitude can affect the timing of phenological events in some species (e.g. Bartomeus *et al.*, 2011). For *P. icarus,* the breakdown of peak abundance in recent years (using UKBMS data) showed that the timings of peak abundance can fluctuate. Although, the overall trend in this period was for earlier peak abundance, in some year groups the peak abundance was later than the average for the years covered by the museum data. Changes in phenology have been linked to increasing temperature (e.g. Roy and Sparks, 2000; Bartomeus *et al.*, 2011; Polgar *et al.*, 2013; Fenberg *et al.*, 2016; Brooks *et al.*, 2017); this may be reflected in the patterns of timing of peak abundance here as temperatures also fluctuate between years, but with an overall increase in temperature over more recent decades.

Museum specimens can also be used to track changes in spatial abundance of a species by comparing specimen data to modern field data. This works well for species which are relatively immobile within their life cycle or a specific life stage (e.g. rocky shore gastropods that stay on one shore during their adult stage) so that migrations do not affect the results. Previous research has used museum data to show that the range edges of gastropods species have contracted or expanded in recent years (Fenberg and Rivadeneira, 2011; Fenberg, Posbic and Hellberg, 2014),

while the present study has mainly focussed on spatial changes in peak abundance. The abundance distributions of *L. gigantea* and *M. lugubris* match well between the museum and field data, with the exception of the range contraction of *L. gigantea* (Fenberg and Rivadeneira, 2011). For *F. volcano*, the main peak in abundance occurs in the same location in the museum and field data. However, there are two additional peaks in abundance in the field data; these peaks are not seen in the museum collections, but there are some specimens in these areas in the museum collections. The museum data also goes 2° further north than the field data. This could reflect a potential range contraction, but could also be due to field misidentification with a similar looking species, *Fissurella rubropicta* (Roy *et al.*, 2003). For *T. funebralis*, the larger range in museum data is likely due to the fact that in the field data the species was not distinguished from *T. gallina* below 32°N. It is also possible, therefore, that some of the museum specimens below this latitude may have been misidentified. The mismatch in the overall abundance pattern of *T. funebralis*, may be due to changes in abundance in recent years, or may be due to collection biases for certain locations, resulting in peaks in different locations to where they peak in the field data.

As previously mentioned, there are limitations to data collected from museums specimens such as unknown or inconsistent sampling methods, biases in the specimens collected and biases in the time and/or locations the specimens were taken from (Pyke and Ehrlich, 2010; Bartomeus *et al.*, 2018; Kharouba *et al.*, 2018; Meineke and Davies, 2018; Meineke *et al.*, 2018). This was also the case in this study. For example, the sampling methods used to collect the specimens were unknown, and as the specimens came from a variety of collectors, it is unlikely that the methods were consistent. For the gastropod data, specimens were combined from several museums and collectors, which should have reduced the effect of any sampling inconsistencies or labelling errors on the results. For the butterfly data, however, only specimens from one museum were used. This included multiple collectors and a large number of data points, and therefore, I can still be confident in the overall results. In both cases, I was interested primarily in comparing the timing and location of peak abundance. While the museum specimens may not exactly reflect the actual abundance in the field, I have shown that the general patterns are similar (Figures 5.1-5.6 and 5.8-5.10). Smoothing the spatial data also accounts for missing locality data.

These results show, that while museum abundance data are not a perfect match for field abundance data, the general patterns are statistically similar (especially when known phenological or geographical shifts are taken into account). Therefore, NHCs with complete meta-data can be used with caution as proxies for abundance patterns in the field, as long as biases are acknowledged. Museum collections can, therefore, provide a useful tool for research into various biotic factors such as distribution patterns, range and phenology (e.g. Bartomeus *et al.*, 2011; Fenberg and Rivadeneira, 2011; Brooks *et al.*, 2017).

5.5.1 Conclusions

The results show that NHCs can be used as proxies of the spatial and temporal abundance of species when they were collected. Any offsets between NHC data and modern monitoring data are probably due to modern shifts in phenology or range in response to climate change. Some errors may be due to collection biases or some locations not having any collection data. Yet, when these issues are taken into consideration, and data can be complied from multiple museums or collectors, NHCs provide a useful source of data that can give a clearer picture of long-term changes in spatial or temporal abundance patterns of species by supplementing modern monitoring data.

Chapter 6 General Discussion

6.1 Introduction

The temperature-size rule predicts that ectotherms developing in cooler conditions will be larger as adults than those developing in warmer conditions (Daufresne, Lengfellner and Sommer, 2009; Gardner *et al.*, 2011; Sheridan and Bickford, 2011). As a result, ectothermic species are predicted to decrease in size in response to climate warming (Gardner *et al.*, 2011; Sheridan and Bickford, 2011), and many species have been found to follow this trend (Daufresne, Lengfellner and Sommer, 2009; Irie and Fischer, 2009; Ghosh, Testa and Shingleton, 2013; Bowden *et al.*, 2015; Wilson-Brodie, MacLean and Fenberg, 2017). However, research has now shown that this is not a universal response to climate change (Høye *et al.*, 2009; Horne, Hirst and Atkinson, 2015; Fenberg *et al.*, 2016; MacLean, Kingsolver and Buckley, 2016), and that how species respond to temperature is likely more complicated than a simple 'rule'. The research presented in this thesis uses museum collections and field data to study the changes in the adult sizes of species from two representative ectothermic taxa (marine gastropods and butterflies) over time and the ecological factors that may have shaped these responses. In this discussion, I will summarise how these results have improved our knowledge of temperature-size responses in ectotherms and how museum collections provide a useful tool for ecological research. I will also discuss the wider implications and limitations of this research, and future research that may be conducted.

6.2 Environmental factors

As mentioned in the introduction to this thesis, there are a number of environmental factors that may be influencing the temperature-size response of ectotherms. These factors include, but are not limited to, whether the species is living in an aquatic or terrestrial environment, latitude, and food quantity and quality. Below, how environmental factors can impact temperature-size responses are discussed in light of the results presented in this thesis.

6.2.1 Aquatic versus terrestrial species

Aquatic species are thought to be particularly vulnerable to rising temperatures due to oxygen becoming an increasingly limiting factor at higher temperatures, especially compared to terrestrial environments (Verberk *et al.*, 2011; Forster, Hirst and Atkinson, 2012; Ghosh, Testa and

Shingleton, 2013; Ohlberger, 2013; Horne, Hirst and Atkinson, 2015). Generally, this has led to many aquatic ectotherms being found to decrease in size with warming temperatures, while terrestrial ectotherms have shown a much greater variation in response (Høye *et al.*, 2009; Horne, Hirst and Atkinson, 2015; Fenberg *et al.*, 2016; Ortega *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017; Baar *et al.*, 2018; Tseng *et al.*, 2018). This is not always the case, however, as shown by a study on reef fishes, which found that only a marginally higher percentage of the 335 species studied decreased in size as temperatures increased (55% decreasing in size to 45% increasing in size; Audzijonyte *et al.*, 2020).

The results presented in chapter 4 also go against this general trend. The seven species of intertidal gastropods studied showed no sign of decreases in size over time, which includes the recent onset of climate warming. As it is not known whether there were any larger specimens that were uncollected in the past, and as individuals measured were not likely at their maximum size, it is impossible to determine, however, whether these species have increased in maximum size or stayed the same. This is in contrast to results of a similar study on *Nucella lapillus*, which found that dogwhelks had decreased in size with increasing temperatures over the same period. Additionally, it was clear in that study that the largest size classes had completely disappeared from the study area (Wilson-Brodie, MacLean and Fenberg, 2017).

The results presented in chapters 2 and 3 were consistent with previous research in that they showed there is variation in lepidopteran response to warming temperatures (Horne, Hirst and Atkinson, 2015). In general, the most consistent result was an increase in adult body size in relation to an increase in temperature experienced during the late larval stage (discussed further in section 6.3). This is in agreement with studies on other terrestrial insects, which have shown a reverse TSR response (Høye *et al.*, 2009; Horne, Hirst and Atkinson, 2015; Fenberg *et al.*, 2016; MacLean, Kingsolver and Buckley, 2016; Davies, 2019). However, there are exceptions to this, as shown by research on other insects, including studies of two Arctic butterfly species, *Boloria chariclea* and *Colias hecla,* and on the wasp *Dolichovespula sylvestris,* which all followed the TSR (Bowden *et al.*, 2015; Polidori *et al.*, 2020). Additionally, as shown by *Melitaea cinxia, Favonius quercus, Aglais io, Thecla betulae*, *Papilio machaon and Pararge aegeria* in chapter 3, some species do not appear to respond to temperature by changing size. This suggests that for terrestrial species, other factors need to be considered to determine why some species follow the TSR, some show the reverse response and others do not respond at all.

The results presented in this thesis show that while environmental conditions may be a factor in determining temperature-size responses of ectotherms, it is not the only factor and it cannot be presumed that species will decrease in size with warming temperatures. Other

environmental factors that may be contributing to responses are discussed below, and important ecological/biological factors are discussed in section 6.3.

6.2.2 Other environmental factors

In chapters 2 and 3, I considered the role of habitat type in influencing temperature-size responses of butterflies. Two of the species, *Polyommatus bellargus* and *Polyommatus coridon*, were from chalk hill grasslands, whereas *Plebejus argus* primarily lives in heathland. All three species showed relatively similar temperature-size responses and, therefore, in this case, habitat type is unlikely to be affecting the results. Interestingly, *Hesperia* comma, is also from a chalk grassland habitat and also followed the same general pattern as the three Lycaenidae species (chapters 2&3; Fenberg *et al.*, 2016). This is consistent with results of the multi-species analyses presented in chapter 3, which showed that habitat type was not a major factor in determining temperature-size responses. There was, however, some difference in how adult body size changes in relation to changes in temperature during the pupal stage for species living in different habitat types. It is likely that each life stage lives in different microclimates (Kingsolver *et al.*, 2011), and that this microclimate may be important for pupal development, potentially by affecting metabolic processes during this stage (Davies, 2019). In chapter 4, which considered temperature-size responses in seven species of intertidal gastropods, all species were from intertidal rocky shores, but the exact environmental conditions can vary between shores (e.g. extent of wave exposure). As the precise conditions cannot be determined from museum specimen locations, this could not be considered as part of this study. It is, however, reasonable to expect that some variation in the results was caused by the conditions on each individual shore (Jenkins and Hartnoll, 2001; Cotton, Rundle and Smith, 2004; Cabral, 2007; Queiroga *et al.*, 2011). Given that there was no clear size decline, unlike that seen in *Nucella lapillus* (Wilson-Brodie, MacLean and Fenberg, 2017), and there was clear spatial variation in both shape and size of shells, it can be hypothesised that the morphology of these species is a product of the local environment rather than temperature.

In addition to habitat type, it is also important to consider the food quality and quantity available to species at their location as this can impact the amount of energy available for growth. Some previous studies have found that the food available can alter temperature-size responses (Diamond and Kingsolver, 2010; Horne *et al.*, 2016) or that species tended towards smaller individuals in locations where resources were thought to be limiting (Reuman, Holt and Yvon-Durocher, 2014; Classen *et al.*, 2017). This cannot be assessed for museum specimens, but *in situ* or experimental studies may be able to shed further light on the effects of food availability on temperature-size responses. Such studies would also allow for species to be studied alongside

their prey and/or predators. If one species gets smaller in size or decreases in abundance in that area, then this will likely have a knock-on effect on other species in the food chain (Yvon-Durocher *et al.*, 2011; Ohlberger, 2013; Nelson *et al.*, 2017). In chapter 4, the gastropod grazers studied did not decrease in size over time, and this is likely partially due to a high abundance of food being available. This was not the case for the predatory gastropod *N. lapillus*, however, which decreased in size with increasing temperatures. This species was likely not able to compensate for higher energy demands at higher temperatures by increasing food intake (Wilson-Brodie, MacLean and Fenberg, 2017). As oxygen is also potentially a limiting factor for marine predators (Verberk *et al.*, 2011; Ohlberger, 2013; Horne, Hirst and Atkinson, 2015), they are likely to be particularly vulnerable to decreases in size with increasing temperature. At the present time, it is likely that a decrease in predator size is benefiting species lower down the food chain as they are able to graze more freely, but this could change in the future as temperatures continue to rise.

Finally, it is important to consider that some species have also been affected by anthropogenic impacts. This is particularly relevant for marine ectotherms, which are often harvested by humans for food. For example, decreases in size of gastropods in southern California is likely due to a mix of harvesting and temperature increases (Roy *et al.*, 2003), while the clam *Spisula solidissima*, has also decreased in size due to fishing pressure and increasing temperatures (Munroe *et al.*, 2016). On the southern UK coastline, TBT pollution from boats in the 1980's was found to have impacted marine gastropods such as *N. lapillus*, and was suggested to be one of the possible reasons behind the size declines and potential local extinctions in the region (Wilson-Brodie, MacLean and Fenberg, 2017). Terrestrial ectotherms may also suffer from loss of habitat due destruction through human activity. For example, much of the global tallgrass prairie ecosystem has been lost as it is now used for agriculture. This has impacted specialist species such as the Lepidoptera species *Speyeria idalia*, which has resulted in a large decrease in abundance of this species due to poor habitat quality (Henderson, Meunier and Holoubek, 2018). This suggests that in this habitat, conditions are not suitable for living in and therefore, it is likely that the conditions would be poor for growth and result in smaller adult sizes.

6.3 Biological factors

The results presented in this thesis have also shown that biological factors, including life history traits, can influence the temperature-size response of a species. The three most important factors are discussed below.

6.3.1 Voltinism

In short-lived species, such as insects, the number of generations per year was expected to affect the temperature-size response of a species. Species which have obligate voltinism, i.e. do not change the number of generations per year, are thought to take advantage of the increased temperatures by prolonging the growth period and therefore, reaching a larger adult size (Horne, Hirst and Atkinson, 2015). Some species on the other hand, increase voltinism in warmer years and therefore, prioritise squeezing in an extra generation over growing to a larger size (Horne, Hirst and Atkinson, 2015; Van Dyck *et al.*, 2015; Forrest, 2016). In chapters 2 and 3, I studied two bivoltine butterfly species (*P. bellargus* and *A. urticae*) and one multivoltine species (more than 2 generations per year; *P. aegeria*). For the bivoltine species, the first generation responded in the same way as the univoltine species (i.e. there was an increase in adult body size as temperatures increased during the late larval stage), but the multivoltine species showed no temperature-size response in relation to temperatures experienced in any development stage. There were also six species studied that regularly change the number of generations per year (see appendix II), but in four of these species, the temperature-size response was similar to other species (i.e. there was an increase in adult body size as temperatures increased during the late larval stage), and in the remaining two species, there was no significant response. These results suggest that in British Lepidoptera, changes in the number of generations per year does not influence temperature-size responses. This may be because changes in the number of generations per year are linked to an extended growth season with increasing temperature, and with increased growth and development rates, this may not impact in final adult size of the first generation. Additionally, in bivoltine species, the first generation responds in the same way to temperature changes as univoltine species. Analyses of more bivoltine and multivoltine species would be required to determine if this is a general trend amongst Lepidoptera or other taxa and if this trend also applies to species with more than two generations per year.

6.3.2 Life stages and growth strategies

For species with short life cycles (i.e. a year or less), each life stage will likely experience different temperatures. Therefore, where possible, it is important to consider how each stage is responding to temperature. In chapters 2 and 3, I compared monthly temperatures covering the early larval, late larval and pupal stages to adult size to determine whether size was following the TSR in relation to temperatures experienced by each immature stage. For most butterfly species, adult size increased in relation to increasing temperatures experienced during the late larval stage, and this was usually the most significant result (including in the previously studied *H. comma*; Fenberg *et al.*, 2016). This suggests that for short-lived species, warmer temperatures may favour growth

during the late larval stages when there may be favourable conditions for growth, including higher quality and quantity of food and oxygen is not a limiting factor (Suhling, Suhling and Richter, 2015; Kivelä, Lehmann and Gotthard, 2016). This is not always the case, however, as a study of *Anthocharis cardamines* found that size increased with increasing temperatures in the pupal stage (Davies, 2019). This may be because this species overwinters in the pupal stage and spends 9 months in that stage, and therefore, it would not be unexpected that this stage is most important in predicting final size. It is not clear why this is the case but has been suggested that it may be due to the fact that temperatures affect metabolic processes, and for species which take a diapause in the pupal stage, they are not as time pressured to pupate so are likely to be less impacted by temperature in the larval stages than in the pupal stage (Davies, 2019). In Chapter 3, there were two species that overwinter in the pupal stage; *P. malvae* increased in adult size with increasing temperature during the late larval stage but no response to temperatures experienced during the pupal stage, while *P. machaon* showed no significant response to temperature.

Furthermore, my results show that in some species, the temperature-size response is not consistent between stages. In seven of the species studied, there was a mixture of significant increases and decreases in adult size with increasing temperatures across the three immature stages. This means that an overall increase or decrease in size for that generation would depend on which stages were exposed to warmer or cooler temperatures. The advantage of studying short-lived species with determinate growth is that it is easier to assess how temperature affects each development stage and this can be studied easily from museum specimens or field data, without needing to take *in situ* temperature measurements as long as monthly temperature records are available for that period. For indeterminate growers, such as gastropods, this is hard to study without experiments as the adult stage lasts much longer than the juvenile stage and so, is hard to predict which temperature records are appropriate to use. An experimental study of the marine gastropod *Monetaria annulus* found that this species followed the TSR, and therefore, those developing at higher temperatures were smaller at the end of the juvenile stage than those that had developed in cooler conditions (Irie and Fischer, 2009). However, other methods such as sclerochronology to study growth increments and using oxygen isotopes to study how temperature affects the growth of marine molluscs (Schöne *et al.*, 2002; Gröcke and Gillikin, 2008).

6.3.3 Sexual size dimorphism (SSD)

Previous research has shown that temperature-size responses differ between males and females in some species (Høye *et al.*, 2009; Fenberg *et al.*, 2016), but in other species the responses are the same in both sexes (Bowden *et al.*, 2015; McCauley *et al.*, 2015; Davies, 2019; Wonglersak *et*

al., 2020). In chapters 2 and 3, I studied 12 butterfly species in which sex could easily be determined from the images. In the majority of these species, there was a difference in the response of males and females in relation to the temperature experienced by at least one immature stage. Generally, males were more sensitive than females to temperature changes, particularly in the early and late larval stages. Sex and family were both significant random variables in the linear mixed effects models, although family explained more variation in results than sex. Sex may be less important as increasing size with increasing temperature during the late larval stage is a common response regardless of sex.

If sexes are responding differently to changing temperatures, then this could result in a shift towards or away from SSD. A previous analysis of arthropod species found that generally, males and females responded in a similar way to temperature (Hirst, Horne and Atkinson, 2015). The results presented in this thesis, however, show that this is not always the case, especially when temperature-size responses are compared between immature stages. Furthermore, in females, changes in size could have an impact on reproductive outputs as size is related to fecundity (Garcia-Barros, 2000). Therefore, an increase or decrease in female size could be particularly important for predicting the future fitness of the species.

6.4 Wider implications

While I have focussed on two model taxa, other species in the same ecosystems are also likely responding to temperature changes. Additionally, many species are also experiencing advances in phenology and changes in geographic range as a result of ongoing climate warming (Gardner *et al.*, 2011; Sheridan and Bickford, 2011; Fenberg, Posbic and Hellberg, 2014; Fenberg *et al.*, 2016; Brooks *et al.*, 2017). A study of *H. comma*, found that changes in temperature during the immature stages were related to changes in size, phenology and range (Fenberg *et al.*, 2016). These changes could result in changes in fitness, and potential trophic mismatches if other species in the food web are not responding in the same way (Van Dyck *et al.*, 2015).

Furthermore, a change in individual size would likely result in a decrease in mean size at the population level resulting in a decreased abundance of larger size classes. There may also be a decrease in size at specific stages, such as maturity and a shift towards a population of smaller individuals with a higher optimum temperature for growth (Ohlberger, 2013). At the community level, these changes could have effects on predator-prey interactions and result in changes in species composition and size-structure of the community (Yvon-Durocher *et al.*, 2011; Ohlberger,

2013), which in turn may affect ecosystem services (Edeline *et al.*, 2013; Pecl *et al.*, 2017). These changes may also be further exasperated by harvesting of predators (Gardner *et al.*, 2011).

The results in chapter 2 highlight that temperature-size responses are not necessarily linear, and at some point changes in size will plateau, or even reverse. This is likely due to physiological constraints on size and growth. Species have a thermal optimum for growth, and it is thought that below this temperature growth rate increases with increasing temperature until it reaches the optimum value. Above the optimum temperature, however, growth rate starts to decrease, and therefore above this temperature, maximum size will likely decrease (Ohlberger, 2013). Therefore, as temperatures continue to rise, species may be forced to live at temperatures above their thermal optimum and so even species showing reverse TSR responses may start to decrease in size. Additionally, development rate should also be considered as increased temperatures often result in an increase in development rate (i.e. individuals reach maturity faster) and so this should be considered in line with growth rates to determine if species will decrease in size at maturity (or final adult size in determinate growers) as temperatures continue to rise (Ohlberger, 2013).

6.5 Museum collections

Museum collections provide a useful resource for studying morphological changes over time (e.g. Roy *et al.*, 2003; Fenberg *et al.*, 2016; Wilson-Brodie, MacLean and Fenberg, 2017; Baar *et al.*, 2018; Tseng *et al.*, 2018; Wonglersak *et al.*, 2020), and the results presented in chapters 2, 3 and 4 add to this research. Many large national museums house millions of specimens which cover a large spatial and temporal range (Johnson *et al.*, 2011), and if specimens from multiple museums are combined then this provides even better coverage. I used species which had a large number of specimens covering a wide temporal range and appropriate spatial coverage for each study, and in the case of chapters 4 and 5, data was used from across multiple museums. Museum data can be used to create a time series of data that can be compared to modern data, as was done in chapters 4 and 5, and to temperature data over the same time period, as was done in chapters 2 and 3 (Lister *et al.*, 2011). Having specimens cover a wider range of years, and more specimens per year, than is usually possible in laboratory or field studies gives a more expansive view of changes in size over time (and with temperature).

Each taxon, however, presents its own set of challenges. For example, butterfly specimens are fragile and so the best way to measure them is digitally. This either requires digital collections (e.g. the NHM iCollections; Paterson *et al.*, 2016) or taking time to photograph specimens

specifically for that research, so this limits the number of museums with accessible collections for body size research or the number of specimens that can be studied. Equally, when digital specimens have to be measured manually, as was done in chapter 2, this is time consuming and limits the number of specimens that can be included. In chapter 3, using the computer vision pipeline allowed for almost 40,000 specimens to be measured in a relatively short period of time (approximately a quarter of the time it would take to manually measure them). Additionally, museum specimens also often lack ecological context (MacLean *et al.*, 2018), which can be important in assessing the effects of environment on size. This can be overcome to a certain extent by using museum specimen data alongside *in situ* field studies, where the environmental conditions and ecological variables are known. This is not easy to do for butterfly species due to their fragile nature as capturing them in the wild to measure them would likely be too damaging and very time consuming.

Gastropods, on the other hand, are much simpler to study in the wild as they can easily be measured *in situ*. This taxon, however, provides other challenges, such as being an indeterminate grower. This means that when the specimens were collected, they were not likely at their maximum size. Additionally, this means that there is generally a much wider range of adult sizes than in determinate growers such as butterflies. To overcome this problem, I used only the largest size classes so that 'younger' individuals would not be included. This is the same methodology that was used in previous studies, and in those studies it was clear that the largest size classes had disappeared over time (Roy *et al.*, 2003; Wilson-Brodie, MacLean and Fenberg, 2017). A further problem with museum specimens is that it is impossible to tell if there were larger individuals that were not collected, and therefore, it can only be concluded that the species was at least as large as the largest individual in the collections. Large individuals are less likely to be missed during field studies as surveys are methodical and have consistent methods, whereas collections made for museums are usually more haphazard. Despite the fact that for some species studied in Chapter 4, the modern field data included individuals as larger than those found in the museum data, it cannot be concluded that the species have increased in size over time as there may have been larger individuals in the past which were not collected. However, it is reasonable to assume that the species have not decreased in size over time, and thereby not decreased in size with increasing temperature.

Although museum collections have their limitations, when used cautiously they can provide data which is representative of the natural environment. Chapter 5 shows that for well collected and documented species, museum specimens can show ecological patterns, such as patterns in abundance, that are similar to those shown by field surveys. Chapter 5 focuses on abundance data, and suggests that the number of specimens collected is roughly proportional to abundance

in the field. If this is the case, then this suggests that the museum specimens collected are a representative sample of the local populations and, therefore, are useful for other ecological studies, such as those on size change.

6.6 Limitations

As mentioned above, for indeterminate growers, it is hard to measure size changes, especially size increases, as some individuals are not at their maximum size when measured, regardless of whether they are museum specimens in the field. For gastropods, or other shelled species, sclerochronology can be used to study growth increments of individuals at the monthly or seasonal level (Schöne *et al.*, 2002; Gröcke and Gillikin, 2008), which could be compared with temperature data. Such studies, however, would likely be more time and resource intensive than measuring the size of the shells. Furthermore, to use museum specimens in such a study, would require damaging them.

The temperature-size responses discussed in chapters 2 and 3 are based on specimens and temperatures collected from the first half of the 20th century as this is when these species were primarily collected. There was inter-annual variation in temperatures during that period, but this is before the recent onset of climate warming. While this gives an indication of how each species responds to temperature, it does not show if they will continue to respond in this way as temperatures continue to rise. Therefore, it is important that this gap in knowledge is filled by continued field studies and laboratory experiments. Furthermore, temperature-size changes are unlikely to be linear, as size has physiological constraints, so size will likely decrease/increase above or below certain temperature thresholds (Ohlberger, 2013). This may lead to a plateau (e.g. Fig 3, Chapter 2) or an n-shaped size response curve.

Comparing historic size data to temperature requires suitable temperature data that covers the same temporal range as the size data. In the UK, the Central England Temperature Record provides air temperatures from 1669 to the present for an area within the triangle delimited by London, Lancashire and Bristol (Parker, Legg and Folland, 1992). This record provides ample coverage for most UK museum collections, and as mentioned above, this is why these collections were used (Chapter 3). Additionally, this data set can be used for various resolutions from daily to seasonal temperature measurements for each year, and, therefore, can be useful for various studies. Monthly data was useful for chapter 3 as it allowed me to look at temperature changes within different stages of larval development. In contrast, I used more localised regional data in chapter 2 as the specimens for each species were from one specific location or area. The regional

temperature data set, however, does not extend as far back in time as the CET, and so it was not possible to use about 50 specimens for one of the species, which were collected prior to the start of the collection of regional temperature data. Nevertheless, the CET has strong correlations with regional temperature data from around the UK, and therefore, is a good proxy for UK-wide temperatures (Duncan, 1991; Fenberg *et al.*, 2016). However, I believe where possible (for example when available data is spatially restricted), that temperature data with high spatial resolution should be used as this is likely to be closer to the actual temperatures experienced by the individuals being studied.

Manual measurement of butterfly wing length from images is time intensive, which severely limits the number of specimens that can be included in studies. Chapter 3 tested a computer vision pipeline that was specifically designed for measuring butterfly wing lengths in order to overcome this problem. The pipeline version used in Chapter 3 works well for some species (such as *H. comma*), but not others (such as *P. bellargus*). The pipeline was initially developed using *H. comma* so species with similar colours and wing shapes to this species tended to work well. Many of the Lycaenidae species, which are blue, tended to have a high percentage of specimens with measurement errors. Additionally, no species in the Pieridae family were included in the study as the testing stage indicated that these species had a high percentage of errors. Species in this family tend to be white or yellow, unlike *H. comma* which is orange, which may be why they did not work well. The pipeline allowed for thousands of specimens to be analysed in about one quarter of the time that it would take to measure them manually. However, there was still some time intensive work to be completed; images needed to be visually checked as the specimens were pinned in the ventral view in some of the images and could not be used in analyses, some specimens had wing damage, and, sexes of some specimens needed to be determined, which could only be done manually. To reach its full potential, more improvements are required to the pipeline so that it works for all species and specimens, and so that some of the other time-intensive work is not required (see section 6.7).

6.7 Further research and future applications

Future research on temperature-size response in ectotherms should focus on studying more taxa to determine whether the patterns found here occur more widely. Additionally, studies of historical collections should be combined with both *in situ* field studies, to understand how responses may be affected by the environment, and laboratory or mesocosm studies, to understand how species may respond to future climate change. By using more taxa, species from

different trophic levels within the same environment can be studied, which may give a more holistic view of how the community is responding as a whole, and the influence of predator-prey interactions on size response. When making predictions of temperature-size responses, researchers should consider various factors including taxa, trophic level, habitat type and growth strategy. It is also important to consider the response of sex and different generations of a species separately.

As technology advances, more improvements can be made to digital collections and the software used to analyse them. Work is already underway to improve the computer vision pipeline described in Chapter 3 so that it can be used for more species. Initial results of this new version indicate that it will work for more species including the blue Lycaenidae species that did not work previously (e.g. *P. bellargus*) and on some moths in the collections. In an ideal world, museums would digitise their specimens in a standardised way so that specimens could easily be measured using the same methods irrespective of where the collections were housed. This would greatly increase the number of specimens that can be used in studies, as well as increasing the temporal and spatial coverage of data. With a higher coverage of data available, this would allow for responses to be studied over a larger range of temperatures and over a larger proportion of the species' ranges to determine if individuals in any areas or at different latitudes respond differently. Where possible, specimens should continue to be added to collections so that future studies can include more data from the 21st century.

Appendix A Chapter 3 Supplementary Information

Table A.1 Ecological information about the 21 species measured using the automated pipeline, plus the three Lycaenidae species previously manually measured by Wilson *et al*. (2019) and used in multi-species analysis here(*). The general habitat type (given in parentheses) indicates the habitat type used in analyses. For size category, the following groupings were used based on average forewing length: Small=10-20mm, Medium=20-30mm, Large=30-40mm. ‡ Notes species not used in analyses.

Family	Species	Number of generations	Can sexes be identified?	Overwinter stage	Habitat (general type)	Size Category
Hesperiidae	*Erynnis tages*	1 (can be 2 in warm years)	No	Larval	Warm, open areas (Other)	Small
	Hesperia comma	1	Yes	Egg	Chalk grassland (Grass)	Small
	Ochlodes sylvanus	1	Yes	Larval	Sheltered grassland (Grass)	Small
	Pyrgus malvae	1 (can be 2 in good conditions)	No	Pupal	Sparse vegetation (Other)	Small
	Thymelicus lineola	1	Yes	Egg	Rough grass (Grass)	Small
	Thymelicus sylvestris	1	Yes	Larval	Rough grass (Grass)	Small
Lycaenidae	*Favonius quercus*	1	Yes	Egg	Woodland (Wood)	Small
	Plebejus argus *	1	Yes	Egg	Heathland (Other)	Small
	Polyommatus bellargus *	2	Yes	Larval	Chalk grassland (Grass)	Small
	Polyommatus coridon *	1	Yes	Egg	Chalk grassland (Grass)	Small
	Thecla betulae	1	Yes	Egg	Woodland or hedgerows (Wood)	Small

Family	Species	Number of generations	Can sexes be identified?	Overwinter stage	Habitat (general type)	Size Category
Nymphalidae	*Aglais io*	1	No	Adult	Anywhere (Any)	Medium
	Aglais urticae	2 (1 in Scotland)	No	Adult	Anywhere (Any)	Medium
	Apatura iris	1	Yes	Larval	Woodland (Wood)	Large
	Argynnis paphia	1	Yes	Larval	Woodland (Wood)	Large
	Euphydryas aurinia	1	No	Larval	Sunny areas (Other)	Small
	Fabriciana adippe	1	No	Egg	Woodland (Wood)	Medium
	Hipparchia semele	1	Yes	Larval	Sparse vegetation (Other)	Medium
	Melitaea athalia	1 (can be 2 in warm years)	No	Larval	Woodland or heathland (Wood)	Small
	Melitaea cinxia	1	No	Larval	Coastal or chalk grassland (Grass)	Small
	Nymphalis polychloros ‡	1	No	Adult	Woodland (Wood)	Medium
	Pararge aegeria ‡	3 (sometimes 2)	No	Larval or Pupal	Woodland (Wood)	Medium
	Speyeria aglaja	1	No	Larval	Open grassland (Grass)	Medium
Papilionidae	*Papilio machaon*	1 (can be 2)	No	Pupal	Open fens (Other)	Large

Table A.2 Results of the linear models for predicting average forewing length for all species (sex and generations were analysed separately where applicable) using mean monthly temperatures as variables; values are adjusted R^2 (AR^2), the F statistic (F), degrees of freedom (df) and the p-value (p). * Indicates results taken from Wilson, Brooks and Fenberg (2019).

Family	Species	Sex	AR^2	F	df	p
Hesperiidae	*Erynnis tages*	Both	0.0345	4.946	6, 657	<0.001
	Hesperia comma	Male	0.0499	3.231	6, 249	0.00449
		Female	0.0214	1.862	6, 221	0.0951
	Ochlodes sylvanus	Male	0.0576	5.664	7, 527	<0.001
		Female	0.0361	2.728	7, 316	0.00924
	Pyrgus malvae	Both	0.0249	4.452	6, 807	<0.001
	Thymelicus lineola	Male	0.0235	2.868	4, 307	0.0234
		Female	0.0169	2.012	4, 231	0.0936
	Thymelicus sylvestris	Male	0.0130	2.43	4, 429	0.0471
		Female	0.0192	2.563	4, 315	0.0385
Lycaenidae	*Favonius quercus*	Male	0.0123	1.312	5, 120	0.263
		Female	0.000451	1.013	5, 144	0.412
	Plebejus argus *	Male	0.0408	3.38	5, 275	0.00555
		Female	0.0466	2.27	5, 125	0.0514
	Polyommatus bellargus * generation 1	Male	0.147	4.67	3, 61	0.00529
		Female	0.101	5.62	3, 121	0.00122
	Polyommatus bellargus * generation 2	Male	0.00534	1.36	3, 199	0.256
		Female	-0.00820	0.111	3, 325	0.954
	Polyommatus coridon *	Male	0.0624	2.76	5, 127	0.0213
		Female	0.0431	3.55	5, 278	0.00397
	Thecla betulae	Male	0.0443	1.73	4, 59	0.155
		Female	-0.0201	0.630	4, 69	0.643
Nymphalidae	*Aglais io*	Both	-0.000264	0.965	3, 395	0.409
	Aglais urticae generation 1	Both	0.0188	3.995	3, 466	0.00793
	Aglais urticae generation 2	Both	0.0147	2.507	3, 300	0.0591
	Apatura iris	Male	0.0713	1.779	7, 64	0.107
		Female	0.0162	2.573	7, 50	0.0241
	Argynnis paphia	Male	0.00300	1.328	5, 540	0.251
		Female	0.0259	4.251	5, 606	<0.001
	Euphydryas aurinia	Both	0.00953	4.546	6, 2205	<0.001
	Fabriciana adippe	Both	0.0270	4.696	5, 662	<0.001

Family	Species	Sex	AR^2	F	df	p
Nymphalidae	*Hipparchia semele*	Male	0.0307	4.134	7, 685	<0.001
		Female	0.00897	1.741	7, 566	0.0971
	Melitaea athalia	Both	0.0131	2.999	6, 895	0.00661
	Melitaea cinxia	Both	0.00311	1.239	5, 378	0.290
	Pararge aegeria	Both	0.00228	1.216	7, 656	0.291
	Speyeria aglaja	Both	0.027	9.264	4, 1187	<0.001
Papilionidae	*Papilio machaon*	Both	-0.0004926	0.992	6, 87	0.436

Table A.3 Outputs for variables included in significant linear models (listed in Table B) using mean monthly temperatures; t-value and significance (p) for each variable, slope estimate, standard error and upper and lower confidence intervals (CI), importance scores, variance inflation factors (VIF) and whether the variable was selected for the final model after stepwise regression in both directions. Slope estimates, standard error and confidence intervals are based on an average of all candidate models (using the IT-AIC approach), and variables in bold are those retained after nested models were removed. Importance scores are based on the number of candidate models the variable was present in, with a score of 1.0 indicating that the variable was present in all candidate models.

Species	**Month**	***t-value***	***p***	***Slope estimate***	***Standard error***	***Lower CI***	***Upper CI***	***Importance***	***VIF***	***Final model (Y/N)***
E. tages	*June -1*	2.001	0.0458	0.0722	0.0359	0.00182	0.143	0.76	1.247	Y
	July -1	0.205	0.837	0.0144	0.0322	-0.0489	0.0776	0.30	1.479	N
	August-1	1.996	0.0463	0.0855	0.0346	0.0175	0.153	0.89	1.722	Y
	March	-1.842	0.0659	-0.0459	0.0251	-0.0952	0.00339	0.67	1.079	Y
	April	4.264	<0.001	0.118	0.0281	0.0628	0.173	1.00	1.085	Y
	May	0.490	0.624	0.0183	0.0405	-0.0611	0.0978	0.30	1.223	N
H. comma (Males)	*March*	0.980	0.328	0.0374	0.0327	-0.0269	0.102	0.41	1.425	N
	April	0.838	0.403	0.0302	0.0405	-0.0496	0.110	0.32	1.259	N
	May	0.136	0.892	-0.00159	0.0582	-0.116	0.113	0.27	1.591	N

Species	Month	*t-value*	*p*	*Slope estimate*	*Standard error*	*Lower CI*	*Upper CI*	*Importance*	*VIF*	*Final model (Y/N)*
H. comma (Males)	*June*	3.275	0.00121	0.152	0.0420	0.0689	0.234	1.00	1.416	Y
	July	-1.765	0.0788	-0.0745	0.0418	-0.157	0.00792	0.64	1.288	Y
	August	-0.164	0.870	-0.0139	0.0399	-0.0926	0.0647	0.28	1.604	N
O. Sylvanus (Males)	*Aug -1*	1.624	0.105	0.0529	0.0287	-0.0351	0.109	0.68	1.253	Y
	Sept-1	1.618	0.106	0.0552	0.0301	-0.00390	0.114	0.67	1.285	Y
	March	2.124	0.0342	0.0480	0.0219	0.00505	0.0910	0.81	1.183	Y
	April	-1.000	0.318	-0.0298	0.0301	-0.0888	0.0293	0.37	1.172	N
	May	4.358	<0.001	0.141	0.0306	0.0804	0.201	1.00	1.264	Y
	June	0.820	0.413	0.0229	0.0315	-0.0390	0.0849	0.32	1.237	N
	July	-1.037	0.300	-0.0253	0.0253	-0.0750	0.0244	0.38	1.235	N
O. Sylvanus (Females)	*Aug-1*	0.550	0.583	0.0258	0.0345	-0.0421	0.0938	0.32	1.482	N
	Sept -1	0.202	0.840	0.0121	0.0401	-0.0667	0.0910	0.27	1.317	N
	March	0.0300	0.976	0.00635	0.0295	-0.0517	0.0644	0.26	1.361	N
	April	3.208	0.00147	0.139	0.0425	0.0550	0.222	1.00	1.310	Y
	May	2.888	0.00415	0.124	0.0396	0.0463	0.202	1.00	1.317	Y
	June	-0.115	0.909	-0.0157	0.0437	-0.102	0.0703	0.27	1.266	N
	July	-0.914	0.361	-0.0382	0.0377	-0.112	0.0360	0.37	1.351	N

Species	Month	*t-value*	*p*	*Slope estimate*	*Standard error*	*Lower CI*	*Upper CI*	*Importance*	*VIF*	*Final model (Y/N)*
P. malvae	*May -1*	3.198	0.00144	0.0735	0.0243	0.0259	0.121	0.99	1.165	Y
	June -1	3.459	<0.001	0.0990	0.0274	0.0452	0.153	1.00	1.147	Y
	July -1	1.399	0.162	0.0416	0.0242	-0.00581	0.0891	0.62	1.455	Y
	August -1	1.231	0.219	0.0417	0.0264	-0.0101	0.0935	0.57	1.306	N
	March	-0.782	0.434	-0.0151	0.0194	-0.0533	0.0231	0.33	1.056	N
	April	0.567	0.571	0.0110	0.224	-0.0330	0.0550	0.29	1.032	N
T. lineola (Males)	*April*	2.414	0.0234	0.0840	0.0363	0.0127	0.155	0.87	1.144	Y
	May	0.151	0.880	-0.000822	0.0411	-0.0816	0.0800	0.27	1.236	N
	June	-1.441	0.151	-0.0781	0.0418	-0.160	0.00412	0.69	1.463	Y
	July	-1.042	0.298	-0.0465	0.0381	-0.121	0.0284	0.45	1.320	N
T. sylvestris (Males)	*April*	0.936	0.350	0.0241	0.0300	-0.0348	0.0830	0.33	1.102	N
	May	2.109	0.0356	0.0704	0.0312	0.00907	0.132	0.82	1.086	Y
	June	1.639	0.102	0.0542	0.0326	-0.00985	0.118	0.60	1.166	Y
	July	-0.344	0.731	-0.00128	0.0279	-0.0560	0.0535	0.27	1.171	N
T. sylvestris (Females)	*April*	-1.643	0.102	-0.0612	0.0353	-0.131	0.00835	0.62	1.115	Y
	May	1.101	0.272	0.0483	0.0394	-0.0292	0.126	0.44	1.143	N
	June	1.995	0.0469	0.0775	0.0355	0.00759	0.147	0.80	1.244	Y
	July	-0.551	0.582	-0.0257	0.0356	-0.0956	0.0442	0.34	1.247	N

Species	Month	*t-value*	*p*	*Slope estimate*	*Standard error*	*Lower CI*	*Upper CI*	*Importance*	*VIF*	*Final model (Y/N)*
P. argus (Males)	*March*	-2.664	0.00817	-0.0898	0.0403	-0.169	-0.0105	0.86	1.518	Y
	April	1.397	0.163	0.0634	0.0500	-0.350	0.167	0.46	1.476	N
	May	2.336	0.0202	0.124	0.0505	0.0251	0.224	0.89	1.606	Y
	June	1.412	0.159	0.142	0.0886	-0.0319	0.317	0.58	1.312	Y
	July	-0.361	0.718	-0.00468	0.0369	-0.0755	0.0678	0.27	1.253	N
P. bellargus (G1 Males)	*March*	-3.178	0.00254	-0.291	0.0956	-0.482	-0.100	0.94	2.286	Y
	April	3.105	0.00288	0.425	0.143	0.139	0.711	0.94	2.331	Y
	May	2.275	0.0264	0.157	0.0703	0.016	0.297	0.80	1.043	Y
P. bellargus (G1 Females)	*March*	-1.663	0.0989	-0.117	0.0754	-0.266	0.0325	0.55	1.536	Y
	April	0.720	0.473	0.0394	0.0956	-0.150	0.228	0.29	1.285	N
	May	2.660	0.00886	0.159	0.0523	0.0550	0.262	0.97	1.232	Y
P. coridon (Males)	*March*	1.627	0.106	0.202	0.110	-0.0160	0.419	0.66	1.814	Y
	April	-1.396	0.165	-0.102	0.0995	-0.299	0.0942	0.39	1.600	Y
	May	-1.806	0.0733	-0.217	0.162	-0.536	0.103	0.52	3.459	Y
	June	2.495	0.0139	0.240	0.116	0.0110	0.469	0.77	1.573	Y
	July	-1.870	0.0638	-0.164	0.0972	-0.356	0.0278	0.62	1.871	Y
P. coridon (Females)	*March*	0.761	0.447	0.0311	0.0453	-0.0581	0.120	0.31	1.650	N
	April	-0.662	0.509	-0.0229	0.0617	-0.144	0.0985	0.28	1.637	N

Species	Month	*t-value*	*p*	*Slope estimate*	*Standard error*	*Lower CI*	*Upper CI*	*Importance*	*VIF*	*Final model (Y/N)*
P. coridon (Females)	*May*	-2.232	0.0264	-0.131	0.0564	-0.242	-0.0200	0.86	1.729	Y
	June	0.048	0.962	0.00555	0.0580	-0.108	0.120	0.26	1.330	N
	July	-3.932	<0.001	-0.150	0.0405	-0.230	-0.0706	1.00	1.533	Y
A. urticae (G1)	*April*	1.917	0.0559	0.123	0.0702	-0.0147	0.261	0.64	1.111	Y
	May	2.705	0.00707	0.198	0.0841	0.0328	0.363	0.86	1.153	Y
	June	-2.514	0.0123	-0.192	0.0831	-0.356	-0.0288	0.84	1.068	Y
A. iris (Females)	*Aug -1*	0.677	0.502	0.0636	0.217	-0.372	0.499	0.20	1.579	N
	Sep -1	0.0830	0.934	0.0981	0.248	-0.400	0.596	0.21	1.211	N
	March	-1.098	0.277	-0.145	0.164	-0.473	0.184	0.29	1.268	N
	April	-0.799	0.428	-0.0823	0.276	-0.635	0.470	0.20	1.416	N
	May	2.671	0.0102	0.730	0.279	0.173	1.288	0.95	1.207	Y
	June	-1.868	0.0677	-0.463	0.267	-0.999	0.0726	0.58	1.160	Y
	July	2.193	0.0330	0.387	0.189	0.00902	0.765	0.75	1.301	Y
A. paphia (Females)	*March*	-1.153	0.249	-0.0540	0.0487	-0.150	0.0417	0.41	1.147	N
	April	-2.367	0.0182	-0.174	0.0606	-0.293	-0.0548	0.98	1.331	Y
	May	-0.634	0.527	-0.0268	0.0618	-0.148	0.0946	0.28	1.262	N
	June	-1.788	0.0742	-0.157	0.0752	-0.304	-0.00926	0.76	1.183	Y
	July	-0.742	0.459	-0.0268	0.0618	-0.148	0.0946	0.33	1.475	N

Species	Month	*t-value*	*p*	*Slope estimate*	*Standard error*	*Lower CI*	*Upper CI*	*Importance*	*VIF*	*Final model (Y/N)*
E. aurinia	*July -1*	-0.308	0.758	-0.0131	0.0308	-0.0735	0.0473	0.29	1.448	N
	August -1	-0.012	0.991	-0.0127	0.0333	-0.0781	0.0526	0.30	1.710	N
	March	-1.485	0.138	-0.0466	0.0293	-0.104	0.0109	0.57	1.232	Y
	April	0.498	0.618	0.0144	0.0333	-0.0509	0.0797	0.29	1.063	N
	May	4.093	<0.001	0.145	0.0337	0.0785	0.211	1.00	1.132	Y
	June	-3.054	0.00229	-0.109	0.0352	-0.178	-0.0397	1.00	1.083	Y
F. adippe	*March*	-0.741	0.459	-0.0391	0.0445	-0.126	0.0483	0.34	1.099	N
	April	-1.936	0.0533	-0.110	0.0553	-0.218	-0.00120	0.72	1.173	Y
	May	3.927	<0.001	0.217	0.0556	0.108	0.326	1.00	1.065	Y
	June	0.720	0.472	0.0499	0.0780	-0.103	0.203	0.31	1.101	N
	July	2.578	0.0101	0.134	0.0530	0.0301	0.238	0.92	1.144	Y
H. semele (Males)	*Aug-1*	0.721	0.471	0.0255	0.0611	-0.0944	0.146	0.29	1.201	N
	Sep-1	-1.597	0.111	-0.0845	0.0580	-0.198	0.0295	0.51	1.162	Y
	March	-3.233	0.00129	-0.118	0.0400	-0.197	-0.0398	1.00	1.247	Y
	April	2.486	0.0132	0.113	0.0511	0.0130	0.214	0.82	1.187	Y
	May	-0.361	0.718	-0.0219	0.0533	-0.127	0.0828	0.28	1.145	N
	June	3.996	<0.001	0.202	0.0554	0.0932	0.311	1.00	1.105	Y
	July	-1.570	0.117	-0.0803	0.0544	-0.187	0.0264	0.52	1.153	Y

Species	Month	*t-value*	*p*	*Slope estimate*	*Standard error*	*Lower CI*	*Upper CI*	*Importance*	*VIF*	*Final model (Y/N)*
M. athalia	*July-1*	1.793	0.0734	0.0711	0.0526	-0.0320	0.174	0.54	2.375	Y
	Aug-1	-1.923	0.0537	-0.0714	0.0551	-0.180	0.0368	0.52	2.400	Y
	March	2.498	0.0127	0.0596	0.0325	-0.00424	0.123	0.70	1.295	Y
	April	-0.946	0.344	-0.0490	0.0428	-0.133	0.0350	0.42	1.181	N
	May	1.383	0.167	0.0541	0.0443	-0.0330	0.141	0.46	1.309	N
	June	1.732	0.0836	0.121	0.0518	0.0196	0.223	0.86	1.388	Y
S. aglaja	*March*	-1.551	0.121	-0.0459	0.0359	-0.120	0.0210	0.49	1.100	N
	April	1.382	0.167	0.0608	0.0463	-0.0300	0.152	0.47	1.023	N
	May	4.977	<0.001	0.231	0.0441	0.145	0.318	1.00	1.105	Y
	June	1.270	0.204	0.0603	0.0519	-0.0414	0.162	0.42	1.044	N

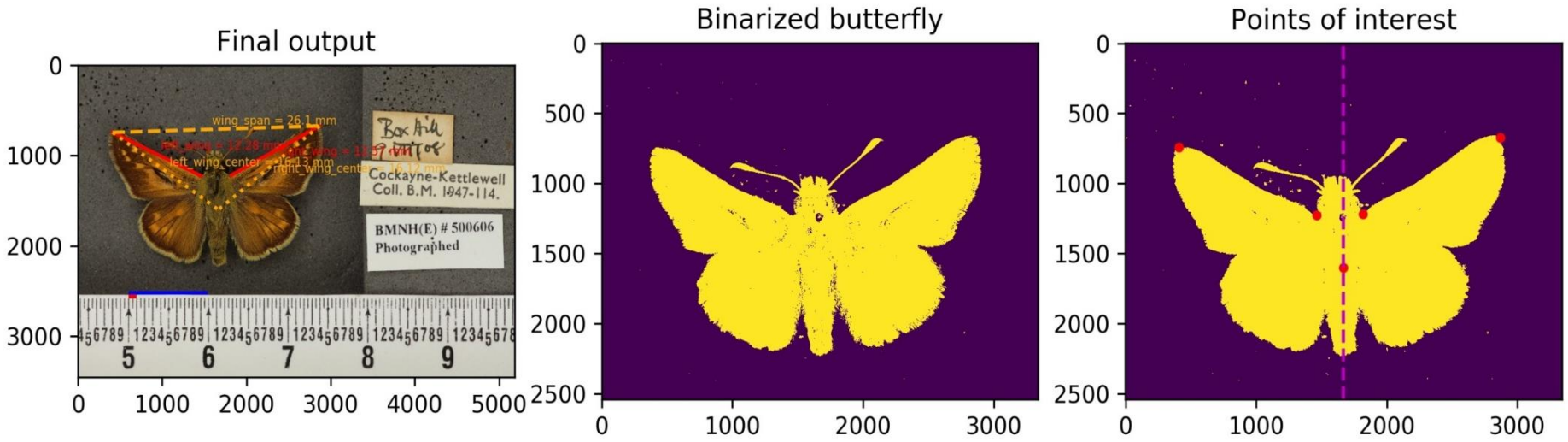

Figure A.1 Output image for a specimen of Hesperia comma showing the stages of taking measurements using the computer vision pipeline. The middle panel shows the first stage (binarisation) where the pipeline differentiates between the butterfly and the background. The right-hand panel shows the points of interest detected by the pipeline (forewing tips, points of attachment of forewings to the thorax, and the centre of the 'body'). The left-hand panel shows the final output, where the pipeline has set the scale using the scale bar in the image and taken measurements using the points of interest.

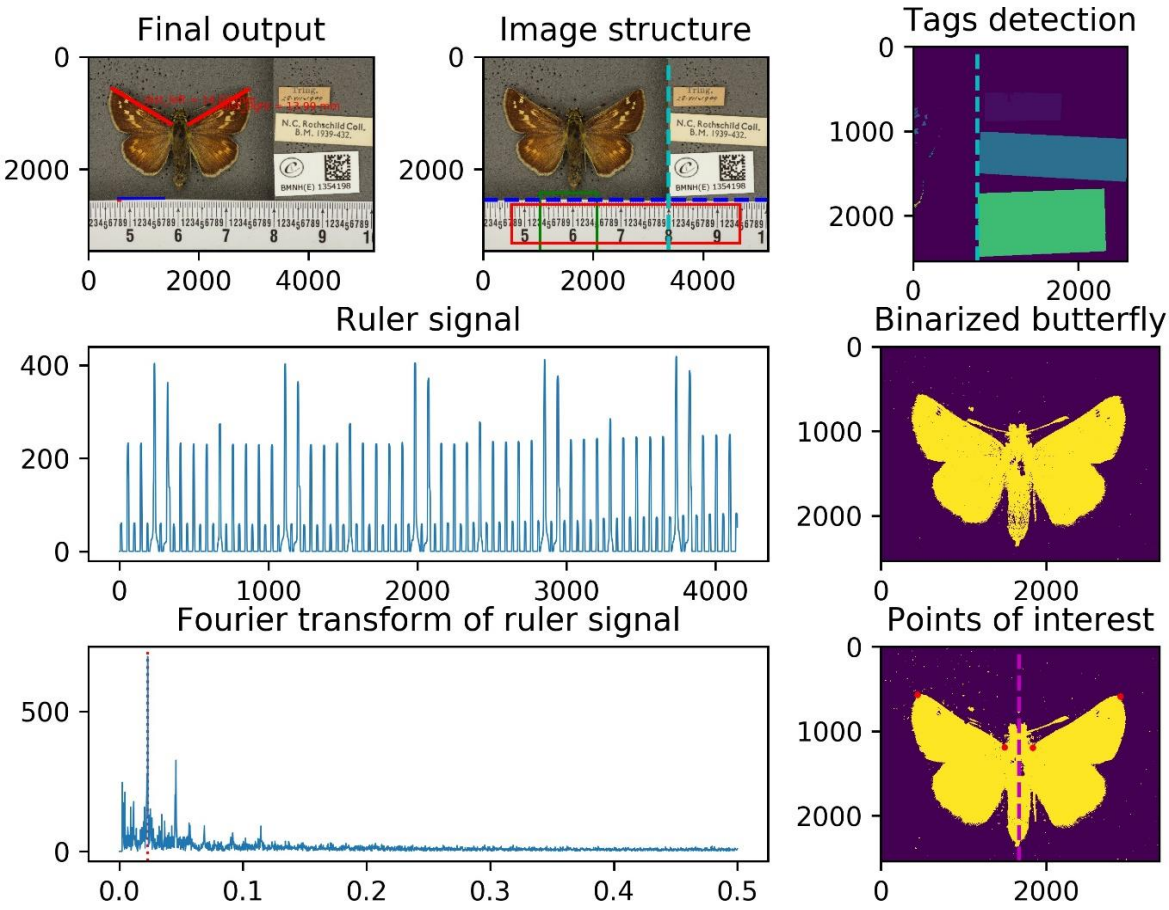

Figure A.2 Detailed output image for a *Hesperia comma* specimen, showing the stages of the pipeline (Scale detection, butterfly location and tracing and measurement).

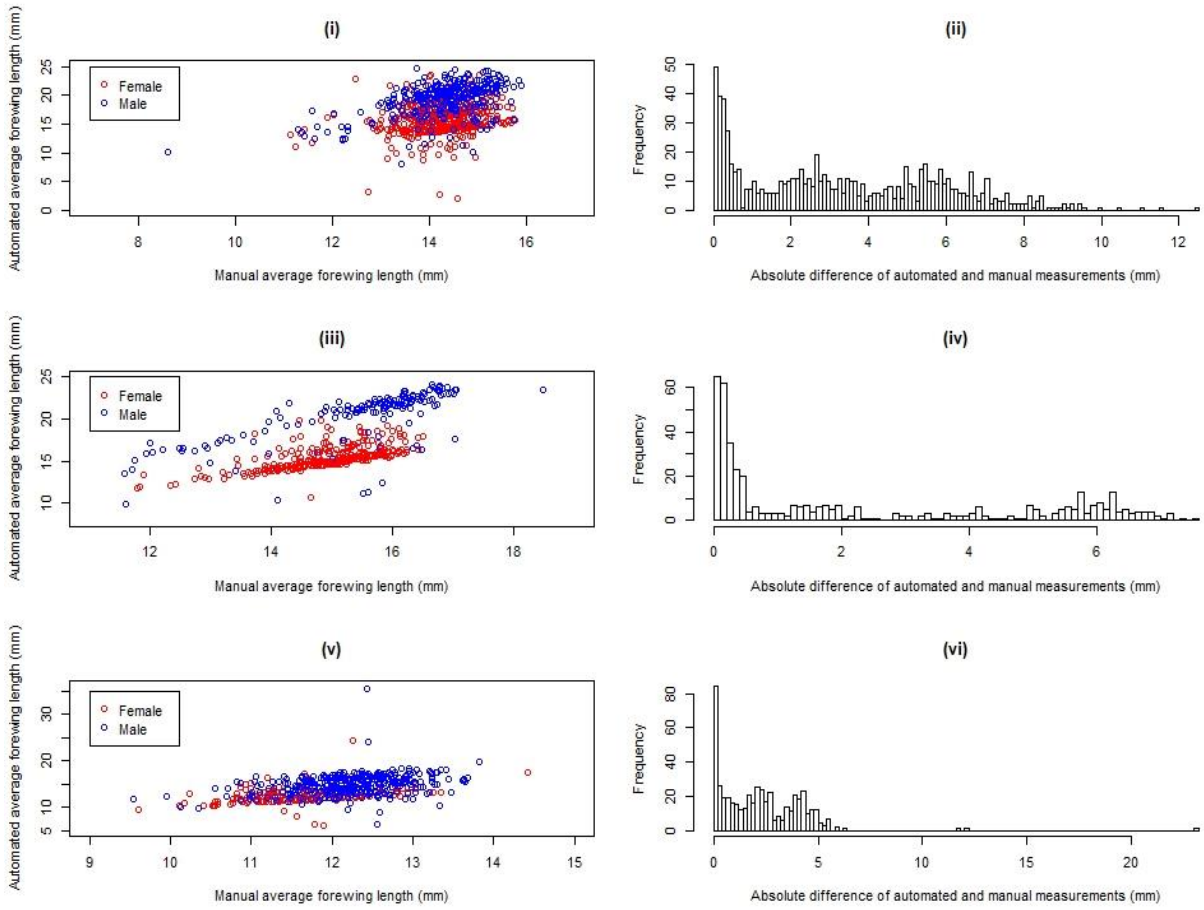

Figure A.3 Scatter plots of manual versus automated measurements for each individual, and frequency plots of the absolute difference in values between the manual and machine measurements for specimens of i and ii) *Polyommatus bellargus*, iii and iv) *Polyommatus coridon*, v and vi) *Plebejus argus*. For the scatter plots, males are indicated by the blue points, and females are indicated by the red points.

Appendix B Chapter 4 Supplementary Information

Table B.1 Meta-data for sites where field data were collected, and notes about other species present at the site. N.B. Species measured: P.v – *Patella vulgata*, P.u – *Patella ulyssiponensis,* P.d – *Patella depressa*, L.l – *Littorina littorea*, S.u – *Steromphala umbilicalis*, S.c – *Steromphala cineraria*, P.l – *Phorcus lineatus*

Region	Site name	Location	Date	Species measured	Notes
North West (Outer Hebrides)	Cille pheadair	57°09.011'N, 007°24.970'W	9/8/18	P.v, L.l, S.u, S.c	Fucus spp. were super abundant, other littorinids were common and *N. lapillus* was abundant
	Cheesebay	57°38.915'N, 007°04.602'W	10/9/18	P.v, L.l, S.u, S.c	Algae was abundant, other littorinids were abundant*, N. lapillus* was frequent
	Lochmaddy	57°35.957'N, 007°09.422'W	11/8/18	P.v, L.l, S.u, S.c	Algae was abundant, other littorinids were common, *N. lapillus* was abundant. Many L.l were dead and shells contained hermit crabs (not measured)
	Eriskay	57°04.680'N, 007°18.595'W	12/8/18	P.v, P.u, L.l, S.u, S.c	Algae common in areas, other littorinids were occasional, *N. lapillus* common.
	Vallay Island	57°39.834'N, 007°24.846'W	13/8/18	P.v, P.u, L.l, S.u, S.c	Very exposed site, some algae present on lower shore, other littorinids occasional, *N. lapillus* was abundant.
	Peter's Port	57°24.023'N, 007°15.929'W	14/8/18	P.v, L.l, S.c	High diversity on lower shore. Algae super abundant, other littorinids frequent, *M. edulis* frequent. At low water, nudibranchs, fish, jellyfish, bryozoans, sea squirts and sponges also present
	Balranald	57°36.569'N, 007°31.435'W	15/8/18	P.v, L.l, S.u, S.c	Algae was abundant, other littorinids were common, *N. lapillus* was common

Region	Site name	Location	Date	Species measured	Notes
North West (Outer Hebrides)	Grimsay	57°28.685'N, 007°12.238'W	16/8/18	P.v, L.l, S.u, S.c	Site next to shellfish fishery, many species found living on shell middens (mainly made of scallops). Other littorinid species were rare-occasional, *N. lapillus* was common, *M. edulis* was frequent
South East (S.E. England)	Bognor Regis	50° 47.149'N, 000° 39.012'W	16/5/19	P.v, P.u, L.l, S.u, S.c, P.l	Algae was abundant, other littorinids occasional, *N. lapillus* was common
	Ramsgate	51° 21.301'N, 001° 26.642'E	4/6/19	P.v, L.l, S.u, S.c	Algae was super abundant, other littorinids were occasional, *N. lapillus* was common
	Folkestone	51°04.953'N, 001°11.666'E	5/6/19	P.v, L.l, S.u, S.c	Algae was common on high and low shore, barnacles on mid shore, other littorinids were rare, *N. lapillus* was common
	Hastings	50°51.064'N, 000°33.789'E	5/6/19	P.v, L.l, S.u	Muddy shore, rocks covered in barnacles, *N. lapillus* was abundant, *M. edulis* was abundant
	Beachy Head	50°44.235'N, 000°15.507'E	6/6/19	P.v, L.l, S.u	Rocks covered in algae and barnacles, *N. lapillus* was rare
	Brighton Marina	50° 48.698'N, 000° 05.502'W	6/6/19	P.v, L.l, S.u, S.c	Algae was abundant, other littorinids were rare, *N. lapillus* was rare, *M. edulis* was frequent
	Seaview	50°43.193'N, 001°06.486'W	31/7/19	P.v, P.u, L.l, S.u, S.c	Algae was abundant, other littorinids were common, *C. fornicata* was common
	Totland Bay	50°40.651'N, 001°33.033'W	1/8/19	P.v, P.d, S.u, S.c	Algae was common, other littorinids were rare. P.v had smooth shells
South West (S.W. England)	Lyme Regis	50°43.510'N, 002°55.889'W	21/3/19	P.v, P.u, P.d, L.l, S.u, S.c, P.l	Algae were super abundant, *N. lapillus* was frequent. Limpets found on sea wall as well as rocks
	Swanage	50°36.422'N, 001°57.656'W	7/5/19	P.v, P.u, P.d, S.u	Algae were dominant on the shore, other littorinids were rare. There were some L.l but too small to measure

Region	Site name	Location	Date	Species measured	Notes
South West (S.W. England)	Paignton	50°26.885'N, 003°33.203'W	30/9/19	P.v, P.u, S.u, P.l	Barnacles and algae both abundant, other littorinids were occasional, *N. lapillus* was occasional
	Bude	50°49.950'N, 004°33.318'W	1/10/19	P.v, P.u, P.d, S.u, P.l	Barnacles were abundant, *N. lapillus* was frequent. There were algae covered rocks on other side of beach, but no large individuals of any species
	Ilfracombe	51°12.580'N, 004°06.671'W	2/10/19	P.v, L.l, S.u, P.l	Algae were abundant, other littorinids were occasional
	Plymouth	50°21.445'N, 004°07.604'W	29/10/19	P.v, L.l, S.u, P.l	Algae were abundant, *N. lapillus* was common. Limpets were tall
	Falmouth	50°08.688'N, 005°03.957'W	30/10/19	P.v, P.u, P.d, L.l, S.u, S.c, P.l	Other littorinids were rare, *N. lapillus* was common. Conditions were stormy so measuring time cut to 30 minutes each for limpets and L.l
	Penzance	50°06.854'N, 005°31.851'W	31/10/19	P.v, P.u, P.d, L.l, S.u, S.c, P.l	Algae and barnacles were abundant, *N. lapillus* was common. Many L.l were hermitted. P.v were round

Glossary of Terms

bivoltine ..... a species that has two generations a year

determinate growth..... growth of the individual stops at maturity

ecdysis..... moulting of the exoskeleton in invertebrates

ectotherm ..... a species which requires an external source to regulate body temperature

endotherm ..... a species which is able to maintain its own body temperature (without the need for an external source)

holometabolous..... species that develop in several life stages: egg, larva, pupa and adult; the larva is distinctly different from the adult in appearance.

indeterminate growth..... growth continues throughout the individual's life

multivoltine..... a species that has three or more generations per year

phenology ..... the timing of recurring biological events

sclerochronology..... the study of the physical and chemical variations in hard tissues of organisms, and the time in which they have formed.

sexual size dimorphism..... individuals of one sex are larger in size than individuals of the other sex

univoltine ..... a species that has one generation per year

voltinism..... the number of generations per year

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