Interplay of physiology, ecology and the environment: the implications of body size and shape

Submitted in partial fulfilment of the requirements of the

Degree of Doctor of Philosophy

Hanrong Tan

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Details of collaboration and publications

Throughout this thesis, I used the term ‘we’ to acknowledge the contribution of all the collaborators involved in this work. This thesis was supported by the Queen Mary Principal’s Studentship.

Chapter 1: Hanrong Tan wrote the chapter.

Chapter 2: Hanrong Tan, Andrew G. Hirst and David Atkinson designed the study and wrote the paper with contributions from Douglas S. Glazier. HT and AGH collected the data. HT performed the statistical analyses. **This chapter has been utilised in publication** (see Appendix).

Chapter 3: Hanrong Tan, Andrew G. Hirst and David Atkinson designed the study and wrote the paper with Pavel Kratina. HT collected the data and performed the statistical analyses.

Chapter 4: Hanrong Tan and Pavel Kratina designed the study and wrote the paper with Andrew G. Hirst and David Atkinson. HT performed the experimental work, collected the data and performed the statistical analyses. Ignacio Peralta-Maraver enumerated the bacterial densities.

Chapter 5: Hanrong Tan and Pavel Kratina designed the study and wrote the paper with Andrew G. Hirst and David Atkinson. HT and PK sampled the regional lakes, and collected the mesocosm samples along with the AQUACOSM Transnational Access 2018 (WasserCluster Lunz) team. András Abonyi identified and counted the phytoplankton species. Csaba Vad and Larysa Samchyshyna identified and counted the zooplankton species.

Chapter 6: Hanrong Tan wrote the chapter.

Interplay of physiology, ecology and the environment: the implications of body size and shape

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Abstract

Body size is a key trait that influences organisms' vital rates. Organisms also differ in their body shape, which may influence the scaling of physiological rates. Metabolic rates commonly scale with body size with a scaling exponent between 2/3 and 1 for reasons still contested. Using meta-analyses, I show that the intraspecific scaling exponents of cephalopods covary positively with metabolic levels (i.e. elevation of the metabolism-mass relationship) across species, contrasting the negative covariation observed in teleost fish. I describe how contrasting energetic and mortality pressures, and the steeper size-scaling of body surface area associated with relative body shape elongation or flattening in epipelagic cephalopods that have rapid, near-exponential growth can explain this difference. I further reveal clear differences in energy use during non-flight activity between insects and spiders. Across species, active metabolic rates scaled more steeply with body mass in insects than spiders, and is associated with greater energetic demands for sustained activity in larger insects, which is accentuated by possessing wings. These findings add to the evidence that metabolic scaling varies systematically with multiple ecological factors. I then show that experimental warming strongly influenced body size of model protist species, while their body shape was affected more by resource availability, indicating their competitive abilities. Finally, an experimental heatwave imposed on freshwater plankton communities reduced the elevation and steepened the slope of the negative relationship between organism abundance and body size, and also decreased total zooplankton abundance. However, using a species introduction treatment, I show that connectivity to the regional species pool buffers such changes in the elevation. As changes in the abundance-body size relationship relate to warming effects on trophic efficiency and increased energy use, understanding the interplay between body size, metabolism and temperature is critical for understanding climate change impacts on ecosystem structure and function.

Contents

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Figures

Tables

Supplementary information (End of thesis)

Figures

Tables

Published paper

Appendix 1. Tan H., Hirst A.G., Glazier D.S. & Atkinson D. (2019). Ecological pressures and the contrasting scaling of metabolism and body shape in coexisting taxa: cephalopods versus teleost fish. *Philosophical Transactions of the Royal Society B* **374**, 20180543. https://doi.org/10.1098/rstb.2018.0543 **(from chapter 2 of this thesis)**.

Supplementary information (online)

Online supplementary information can be accessed via Google Drive with the following link:

https://drive.google.com/drive/folders/1nfy1zfijcYy02FsGeyjc1jKQg0d7mBgl?usp=sharing

Dataset S6.1. Cephalopod respiration rate-body mass scaling relationship, mass-length relationship and lifestyle category collected for chapter 2.

Dataset S6.2. Insect and spider body mass and metabolic rate data collected for chapter 3.

SI 6.3. The compiled insect and spider phylogenetic supertree used for Phylogenetic generalised least squares (PGLS) regression.

Dataset S6.4. Mean length, width and body size measurements obtained for the two experimental protist species (*Paramecium aurelia* and *Blepharisma japonicum*) presented in chapter 4.

Dataset S6.5. Mean body size and abundance of phytoplankton and zooplankton taxa used for constructing the community size spectra presented in chapter 5.

Dataset S6.6. The elevation and slopes of the linear model describing the relationship between organism abundance and body size for chapter 5.

List of abbreviations

Chapter 1: General introduction

M	Body mass
b	Body mass-scaling exponent of metabolic rate
DEB	Dynamic energy budget theory
MLBH	The metabolic-level boundaries hypothesis
L_{mid}	Metabolic level, defined as the mass-specific metabolic rate at the geometric mid-point of the metabolic rate-body mass scaling relationship.
TSR	Temperature-size rule, a commonly observed inverse relationship between intraspecific body size-at-stage and temperature.
N	Organism or species abundance
b_{ss}	Slope of the regression line relating abundance to body mass expressed on log-log transformed axes. The slope describes how rapidly organism abundance decline with body mass increase within a community.
i_m	Elevation of the abundance-body mass relationship calculated as the abundance at the geometric midpoint of the abundance-body mass relationship.

Chapter 2: Ecological pressures, and the contrasting size-scaling of metabolism and body shape in cephalopods and fish

MLBH	The metabolic-level boundaries hypothesis
b_R	Intraspecific scaling exponent of metabolic rate in relation to body mass.
L_R	Metabolic level, defined as the mass-specific metabolic rate at the geometric mid-point of the metabolic rate-body mass scaling relationship.
b_A	Intraspecific scaling exponent of surface area in relation to body mass.
b_L	Intraspecific scaling exponent of body mass in relation to body (mantle) length.

Chapter 3: Insects and spiders have contrasting activity-dependence of metabolic scaling

MLBH	The metabolic-level boundaries hypothesis
R	Metabolic rate

M	Body mass
b_R	Interspecific mass-scaling exponent of resting metabolic rate
b_a	Interspecific mass-scaling exponent of active metabolic rate
L_R	Resting metabolic level, defined as the mass-specific respiration rate at the geometric mid-point of the resting metabolic rate-body mass scaling relationship.
L_a	Active metabolic level, defined as the mass-specific respiration rate at the geometric mid-point of the active metabolic rate-body mass scaling relationship.

Chapter 4: Body size and shape responses to warming and resource competition in protists

TSR	Temperature-size rule, a commonly observed inverse relationship between intraspecific body size-at-stage and temperature.

Chapter 5: Regional diversity provides limited insurance to montane-lake communities influenced by heatwaves

i_m	Elevation of the community size structure, represented by the abundance (individuals L^{-1}) at the geometric mid-point of the abundance-body mass relationship.
b_{ss}	Slope of the regression line relating abundance to body mass expressed on log-log transformed axes. The slope describes how rapidly organism abundance decline with body mass increase within a community.
Δi_m	Differences in the elevation of the community size structure (i_m) between two sampling time points.
Δb_{ss}	Differences in the slope of the community size structure (b_{ss}) between two sampling time points.
t_0	Sampling time point before commencing the experimental heatwave.
t_h	Sampling time point at the end of the experimental heatwave.
t_{ah}	Sampling time point 16 days after the experimental heatwave.

Chapter 1

General introduction

Body size is one of the most conspicuous ways in which organisms differ, and has been a subject of much biological interest (Bergmann, 1847; Rubner, 1883; Haldane, 1928). Organism mass can vary up to 20 orders of magnitude, from the cell size of bacteria to the body size of the largest whales (Andersen *et al.*, 2016; Blanchard *et al.*, 2017). Such variation is highly informative, because body size is considered to be a 'master trait' (Andersen *et al.*, 2016). At the level of an individual organism, body size relates to various vital rates including metabolism (Kleiber, 1932; Hemmingsen, 1960; Peters, 1983; Brown *et al.*, 2004), growth (von Bertalanffy, 1957; Gillooly *et al.*, 2002; Hirst & Forster, 2013; Kiørboe & Hirst, 2014), survival and reproduction (Atkinson & Sibly, 1996; Arendt, 2011). Body size also plays an important role in determining species interactions, such as predation and competition (Hildrew, Raffaelli & Edmonds-Brown, 2007). At the level of ecological communities, body size is a key predictor of species or organism abundance (Damuth, 1981; Peters, 1983; White *et al.*, 2007; Perkins *et al.*, 2019), influencing the size structure of communities. The community size structure provides information on how biomass is partitioned among individuals and functional groups in a community, and has implications for energy flow through ecosystems (Woodward *et al.*, 2005; White *et al.*, 2007). Hence, variations in body size have profound implications that extend across levels of biological organisation (Woodward *et al.*, 2005), and understanding how biological traits vary with body size has become a central focus of animal ecology (Peters, 1983).

Body size scaling of metabolic rates

Body size is often related to many biological and ecological characteristics of an organism through a mathematical power function. These relationships often take the form $Y = aM^b$ (equation 1), where Y is the biological rate of interest, M is body mass, and a and b are empirically derived, or mechanistically predicted terms (Rubner, 1883; Huxley, 1932; Peters, 1983; West, Brown & Enquist, 1997; Allen & Gillooly, 2007). One such relationship that has drawn substantial research interest is that between body size and metabolic rate. Metabolism encompasses the process of transforming energy and material to life-supporting functions and biological structures (Glazier, 2005). Metabolic rate therefore represents the rate of collective biochemical processes of extracting resources from the environment, and converting them to energy for survival, growth and reproduction (Brown *et al.*, 2004). This fundamental biological rate relates to the resource demands an organism places on its environment for growth and reproduction, and is closely linked to population growth and biomass production (Brown *et al.*, 2004).

In general, individual metabolic rate tends to increase with body size: consequently, large organisms require more energy per unit of time than small organisms (Kleiber, 1932). However, metabolic rate tends to increase at a slower relative rate than does body mass, and large organisms often have lower energetic requirements than small organisms per unit of biomass (White, 2010). The power function relating metabolic rate to body mass (equation 1) has a scaling exponent (b) that often ranges from 2/3 to 1. Empirical observations across various organisms including endotherms (Brody & Procter, 1932; Kleiber, 1932), ectotherms and unicellular organisms (Hemmingsen, 1960), and plants (Savage *et al.*, 2004) suggest an average mass-scaling exponent of about 3/4. This led to the assertion of a universal '3/4-power law', which have been explained to be the result of an optimised fractal resource-transport network (West *et al.*, 1997; West, Brown & Enquist, 1999; Banavar *et al.*, 2002, 2010; Savage *et al.*, 2004). This 3/4-scaling law underlies the Metabolic Theory of Ecology (MTE) (Brown *et al.*, 2004), which predicts individual metabolic rate from their body size and body temperature. However, explanations based on resource transport networks have limited application in organisms without a closed circulatory system (Glazier, 2010). Claims of universality are further challenged by substantial variation in *b* values related to taxonomic affiliation, developmental stage, physiological state and environmental conditions (Glazier, 2006; DeLong *et al.*, 2010; Killen, Atkinson & Glazier, 2010; Glazier, 2014a; Hirst, Glazier & Atkinson, 2014; Carey & Sigwart, 2014; Glazier & Paul, 2017; Naya, Naya & White, 2018). Hence, further investigations are needed to explain the mechanisms contributing to the variation in body size-scaling of metabolic rates.

Alternatives to the MTE often relax the prediction that $b = 3/4$, and suggest that 3/4-scaling is not driven by a single universal mechanism. Instead, it is the combined outcome of different processes that scale with surface area or volume (Glazier, 2010; Kooijman, 2010). The dynamic energy budget theory (DEB) partitions body mass into 'reserve' and 'structure' (Kooijman, 2010). Maintenance cost is set by structure, which is assumed to be proportional to body volume, while the mobilisation of reserve, which fuels the metabolic needs of an individual (Kooijman, 2010) is assumed to scale with surface area (Van Der Meer, 2006). According to the DEB theory, the scaling exponent is the result of multiple processes that scale with either surface area or with volume (Glazier, 2010; Sibly, Brown & Kodric-Brown, 2012), and variations in the size-scaling of metabolic rates is associated with differences in the proportion of reserve to structure. The metabolic-level boundaries hypothesis (MLBH) also recognises the contribution of both surface area- and volume-related scaling processes for explaining variation in metabolic scaling (Glazier, 2005, 2010). However, the MLBH suggests that metabolic scaling is limited by surface area- and volume- related boundary or transition constraints. Surface area limits the supply of resources, or the elimination of metabolic waste and heat, while volume limits maintenance costs of the living tissue, or power production during activity, both of which may scale with mass to the power 1 (Weibel, 2005; Glazier, 2010). The relative influence of surface area- or volume-related constraint underlies the observed *b* value according to MLBH, and are set by the metabolic level (L_{mid}), which can be estimated as the mass-specific metabolic rate at the geometric midpoint of the metabolic scaling relationship with body mass (Figure 1.1a, Glazier, 2010; Killen *et al.*, 2010). This elevation of metabolic rate is related to the physiological state, and ecological lifestyle of organisms (Heusner, 1991; Killen *et al.*, 2010). Hence, environmental conditions can influence *b* by affecting the metabolic rate required for activity (Figure 1.1b), which is represented as a change in metabolic level (Glazier, 2010; Killen *et al.*, 2010; Glazier, 2014a). The relationship between metabolic level and *b* (Figure 1.1) therefore offers a link for understanding how ecological constraints could influence the size-scaling of metabolic rates (Glazier, 2010).

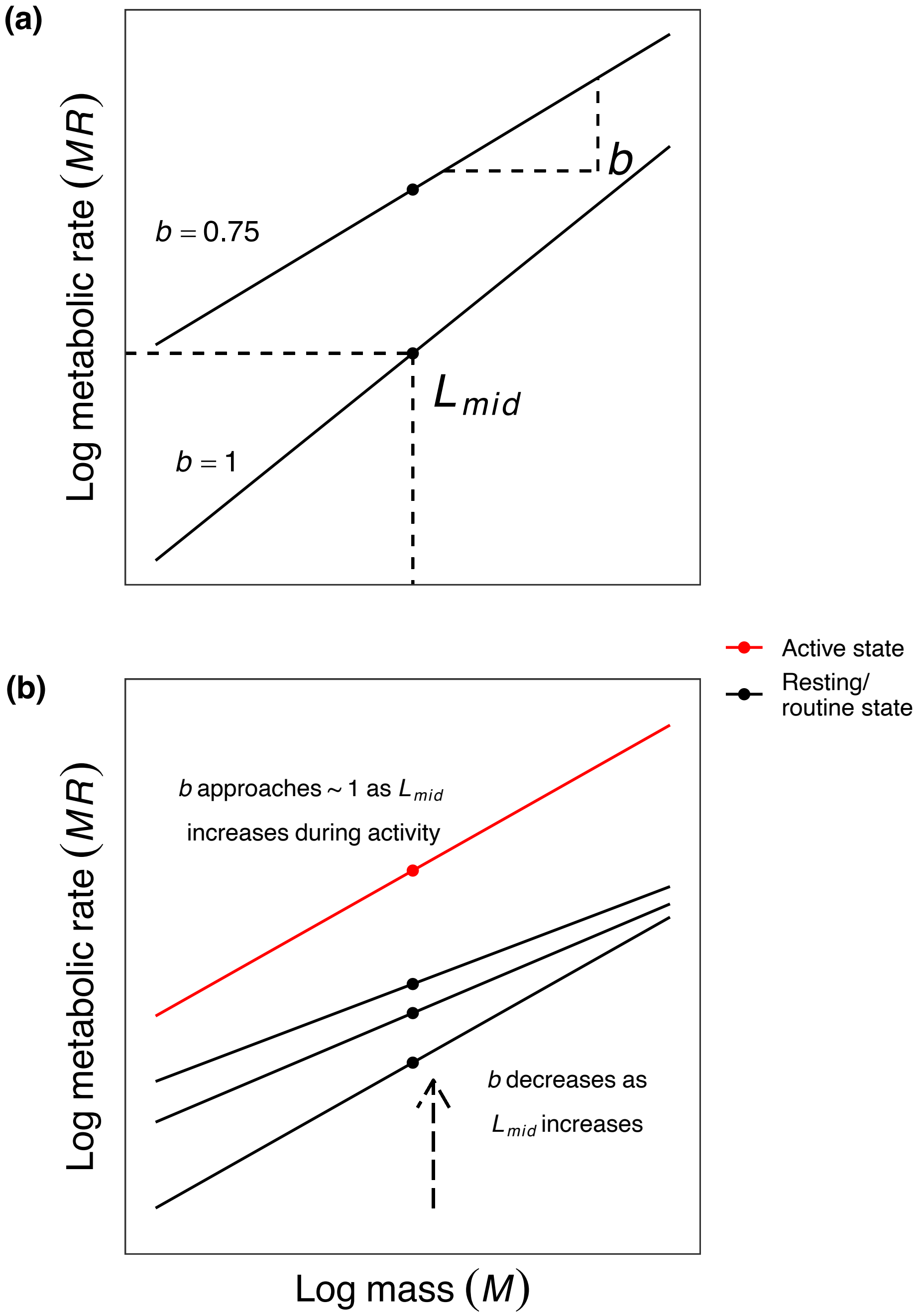


Figure 1.1. (a) A schematic illustrating the parameters in the metabolic-level boundaries hypothesis (MLBH); the metabolic level (L_{mid}) and the metabolic scaling exponent, *b*. L_{mid} is defined as the mass-specific metabolic rate at the geometric midpoint of the metabolic rate-body mass relationship, as indicated by the black points. The body mass-scaling exponent, *b*, refers to the slope of this metabolic rate-body mass relationship. This relationship can be intraspecific (obtained within species) or interspecific (obtained across species of different body size). Intraspecific scaling describes how metabolic rate changes over ontogeny in a species, whereas interspecific

scaling relationships describes changes in metabolic rate as species increase in size. **(b)** For both intraspecific and interspecific scaling relationships, the MLBH predicts that environmental factors and activity state that influences L_{mid} could in turn influence *b*. As L_{mid} increases due to activity, *b* is predicted to increase, and approaches ~1 due to the increased influence of power production on *b*.

Body shape as a functional trait

Besides body size, organisms also differ greatly in their body shape, which has been often ignored in physiological studies. Organisms that retain the same shape as size is increased are termed isomorphic, whereas those that change shape are non-isomorphic. In skin-breathing invertebrates, changes in body shape could influence resource uptake and the size-scaling of physiological rates (Okie, 2013; Hirst *et al.*, 2014, 2017; Glazier, Hirst & Atkinson, 2015). More generally, organisms that depend on body surface for the direct exchange of oxygen and waste material could increase their surface area relative to body mass through changes in body shape in which one or more body dimensions increase more than other dimensions. Extreme body shape changes include relative elongation (greater increase in the long dimension, relative to other dimensions) or flattening (greater growth in the two longest dimensions relative to the third dimension) during growth (Okie, 2013; Hirst *et al.*, 2014). Consequently, body elongation or flattening can increase surface area relative to that of a body that does not change shape (Hirst *et al.*, 2014). Moreover, varying degrees of body-shape change as organisms increase in size are correlated with variation in the body-size scaling of respiration and excretion rates in pelagic invertebrates (Hirst *et al.*, 2014, 2017; Glazier *et al.*, 2015).

In addition to influencing physiological scaling, body shape also modifies biological functions such as motility (Beveridge, Petchey & Humphries, 2010; Lee, Skinner & Camacho, 2013; Gibert *et al.*, 2017; Pennekamp, Clobert & Schtickzelle, 2019), which could potentially alter encounter rates (Berger, 1980) and species interactions. Body shape is also influenced by environmental factors such as temperature and resource availability (Young, 2006; Jezberová & Komárková, 2007; Naselli-Flores & Barone, 2011; Gibert *et al.*, 2017). For example, the body shape of terrestrial arthropods varies systematically in relation to temperature and ecosystem productivity (Ruiz-Lupión, Gómez & Moya-Laraño, 2019), and possibly also to predation pressure (Martin, Proulx & Magnan, 2014). More productive and warmer ecosystems likely favour faster growth in length and shorter development times, while weakening selection for energy storage, resulting in relatively elongate body forms (Ruiz-Lupión *et al.*, 2019). As insect running speed increases with body length (Full & Tu, 1991), increased predation pressure in the tropics (Johnson & Strong, 2000) could also favour disproportionately slender insects that could better evade predators (Martin *et al.*, 2014). These findings suggest that body shape is an important ecological trait that is strongly influenced by the environment. However, studies on body shape responses to environmental changes are rare, and the direct effects of warming and resource availability on body shape remains poorly understood.

Body size patterns

In contrast to body shape, the influence of environmental changes on body size has attracted substantial research attention. Systematic variation in body size has been related to environmental conditions, and remains a subject of on-going research (Bergmann, 1847; Atkinson, 1994; Horne, Hirst & Atkinson, 2015; Evans *et al.*, 2019; Gardner *et al.*, 2019). One prominent geographical pattern in body size is known as Bergmann’s rule (Bergmann, 1847). This rule describes the inter-specific latitudinal cline, where larger bodied species inhabit higher, colder latitudes. While originally derived from endotherms (organisms that maintain a constant body temperature), this relationship has been widely observed in ectotherms (Angilletta, Steury & Sears, 2004; Sommer *et al.*, 2017; Evans *et al.*, 2019). James' rule describes a similar latitudinal size-cline but applies to intra-specific differences, where individuals of a species tend to have larger body sizes at higher, colder latitudes (James, 1970). Because environmental temperature covaries with latitude, temperature is suggested to be the primary mechanistic driver of these latitudinal size-clines. Conspecifics growing in warmer conditions also usually mature at a smaller adult body size than when growing in colder conditions (Figure 1.2, Atkinson, 1994). This phenomenon is commonly referred to as the temperature-size rule (TSR; Atkinson, 1994), and is observed in more than 80% of ectotherms, making it one of the most commonly observed relationships in biology (Angilletta *et al.*, 2004; Kingsolver & Huey, 2008). While important exceptions to all these rules exist (Adams *et al.*, 2013; Teplitsky & Millien, 2014; Riemer, Guralnick & White, 2018), the prevailing inverse relationship between temperature and body size underlies the widespread prediction of reduced body sizes in response to climate warming (Daufresne, Lengfellner & Sommer, 2009; Gardner *et al.*, 2011; Sheridan & Bickford, 2011). Changes to the mean community body size under warming could thus result from reductions in intraspecific individual body sizes, as predicted by James' rule and the TSR, or from increased proportion of small to large species (Daufresne *et al.*, 2009; Morán *et al.*, 2010; Evans *et al.*, 2019), as predicted by Bergmann's rule.

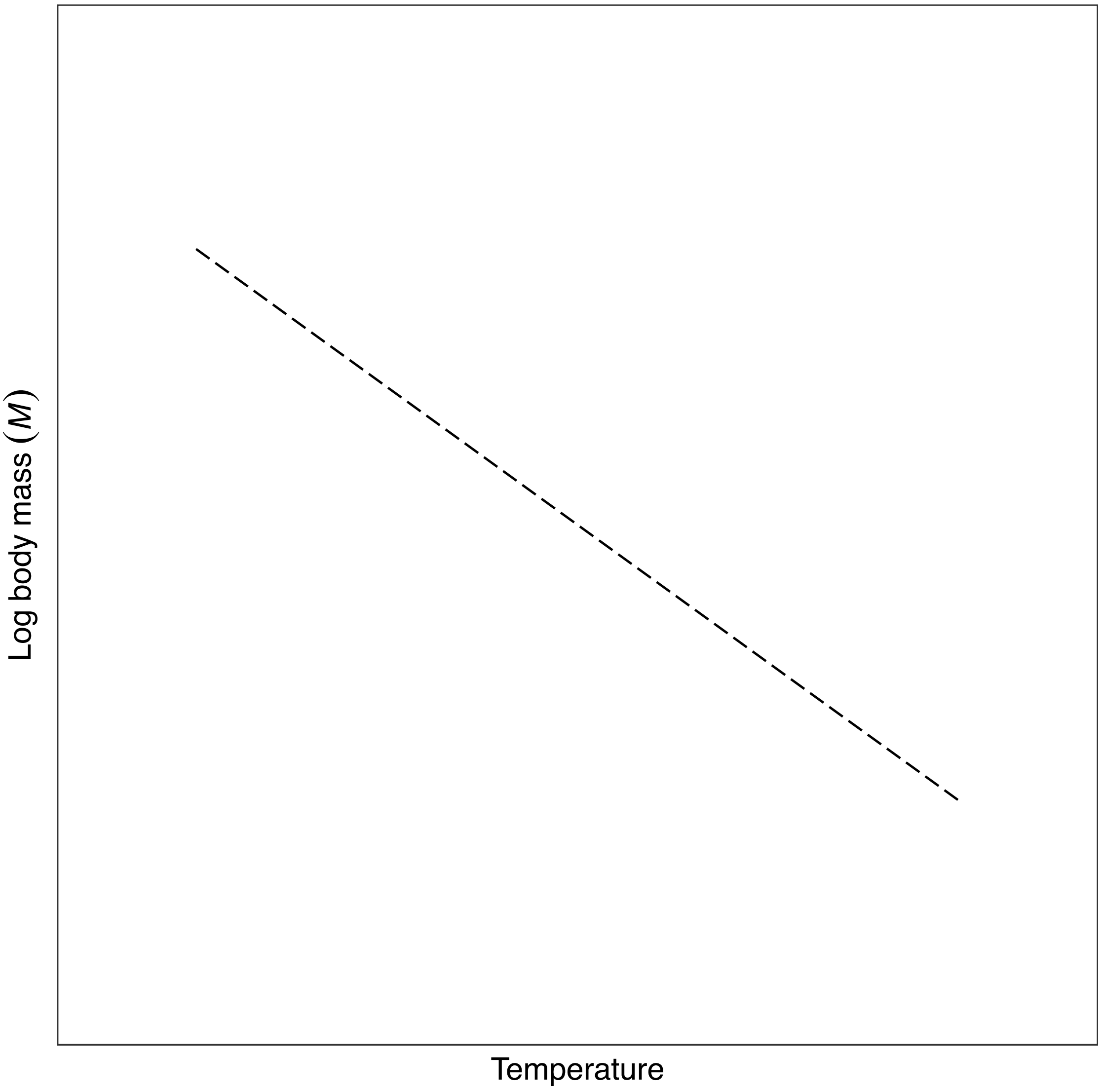


Figure 1.2. A schematic illustrating the commonly observed inverse relationship between body size and temperature. The phenomenon is often referred to as the temperature size rule (TSR), and is observed in over 80% of ectotherm species studied (Atkinson, 1994).

‘Connecting the dots’: TSR, metabolic rates and body shape

Higher, non-lethal temperatures often increase metabolic rates (Schmidt-Nielsen, 1997), but have little effect on the diffusion coefficient of oxygen in aquatic environments (Woods, 1999). Warming therefore increases oxygen demands more strongly than oxygen supply (Verberk *et al.*, 2011), and could result in oxygen limitations. Such effects are potentially greater in aquatic environments, because oxygen concentration and diffusion rate are lower in water than air (Verberk *et al.*, 2011). Body size, as a major determinant of metabolic rates, may therefore decrease in warmer conditions in order to reduce individual metabolic demands under oxygen limitation. Correspondingly, the TSR responses are stronger in aquatic than terrestrial environments (Forster, Hirst & Atkinson, 2012; Horne *et al.*, 2015), supporting the importance of oxygen availability as a driver of the TSR. Besides decreasing metabolic demands, size reduction would also increase surface area to volume ratio, and improve resource uptake (Atkinson, Ciotti & Montagnes, 2003). However, as body-shape changes likely alter how surface area scales with size and hence the uptake of resource, body shape response to warming could potentially modulate temperature-size response. Furthermore, as body shape also influences motility and food acquisition (Berger, 1980; Lee *et al.*, 2013; Gibert *et al.*, 2017), climate warming should favour body forms with increased motility that improve prey capture or dispersal to track climatic niches. For these reasons, the responses of body shape and size should be both studied together.

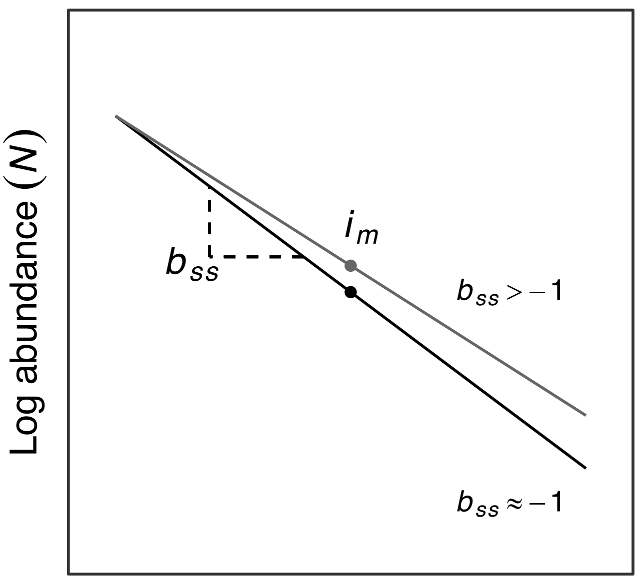
Abundance-body size relationships

In addition to being a major determinant of many physiological and life-history characteristics, body size also relates to species or organism abundance in ecological communities (Peters, 1983; Brown *et al.*, 2004; White *et al.*, 2007). Body size therefore provides a link between individuals, populations and communities. The composition of species with different body sizes in a community is therefore critical for understanding the structure and function of ecological communities (Hildrew *et al.*, 2007; Rossberg, Gaedke & Kratina, 2019). Communities are commonly composed of many small but few large individuals. This relationship between species or individual abundances and body size is described as a power function, $N \propto M^{b_{ss}}$ (Sheldon, Prakash & Sutcliffe, 1972; Peters, 1983; White *et al.*, 2007), which relates species or organism abundances (N) to body-size (M), and b_{ss} is the N-M-scaling exponent that describes how strongly abundance decreases with body size increase (Figure 1.3a). In multi-trophic communities, the b_{ss} thus indicates trophic efficiency and the flow of energy from basal resources to top predators (Heneghan, Hatton & Galbraith, 2019). Steeper, more negative b_{ss} values indicate lower trophic efficiency, with less energy transferred to consumers across each trophic level. In contrast, shallower (less negative) b_{ss} suggests more efficient energy flow through food webs, supporting more large consumers (Figure 1.3a). The relationship between abundance and organism body size also describes the partitioning of energy and nutrients among different community compartments. For instance, communities with steeper b_{ss}, and hence a higher relative abundance of small organisms have a higher proportion of energy partitioned in these organisms. Because metabolic rate scales allometrically with body size, such communities can have higher biomass-specific metabolic rates, which potentially influence ecosystem functions such as carbon cycling.

Population density of organisms that share a common resource (Figure 1.3b) has been found to scale with an N-M-scaling exponent of -3/4 (Damuth, 1981; Peters, 1983; Enquist, Brown & West, 1998). This agrees with theoretical predictions of the MTE, proposing that the decline of large organisms is explained by the allometric scaling of metabolic rate. As metabolic rate is often assumed to scale with $M^{3/4}$ (Brown *et al.*, 2004), this gave rise to the energetic equivalence hypothesis, proposing that population energy use is independent of body size, given a common energy source. Empirical support for this hypothesis highlights the importance of energetic constraints in structuring ecological communities (Huete-Ortega *et al.*, 2012; Perkins *et al.*, 2019).

However, in food webs, organisms do not always share a common resource, and energy transfer from smaller to larger organisms is constrained by low energy transfer efficiency (Lindeman, 1942; Cyr, 2000). As aquatic communities are often strongly size-structured (Hildrew *et al.*, 2007), and body size is a strong predictor of trophic level, size-based analysis of food web size-structure is often used over species-based approaches (Heneghan *et al.*, 2019). The resultant relationship, commonly referred to as the size spectrum (Sheldon *et al.*, 1972; Kerr & Dickie, 2001), is constructed by taking the density of all individuals within logarithmically spaced bins (Blanchard *et al.*, 2017). Empirical observations of size spectra slope often approximates -1 (Sheldon *et al.*, 1972; Jennings & Mackinson, 2003; Sprules, Barth & Giacomini, 2016), supporting predictions of steeper *N*-*M*-scaling exponents based on low energy transfer efficiency through food webs (Brown & Gillooly, 2003).

(a) Community



(b) Population / Within trophic level

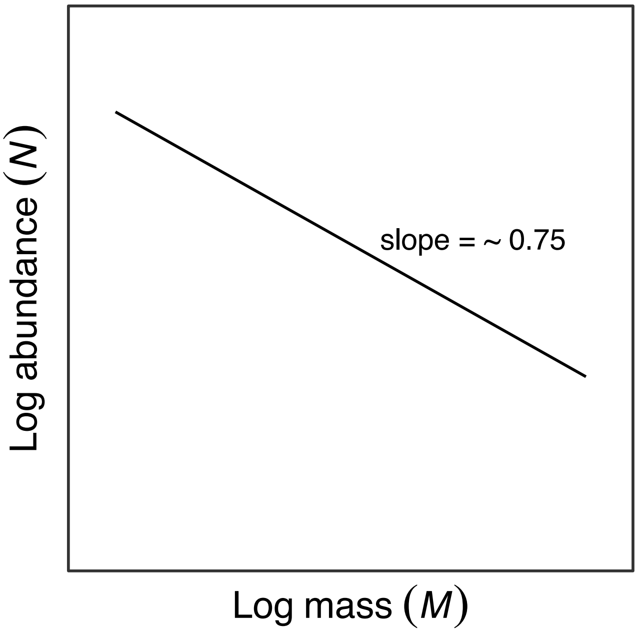


Figure 1.3. The abundance-body size relationship in **(a)** communities. The slope of this relationship (b_{ss}) describes how quickly organism abundance declines with body size. The elevation (i_m) of this relationship indicates the productivity, or carrying capacity of the community, and can be approximated as the abundance at the geometric midpoint of this relationship, as highlighted by the solid points. Different b_{ss} values could indicate differences in trophic efficiency. **(b)** In populations of organisms sharing a common energy source, the slope of the abundance-body mass relationship is commonly hypothesized to be -0.75.

While the size spectrum is highly regular (Heneghan *et al.*, 2019), empirical evidence shows that factors such as habitat complexity (Schmid, Tokeshi & Schmid-Araya, 2000; Jinks *et al.*, 2019), resource or nutrient availability (O'Gorman *et al.*, 2017; Peralta-Maraver, Robertson & Perkins, 2019; Rossberg *et al.*, 2019), and anthropogenic stressors alter the slope of the community size structure (Pauly *et al.*, 1998; Jennings & Blanchard, 2004; Yvon-Durocher *et al.*, 2011). For instance, experimental warming in mesocosms steepened the slope of the abundance-body size relationship (Yvon-Durocher *et al.*, 2011). This was caused by an increase in the relative abundance of small individuals under warming, which shifted the distribution of community biomass towards smaller individuals. A steeper slope indicates that warming reduces energy transfer efficiency through the community (Ullah *et al.*, 2018; Heneghan *et al.*, 2019), and more small individuals are needed to support fewer large predators. Such changes in the size distribution could arise due to at least two general mechanisms. The first mechanism relates to the TSR, which predicts that intraspecific individual size would decrease under warming (Atkinson, 1994; Daufresne *et al.*, 2009; Peter & Sommer, 2013). This could occur to compensate for temperature-enhanced resource demands (DeLong, 2012; Forster *et al.*, 2012) during warming. The second mechanism relates to species turnover and shifts in community composition (Yvon-Durocher *et al.*, 2011, 2015; Rasconi *et al.*, 2015) due to changes in resource competition in warmer environments (Lewington-Pearce *et al.*, 2019). This could favour small species with higher surface area to volume ratio, that are more efficient at acquiring resources than large species (Litchman, Klausmeier & Yoshiyama, 2009; Burson *et al.*, 2018), resulting in the competitive exclusion of larger species.

A steeper community size spectrum slope resulted from an increased abundance of smaller species under experimental warming (Yvon-Durocher *et al.*, 2011), and is consistent with predictions that warming favours small species (Daufresne *et al.*, 2009; Gardner *et al.*, 2011). However, following prolonged warming, an increased abundance of large, colonial phytoplankton taxa was instead observed (Yvon-Durocher *et al.*, 2015). This suggests potentially opposite responses to short-term versus long-term warming, and indicates that continued warming increases the strength of zooplankton grazing. This can in turn reduce the competitive advantage of small phytoplankton species (Yvon-Durocher *et al.*, 2015) and favour larger phytoplankton taxa that are more resistant to grazing (Sauterey *et al.*, 2017; Branco *et al.*, 2020). These findings highlight the importance of species turnover in mediating community-level response and suggest that connectivity and dispersal from regional species pools (Loreau, Mouquet & Gonzalez, 2003; Thompson & Shurin, 2012; Yvon-Durocher *et al.*, 2015) can play an important role in governing the body size structure of ecological communities.

While such findings improve our understanding of community size structure under the influence of mean increases in temperature, they might not be directly applicable to impacts of changes in temperature variability and instability (Vasseur *et al.*, 2014; Romero *et al.*, 2018). This is a pressing concern, because global climate change is also predicted to increase temperature variability, and the frequency of extreme climatic events (Easterling *et al.*, 2000; IPCC, 2012). Such extreme events could preclude evolutionary adaptations, causing local extinctions even when there is potential for evolutionary changes (Gutschick & BassiriRad, 2003). As community level responses are often the result of species turnover (Yvon-Durocher *et al.*, 2015; Salo, Mattila & Eklöf, 2019), local extinctions could in turn accentuate the role of species dispersal for modulating responses in the community size structure. It is therefore essential to understand whether and how regional diversity alters the community size spectra over short timescales, especially as most previous research has focused on the impacts of mean temperature increase (Vasseur *et al.*, 2014; Woodward *et al.*, 2016).

Research aims and thesis organisation

My thesis aims to better understand mechanisms underlying the variation in the body size-scaling of metabolic rates, and how the environment influences body size, body shape, and community size structure. The thesis specifically investigates (1) variation in the size-scaling of metabolic rates; (2) the influence of temperature and resources on body size and shape; and (3) how temperature and connectivity affect the size-scaling of organism abundance. As body shape could also influence physiological processes, understanding the interplay between body size, body shape, metabolic rates and the environment could improve our understanding of the future responses of organisms to climate warming, and how such changes may mediate community-level responses.

Through my research, I carried out meta-analyses and performed laboratory microcosm and outdoor mesocosm experiments to explore the theories and hypotheses outlined above. Meta-analysis has the advantage of drawing on existing data for testing existing theories (Koricheva, Gurevitch & Mengersen, 2013) and identifying broad patterns that persist despite experimental differences among individual studies (Gurevitch, Curtis & Jones, 2001). However, this approach lacks the fine control of laboratory experiments, or the resolution of small-scale studies. Hence, details are traded-off for generality (Gurevitch *et al.*, 2001). In contrast, controlled laboratory microcosm experiments allow the isolation of multiple mechanisms, but they are limited by the lack of realism (Altermatt *et al.*, 2015). Hence, while laboratory experiments are crucial for fundamental mechanistic insights about trait responses, the highly controlled conditions make it difficult to extrapolate the findings to variable natural ecosystems (Carpenter, 1996). This limitation is partially addressed by mesocosm experiments, which represent a compromise between the precision of control of laboratory experiments and the complexity of the natural world (Brown *et al.*, 2011; Kratina *et al.*, 2012; Stewart *et al.*, 2013). By using a combination of these methods, I was able to investigate the implications of body size and body shape at multiple levels of details, and across levels of biological organisation.

In chapter 2, I test how body shape changes and ecological differences influence metabolic scaling in cephalopods. As cephalopods change body shape (i.e. are non-isomorphic) as they grow, they represent an ideal group to investigate whether body shape change through ontogeny could influence the body mass-scaling of metabolic rates. I further compared the ontogenetic metabolic scaling relationship of cephalopods and teleost fish, which display contrasting life histories, despite coexisting in the same habitats. This comparison enables the evaluation of how contrasting energetic pressures associated with ecological circumstances between organisms can influence the size-scaling of metabolic rates.

In chapter 3, I compare the energetics of terrestrial insects and spiders. This allowed me to resolve contradictory findings from previous comparisons between the two arthropod groups. The broadly different ecological characteristics of insects and spiders also enable the evaluation of how such differences influence their metabolic-scaling relationships. Given that insects comprise over 60% of extant arthropod species, the size-scaling of metabolic rates in insects has broad implications for understanding energetics in terrestrial arthropods.

In chapter 4, I investigate both the body size and body shape responses of heterotrophic protists to experimental warming and resource competition. Warming is expected to reduce body size via increasing resource demands. However, such changes could potentially also influence body shape, which could influence resource uptake. Hence, body shape and body size responses should potentially be investigated together in order to improve our ability to forecast responses of individual species and entire communities to climate warming.

In chapter 5, I investigate the effects of a heatwave on the abundance-body size relationship of planktonic communities in outdoor mesocosms. Warming often increases metabolic demands but decreases body size, which potentially alters the community size structure. Since small organisms tend to have higher mass-specific process rates, such changes could have implications for ecosystem processes, and decrease the community biomass. In chapter 5, I therefore connect individual body size response to community-level effects, and seek to improve the understanding of short-term heatwave impacts on planktonic communities, extending body size changes beyond an organism to higher levels of biological organisation. Finally, I summarise my findings in chapter 6, discuss the implications of my research and suggest possible future research.

Chapter 2

Ecological pressures, and the contrasting size-scaling of metabolism and body shape in cephalopods and fish

Abstract

Metabolic rates are fundamental to many biological processes, and commonly scale with body size with an exponent (b_R) between 2/3 and 1 for reasons still debated. According to the 'metabolic-level boundaries hypothesis', b_R depends on metabolic level (L_R). We test this prediction and show that across cephalopod species intraspecific b_R correlates positively with not only L_R, but also the scaling of body surface area with body mass. Cephalopod species with high L_R maintain near constant mass-specific metabolic rates, growth and probably inner-mantle surface area for exchange of respiratory gases or wastes throughout their lives. In contrast, teleost fish show a negative correlation between b_R and L_R. We hypothesize that this striking taxonomic difference arises because both resource supply and demand scale differently in fish and cephalopods, as a result of contrasting mortality and energetic pressures, likely related to different locomotion costs and predation pressure. Cephalopods with high L_R exhibit relatively steep scaling of growth, locomotion, and resource-exchange surface area, made possible by body-shape shifting. We suggest that differences in lifestyle, growth and body shape with changing water depth may be useful for predicting contrasting metabolic scaling for coexisting animals of similar sizes.

Introduction

As emphasized in chapter 1, metabolism is the biochemical transformation of material and energy from the environment into biological structure and functions, and is therefore important for understanding ecological and physiological processes (Humphries & McCann, 2014). As aerobic respiration is the main contributor to metabolic energy production in heterotrophic organisms, and is strongly related to body size (Brown *et al.*, 2004; Glazier, 2005), emphasis continues to be placed on quantifying and explaining the relationship between body mass (M) and aerobic respiration rate (R) (Kleiber, 1932; Peters, 1983). This relationship is most commonly described as a power function, $R = aM^{b_R}$ (Kleiber, 1932; von Bertalanffy, 1957), where a is the scaling coefficient, and b_R is the scaling exponent that describes how respiration rate changes with body mass. The assertion of a universal value of b_R, and hence a scaling 'law' (West *et al.*, 1997), has come under serious scrutiny due to the growing evidence that b_R varies extensively both between and within species in relation to taxonomic affiliation, lifestyle, developmental stage, physiological state and ecological factors (Seibel, 2007; DeLong *et al.*, 2010; Glazier, 2010, 2014a; Hirst *et al.*, 2014).

To help explain variation in metabolic scaling, the 'metabolic-level boundaries hypothesis' (MLBH) (Glazier, 2005, 2010, 2014b) proposes that b_R is affected by the relative influence of surface-area-related and volume-related metabolic processes. The relative influence of either processes is mediated by metabolic level (L_R), as represented by the elevation of a metabolism-mass relationship (Glazier, 2005, 2010), which can be estimated as the mass-specific metabolic rate at the geometric midpoint of a metabolic rate-body mass scaling relationship (Glazier, 2009, 2010; Killen *et al.*, 2010). Specifically, b_R is predicted to vary inversely with L_R at resting or low routine levels (Glazier, 2005, 2010, 2014b). According to the MLBH, the metabolic scaling of organisms with relatively high maintenance and routine activity costs is limited primarily by surface-area related fluxes of resources and wastes or by internal transport of resources to metabolizing cells. In such organisms, if size increases isomorphically (without changing shape), surface-dependent processes (e.g. material and heat exchange) cause b_R to approach 2/3 (Rubner, 1883), or 3/4 when limitations of internal resource-distribution networks predominate (West *et al.*, 1997; Brown *et al.*, 2004). In contrast, organisms with low maintenance costs meet metabolic demands amply by surface-related processes, and the influence of volume-related processes should increase, resulting in b_R approaching 1.

The MLBH is supported by observations across species of teleost fishes whose ontogenetic b_R values during rest or minimal routine activity are inversely related to L_R, which is in turn correlated with lifestyle and ambient temperature (Killen *et al.*, 2010). Fish species living at increasing oceanic depths, including pelagic, benthopelagic, benthic and bathypelagic lifestyles, exhibit decreasing L_R and increasing b_R (Killen *et al.*, 2010). Hence, the MLBH mechanistically links ecology with metabolic rates and metabolic scaling (Glazier, 2005, 2010, 2014a b), as ecological and environmental factors that influence L_R can also help to explain variation in b_R.

However, besides surface area limitations proposed by the MLBH, whole-body metabolic demands at routine levels may also result in b_R values that approach 2/3 or 3/4. For instance, relatively low b_R values may occur when the body-size scaling of energy-expensive growth is non-isometric (von Bertalanffy, 1957; Glazier *et al.*, 2011), thus suggesting that a decrease in mass-specific metabolic demand with size may also contribute to lower b_R. We will later discuss how specific metabolic demands (e.g. from growth) may help explain our observations of cephalopod metabolic scaling.

In pelagic invertebrates, metabolic rates typically decline with increasing water depth (Ikeda, 1988; Seibel *et al.*, 1997; Seibel & Drazen, 2007). However, within a phylum (e.g. cnidarians, molluscs, arthropods and chordates), and in contrast to teleost fish, pelagic species often have higher intraspecific b_R values than those of related benthic counterparts (Glazier, 2006; Seibel, 2007). Among cephalopods, epipelagic families tend to have higher inter- and intra-specific b_R values than deep-living families (Seibel, 2007), which contrasts with the pattern observed in teleost fish. This difference in how ecological lifestyle influences b_R suggests that metabolic scaling might not vary in similar ways when comparing coexisting taxa in similar marine environments. However, no published study has quantitatively investigated the effect of L_R on b_R among closely related marine invertebrate species within a taxonomic clade.

Variation in b_R among diverse pelagic invertebrates has been shown to correlate closely with body-shape change and surface-area enlargement during growth (Hirst *et al.*, 2014; Glazier *et al.*, 2015). In organisms that exchange materials across the external body surface (e.g. skin or cuticle – which can include partially internalised gills and inner mantle surfaces), the correlation arises because body-shape change during ontogeny affects relative surface area. Changes in surface area, expressed as the scaling exponent of surface area in relation to body mass (b_A), could influence body-surface-related material exchange capacity required for metabolism.

As b_A is rarely quantified within species, body mass-length scaling exponents (b_L) have been used to formulate Euclidean predictions of b_A, assuming constant mass-density (Hirst, 2012; Okie, 2013; Hirst *et al.*, 2014; Glazier *et al.*, 2015). The mass to length relationship is commonly described by: $M = xL^{b_L}$ where M is body mass, L is length, often measured as the longest linear dimension of the body, and x and b_L are empirically determined constants. When shape remains constant during growth, then $b_L = 3$ and surface area should scale as $M^{2/3}$ (and $b_A = 2/3$), resulting in a predicted b_R of 2/3. However, relative elongation or flattening of the body shape during growth results in b_L values < 3. At the extremes, pure elongation or pure flattening in body shape during growth results in b_L values of 1 or 2 respectively. In both cases, surface area will scale as M^1 (Hirst *et al.*, 2014). As a result, body-shape changes during growth that result in $b_L < 3$ will cause b_A to shift from 2/3 towards 1 (Hirst *et al.*, 2014). If surface area affects metabolic supply capacity, b_R may predictably increase in the same way.

Many cephalopods have b_L values centred around 2.5 (Hirst, 2012), implying non-isomorphic growth ($b_A > 2/3$). Although cephalopods also utilise gills for exchange of respiratory O_2 supply (O'Dor & Hoar, 2000; Pörtner, 2002), more than 50% of O_2 uptake in cephalopods may be cutaneous (Pörtner, 2002). Hence, shape-shifting potentially increases the scaling slope for resource-supply capacity across the body surface above 2/3, which is likely the case in epipelagic squids (O'Dor & Hoar, 2000; Seibel, 2007). A next step is therefore to test whether b_L and b_R covary among cephalopod species with different body plans and lifestyles. However, recent experimental work on physically constrained adult squid has questioned the importance of the outer mantle surface for respiration (Birk, Dymowska & Seibel, 2018). Later, we discuss the implications of those findings for interpreting our own results.

Cephalopod species co-occur with fish from polar to tropical environments, at various depths including surface waters, bathypelagic and benthic habitats. However, despite their coexistence in similar habitats, cephalopods and fishes exhibit very different life histories. Many of the most active pelagic cephalopod species are semelparous, exhibit exponential mass increase over much of their short lifespans (O'Dor & Webber, 1986; Forsythe & Van Heukelem, 1987), and lack a distinct asymptotic growth phase (Jackson, 2004; Moltschaniwskyj, 2004). In contrast, fishes are typically iteroparous, show an asymptotic von Bertalanffy growth trajectory (Pauly, 1980), and generally live longer lives (Winemiller, Rose & Rose, 1992). These and other biological differences between these taxa prompted us to compare their ontogenetic metabolic scaling relationships, particularly any differences in responses to metabolic level, activity demands, water temperature and depth, relevant life-history traits, and ontogenetic changes in body shape.

Specifically, we collated literature data on cephalopods to test the MLBH and growth-scaling prediction that the metabolic scaling exponent (b_R) should correlate negatively with metabolic level (L_R). Alternatively, b_R may positively covary with L_R if shape-shifting (measured as reduced b_L) correlates with increasing b_R. We further investigated whether L_R, b_R and b_L show systematic differences among ecological lifestyles. Our study reveals a correlation between L_R and b_R that is associated with shape-shifting, and further compares and contrasts these results with those obtained previously for teleost fish (Killen *et al.*, 2010).

Materials and methods

Data collection

Cephalopod respiration-mass scaling exponents (b_R-values) were obtained by searching the literature for Ordinary Least Squares (OLS) regressions of log-respiration rates vs. log-body mass. Literature searches were carried out with Web of Science and Google Scholar using a range of search terms such as, cephalopod+oxygen+consumption, cephalopod+respiration and [<species or taxon name>]+respiration. We also identified related studies from reference lists in relevant papers. All regressions were based on wet body mass, with the exception of one study where dry mass was used (Grigoriou & Richardson, 2009). In this case, dry mass was converted to wet mass using conversions described in the original study.

We excluded any measurements made under known stressors, such as unnatural salinities, extreme temperatures or hypoxic conditions. At extreme temperatures, cephalopods are unable to increase their oxygen consumption to match oxygen demands. However, none of the studies included here reported instances where animals encountered hypoxic stress caused by limitations in meeting oxygen demands due to high temperatures (Melzner, Bock & Pörtner, 2007). Additionally, we only included those results from experiments performed on animals which had been fasted beforehand for periods of between 6 - 24 h to minimise the effect of feeding on oxygen consumption, as respiration is typically elevated post-feeding due to specific dynamic action (Cerezo Valverde & García García, 2004). Experiments in which animals were fed during the measurement period were thus excluded (see Dataset S6.1, Table S1). Stress effects from handling were minimised by only accepting data for animals that were allowed an acclimation period of 0.25 – 168h in the respiratory chamber. The lower acclimation time limit was sufficient for *Octopus vulgaris* to settle on the experimental chamber floor and achieve a constant rate of oxygen consumption (Maginniss & Wells, 1969). The upper time limit allowed acclimation of field-captured octopus to their new environment in substrate-containing (i.e. natural den-simulating) holding tanks where respiration measurements were subsequently carried out (Katsanevakis *et al.*, 2005). In one species (*Illex illecebrosus*), the b_R values were obtained from the equation relating oxygen consumption rates to body mass and activity by extrapolating to a zero activity (Webber & O’Dor, 1985, 1986). When mass-specific respiration rate-body mass relationships were reported, rates were converted to respiration rates per individual. If multiple studies on a single species combined data together for calculating b_R, only b_R calculated from the most recent study was included in the data set. This occurred when new measurements for a species were integrated with previously published data to update the regression for the most recent study. However, we avoided aggregating data from multiple studies for single species into new regressions ourselves to avoid combining data from different conditions. When necessary, raw data for the regressions were extracted from published figures using a web-based plot digitiser, WebPlotDigitizer (Rohatgi, 2018). Any b_R values in which the reported correlation coefficients (*r*) were less than 0.8 were subsequently excluded from our analyses (S6.1, Table S2), given the greater variability associated with lower correlation coefficients (Hirst *et al.*, 2014).

The distinction between standard metabolic rates (R_{standard}) and routine metabolic rates (R_{routine}) arises because R_{routine} includes spontaneous movement in experimental chambers (Killen *et al.*, 2010). After temperature correction to 15°C using residuals from the Arrhenius plot (see *Data analysis*), we found no statistical difference between $\mathrm{R}_{\text{standard}}$ and $\mathrm{R}_{\text{routine}}$ values in either b_R or metabolic levels (L_R, unpaired t-tests, $t = 0.833$, $df = 9.031$, $p = 0.427$ for b_R; $t = 0.748$, $df = 10.784$, $p = 0.471$ for L_R) across all species and within or across lifestyles. Hence, we included both measurement types, and conservatively refer to the respiration rates reported as R_{routine}, as spontaneous activity during the experiments was possible (Seibel, 2007). Variation in spontaneous activity levels during measurement may therefore contribute to differences in L_R.

Mass-length exponents (b_L values) were obtained from published OLS regressions between the wet mass (WM) and dorsal mantle length of cephalopods. Wet mass was chosen for determining b_L for two reasons: (i) b_L values from wet mass come closest to representing volume-length relationships than other mass units, and will therefore better capture body-shape changes; and (ii) wet mass matches the units used to determine respiration rate to mass relationships, which improves comparability. Some of the values were previously compiled in the data set of Hirst *et al.*, (2014). Additional values were obtained from the literature using Web of Science and Google Scholar, adopting search terms including cephalopod+length+weight, [<species or taxon name>]+length+mass, squid+length+mass, and from personal communications with authors. Following the screening criterion for b_R values, we excluded regressions between mass and length with reported correlation coefficients less than 0.8 (see Dataset S6.1, Table S3). When multiple b_R and b_L values were available for a single species, we determined arithmetic means of each of these values to avoid over-representation of more commonly measured species and to improve accuracy of the parameter assessment. For lifestyle comparisons, we categorised cephalopods into pelagic, benthopelagic, benthic or bathypelagic lifestyle (see Dataset S6.1, Table S4 for categorisation details). As species may undergo ontogenetic depth migrations, or exhibit lifestyle transitions between life stages, our lifestyle classification, based on readily available data and descriptions, is simplistic. Although ontogenetic variation may obscure some lifestyle differences, our classification is still useful for identifying broad patterns and making comparisons with fish (Killen *et al.*, 2010).

Data analysis

All respiration rates (R) were converted to µl O_2 ind^{-1} h^{-1} for comparability. Metabolic level (L_R, µl O_2 gWM^{-1} h^{-1}) was defined as the mass-specific respiration rate at the geometric midpoint of the body mass range of the respiration-mass relationship (Glazier, 2010; Killen *et al.*, 2010). As the scaling coefficient a and the scaling exponent (b_R) are not independent of each other, the use of L_R to represent metabolic level avoids this problem and is more appropriate (Glazier, 2009, 2010, 2018a).

Statistical analyses were performed using the statistical software package R. The level of significance was set at $p \leq 0.05$ for all tests. OLS regression was performed to investigate the relationships between ln L_R and $1/kT$, and between b_R and $1/kT$, where T is temperature in Kelvin and k is the Boltzmann constant (8.62 x 10^{-5} eV K^{-1}). Reduced Major Axis (RMA) regressions were performed with the RMA software version 1.21 (Bohonak & van der Linde, 2004) to investigate the relationships between b_R and ln L_R, and between b_R and $1/b_L$ in the non-benthic cephalopods. Benthic octopuses were excluded because these typically have less permeable body surfaces and are more reliant upon gills for respiratory gas exchange, as compared to pelagic cephalopods

(Wells *et al.*, 1988; Pörtner, 1995). RMA regressions were used, as these do not rely on definite dependent and independent variables, and ln L_R, b_R and b_L are likely measured with similar error. The analysis was repeated with phylogenetic generalised least squares (PGLS) to control for phylogenetic non-independence. We compiled a tree based on published phylogenies (see SI 2, Figure S2.1). In both PGLS regressions, λ was not significantly different from 0 ($p = 1$ and $p = 0.629$), but significantly different from 1 ($p < 0.0001$ and $p = 0.003$), indicating that the residuals of the model had a weak phylogenetic signal. With the additional phylogenetic correction, the relationship between b_R and $1/b_L$ is marginally non-significant ($p = 0.054$), but the result of the relationship between b_R and ln L_R is not altered. As the PGLS regressions produced very similar conclusions, we report only the results of the RMA regression.

Linear mixed effects models (LME) using the package 'lme4' were constructed to investigate the effect of lifestyle on ln L_R and b_R, with these as dependent variables, and lifestyle, temperature and the interaction between lifestyle and temperature as fixed variables. Taxonomy (order, family and species) was included as a nested (hierarchical) random effect, as species share evolutionary histories and are not completely statistically independent. This phylogenetically informed method was used instead of phylogenetic contrasts (Felsenstein, 1985), because the phylogenetic relationships among higher cephalopod taxa are still unresolved (Allcock, Lindgren & Strugnell, 2014). For additional comparisons of lifestyle effects on ln L_R without the influence of measurement temperature, the residuals of the Arrhenius plots for L_R were expressed relative to the fitted equation value at 15 °C to standardise L_R to 15 °C (following Killen *et al.*, 2010). To investigate the effect of lifestyle on b_L, we used b_L as a dependent variable, lifestyle as a fixed variable, and taxonomy as a nested random effect in an LME model. We compared LME models with linear models without taxonomy as a random effect to determine the importance of taxonomic differences. We used the AICc function in the 'MuMIn' package for model comparisons, and we regarded the best model as the one with the lowest AIC (AICc) score, corrected for small samples. We estimated *p*-values of LME models using the Satterthwaite approximation in the 'lmertest' package. We also carried out multiple pairwise comparisons between lifestyles using the 'multcomp' package, with Bonferroni adjustments to *p*-values. PGLS was carried out with the 'caper' package.

Results

Metabolic exponents, b_R, ranged from 0.616 to 1.005 (mean = 0.824 ± 0.019 s.e.) among all 24 sampled cephalopod species. Cephalopod body mass ranged over 6 orders of magnitude, from 0.01 gWM to 12200 gWM, which was also the mass range for the largest species, *Dosidicus gigas*. Measurement temperature affected metabolic level (as ln $L_{R,}$ Figure 2.1a) with an Arrhenius activation energy of 0.994 eV (± 0.14 s.e.). However, there was no significant relationship between measurement temperature and b_R (Figure 2.1b). Without temperature correction, L_R across 23 species varied 417-fold, from 1.66 to 693.07 µl O_2 gWM^{-1} h^{-1}. For one species, L_R could not be calculated, as the mass range for the scaling relationship was not reported.

We found a significant positive relationship between b_R and ln L_R among all sampled species (Figure 2.2, $r^2 = 0.194$, $p = 0.035$). As L_R values increased 417-fold, b_R increased approximately 1.5 times, from 0.64 to 0.93. We also found a significant positive relationship between b_R and $1/b_L$ (Figure 2.3a, $r^2 = 0.392$, $p = 0.030$), and therefore a positive body mass-scaling relationship between metabolic rate and body shape, across the 12 non-benthic cephalopod species for which we had both b_L and b_R values. The 95% confidence interval of this RMA regression slope (slope = 1.437, 95% CI: 0.647, 2.227) was significantly different from the lower boundary slope (slope = 0.5) that predicted metabolic scaling based on different degrees of body elongation.

However, it was not significantly different from the upper boundary slope (slope = 2) that predicted metabolic scaling from body shape flattening only. In non-benthic cephalopods, the relationship between ln L_R and b_L was also significant (Figure 2.3b, $r^2 = 0.367$, $p = 0.048$). An additional screening step that excluded b_R values for regressions when the mass range covered less than one order of magnitude or was not reported, excluded two species and one family, and did not significantly alter the results (see SI 2, *The mass range for* b_R).

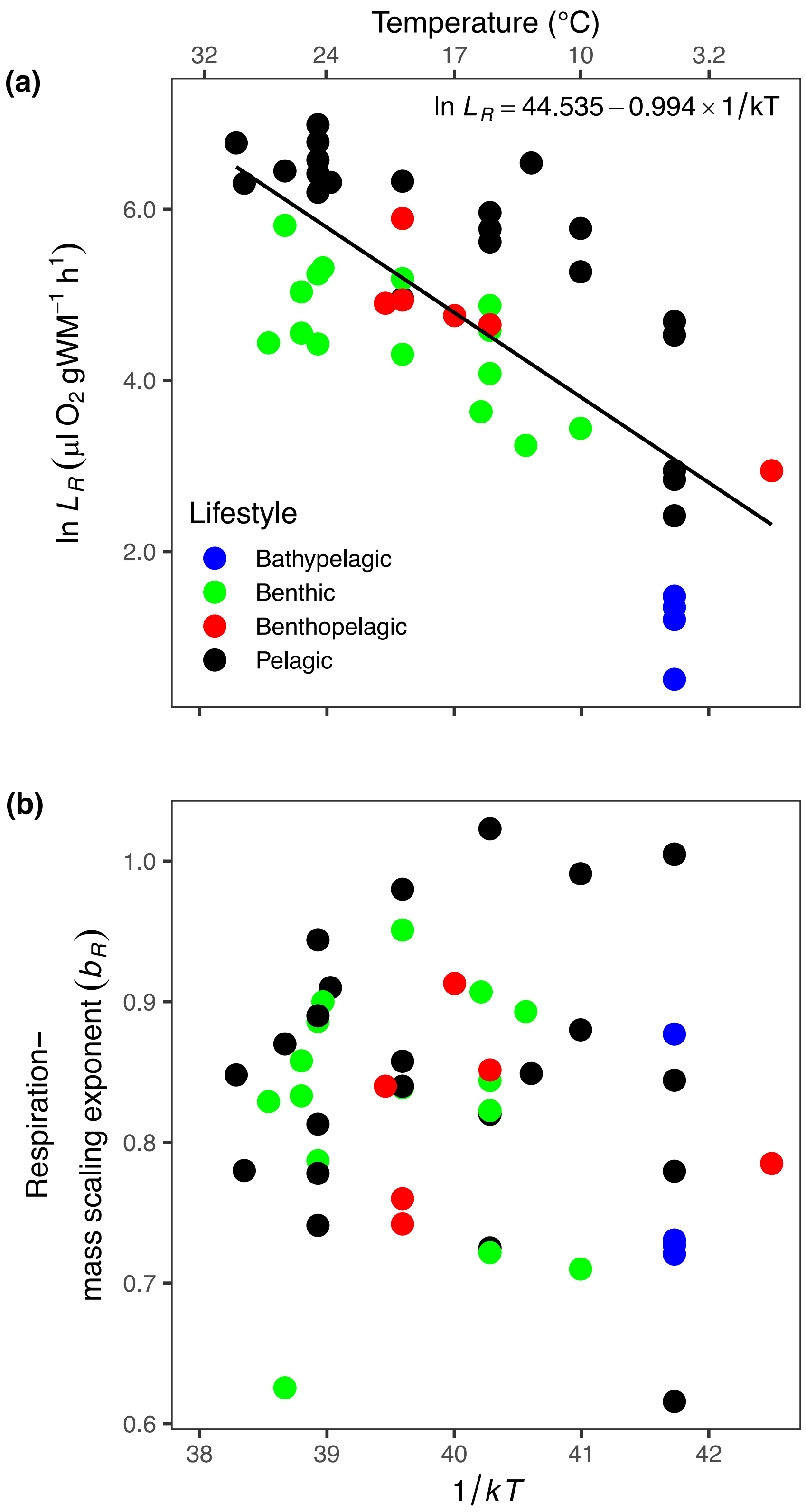


Figure 2.1. Arrhenius plots between **(a)** metabolic level as ln L_R and $1/kT$ ($r^2 = 0.528$, $p < 0.0001$, $n = 47$), and **(b)** metabolic scaling exponent b_R and $1/kT$ ($r^2 = 0.003$, $p = 0.716$, $n = 48$), where T is the measurement temperature in Kelvin and k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}). Four ecological lifestyles are color-coded.

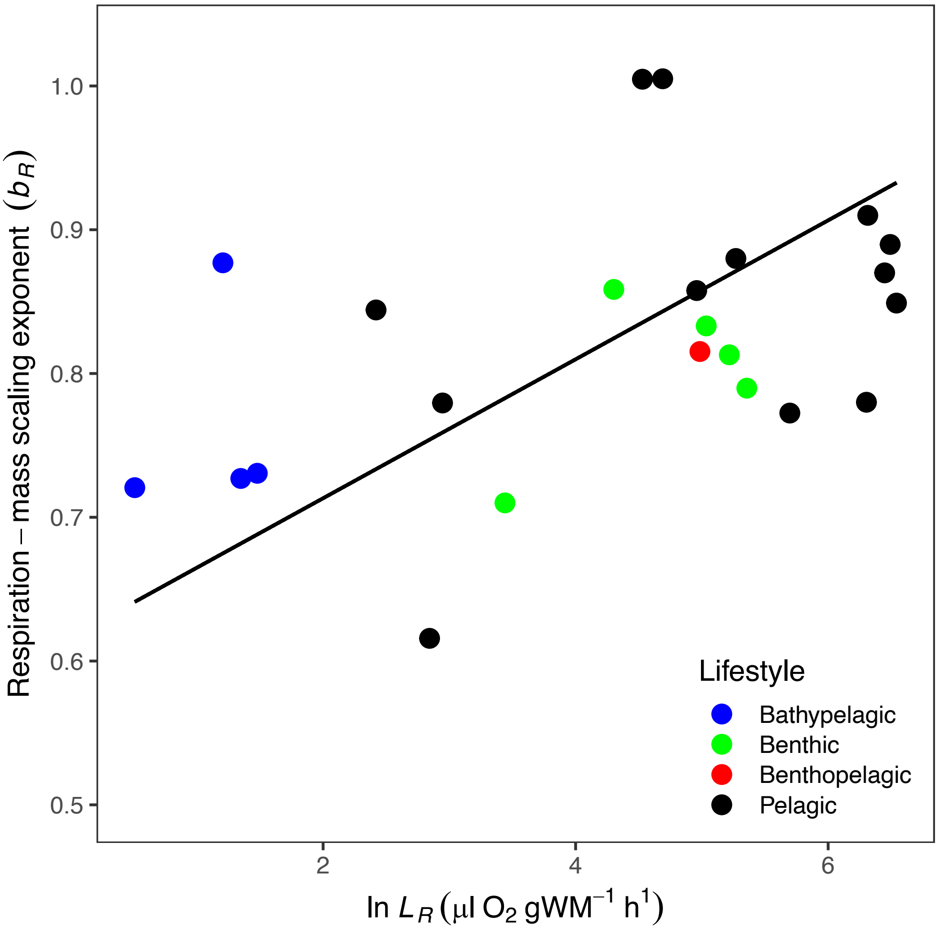


Figure 2.2. Reduced major axis (RMA) regression comparing metabolic scaling exponent b_R and metabolic level as ln L_R for 23 cephalopod species in this study. All L_R values are without temperature corrections. [RMA regression, $r^2 = 0.194$, $p = 0.035$, $b_R = 0.617$ (95% CI: 0.525, 0.708) + 0.048 (95% CI: 0.029, 0.068) x ln L_R]. Four ecological lifestyles are color-coded.

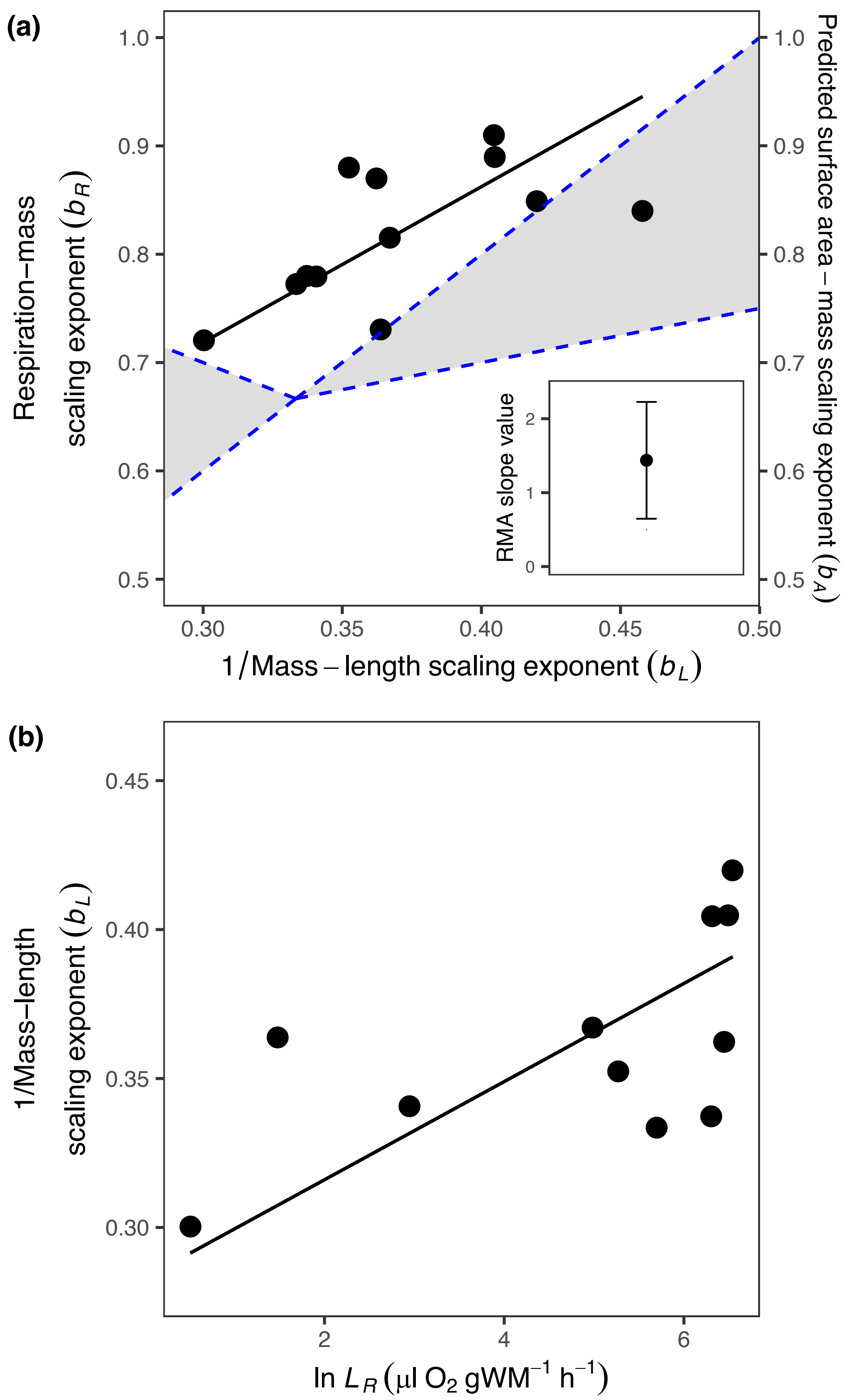


Figure 2.3. Relationships between metabolic scaling exponent (b_R) and the inverse of the mass-length scaling exponent ($1/b_L$), and between $1/b_L$ and metabolic level as ln L_R. **(a)** RMA regression comparing b_R and $1/b_L$ for non-benthic cephalopod species [RMA regression, $r^2 = 0.392$, $p = 0.030$, $b_R = 0.287$ (95% CIs: -0.007, 0.582) + 1.437 (95% CIs: 0.647, 2.227) x $1/b_L$]. The blue dashed lines encloses the prediction envelope for

the surface area to mass scaling powers (b_A) based on b_L values from the Euclidean model from Hirst *et al.*, (2014). The inset shows the slope (± 95% CI) of the RMA regression between b_R and $1/b_L$, and the slopes for the lower [$b_A = 0.5 + 0.5(1/b_L)$], and upper [$b_A = 2 \times (1/b_L)$] boundaries of the prediction envelope. **(b)** RMA regression comparing $1/b_L$ and ln L_R for non-benthic cephalopod species [$r^2 = 0.367$, $p = 0.048$, $1/b_L = 0.283$ (95% CIs: 0.231, 0.335) +0.016 (95% CIs: 0.007, 0.026) x ln L_R].

Metabolic level (as ln L_R) differed between lifestyles (Figure 2.4a). The best model describing variation in ln L_R, which had the lowest AICc score, incorporated lifestyle as a factor and temperature as a covariate, but not the interaction term between lifestyle and temperature. This indicates that the positive effect of temperature on L_R is similar across lifestyles. Both lifestyle ($p = 0.001$) and temperature ($p < 0.0001$) had significant effects on ln L_R, even when taxonomy was included as a random effect. In general, ln L_R decreased across pelagic, benthopelagic, benthic and bathypelagic lifestyles. Mean L_R was lowest in bathypelagic species (3.31 ± 0.59 µl O_2 gWM^{-1} h^{-1}, s.e.), which was significantly lower than that for the other three lifestyles (Figure 2.4a, vs. benthic: $p = 0.006$; vs. benthopelagic $p = 0.006$; vs. pelagic: $p < 0.0001$). At the extreme, mean L_R observed in bathypelagic species was less than 1/100th of the mean value for pelagic species (433.59 ± 64.76 µl O_2 gWM^{-1} h^{-1}, s.e.). However, mean ln L_R did not differ significantly between pelagic and benthopelagic species ($p > 0.9$), nor between pelagic and benthic species ($p > 0.9$). Pelagic species had a significantly higher mean ln L_R (266.63 ± 45.43 µl O_2 gWM^{-1} h^{-1}, s.e.) than that of benthic species (54.31 ± 8.31 µl O_2 gWM^{-1} h^{-1}, s.e.) when ln L_R was corrected to a common temperature of 15 °C ($p = 0.005$).

We found no significant lifestyle ($p = 0.19$) or temperature ($p = 0.74$) effect on b_R. However, mean b_R values decreased across pelagic (mean $b_R = 0.860 \pm 0.021$, s.e.), benthic (0.827 ± 0.022, s.e.), benthopelagic (0.815 ± 0.026, s.e.) and bathypelagic (0.764 ± 0.038, s.e.) species (Figure 2.4b).

Across 60 species, lifestyle appeared to have an effect on the scaling of body shape, measured as b_L, although this was not statistically significant (Figure 2.4c, $p = 0.079$). We found no significant pairwise differences between b_L values among lifestyles following a Bonferroni correction for multiple comparisons.

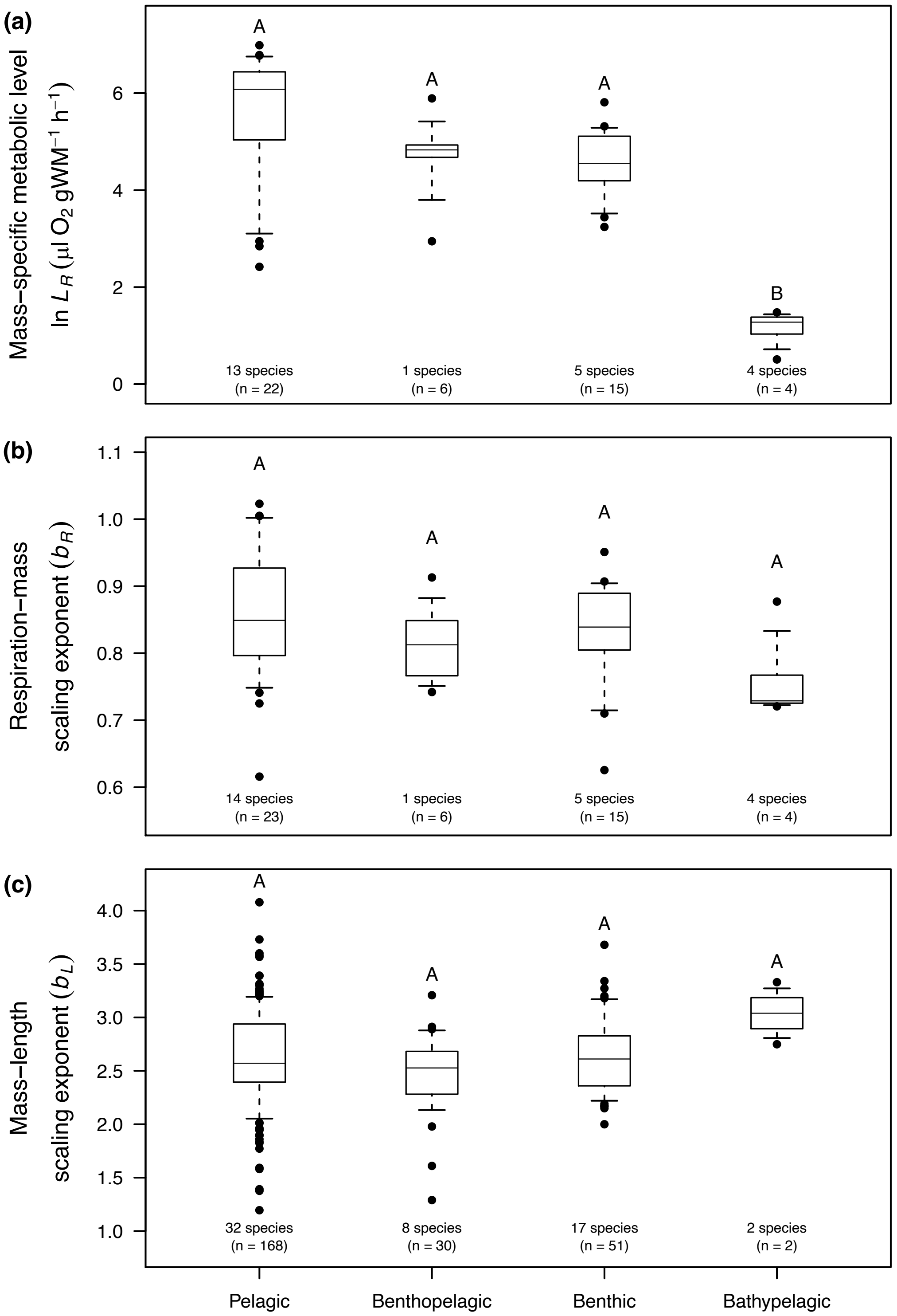


Figure 2.4. The effect of lifestyle on **(a)** metabolic level as ln L_R, (23 species, $n = 47$), **(b)** metabolic scaling exponent b_R (24 species, $n = 48$), and **(c)** mass-length scaling exponent b_L (59 species, $n = 251$). The lower and upper edge of the boxes represents the 25th and 75th percentile respectively, and the black line within the boxes represents the median. The error bars extend to the 10th and 90th percentiles. Values beyond the 10th

and 90th percentiles are indicated as individual points. Different letters indicate significant differences between the lifestyles ($p \leq 0.05$ after Bonferroni adjustment for multiple comparisons).

Discussion

Across a diverse range of cephalopod species with differing lifestyles, the ontogenetic body-mass scaling exponent for respiration (b_R) correlates positively with metabolic level (L_R, Figure 2.2). This positive relationship contrasts with the negative relationship observed among teleost fish (Figure 2.5; Killen *et al.*, 2010), despite both taxa having broadly overlapping body-size ranges, and co-occurring in the same habitats. Moreover, across the non-benthic cephalopods, b_R correlates positively with increasing body shape elongation or flattening, and hence increased relative surface area (as quantified by $1/b_L$, Figure 2.3). Indeed, the RMA slope relating b_R with $1/b_L$ is statistically indistinguishable from predictions of a Euclidean body-surface-area model based on body-shape flattening. This relationship with body shape reinforces existing evidence that across a diverse taxonomic range of open water invertebrates that utilise cutaneous exchange of respiratory gases, nutrients and (or) metabolic wastes, the body-mass scaling exponents for rates of metabolism (including both respiration and soluble nitrogen excretion) are correlated with shape change and associated surface-area enlargement (Glazier *et al.*, 2015; Hirst *et al.*, 2017, 2014).

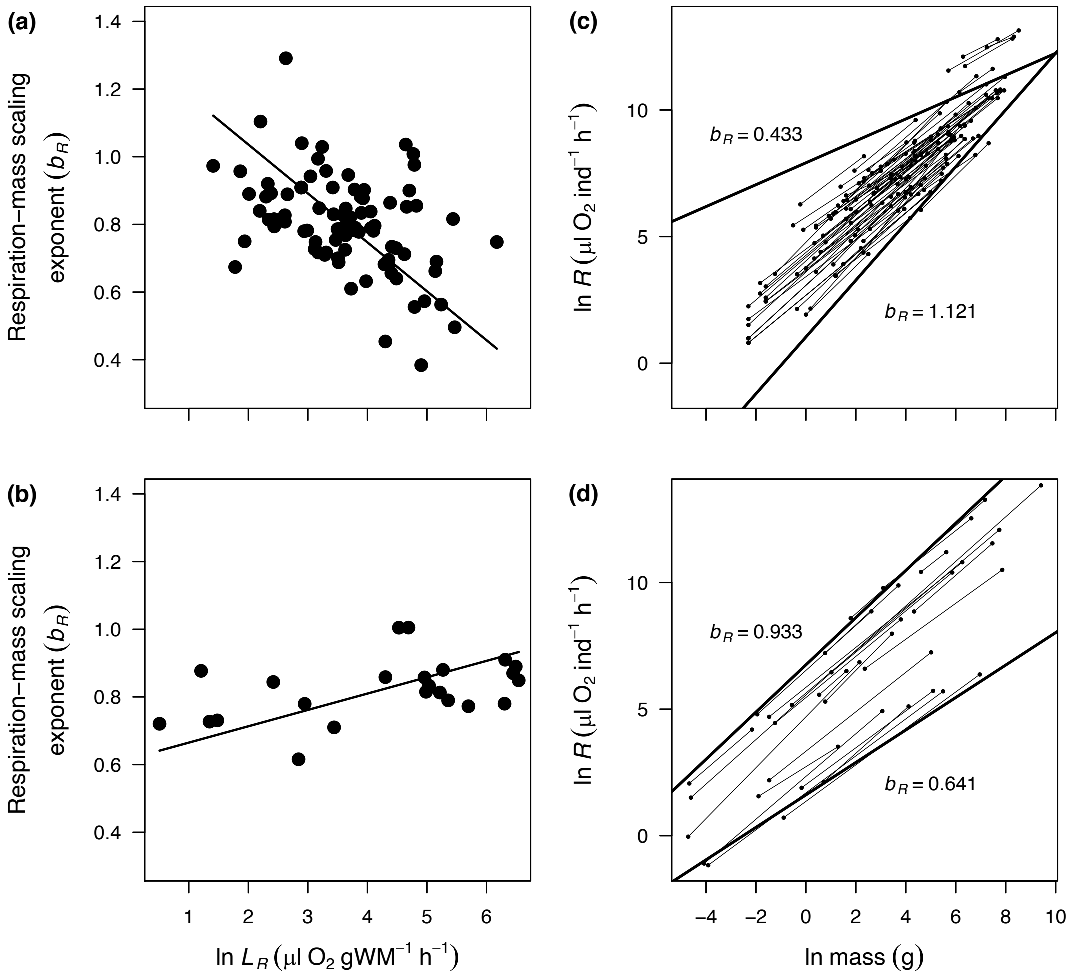
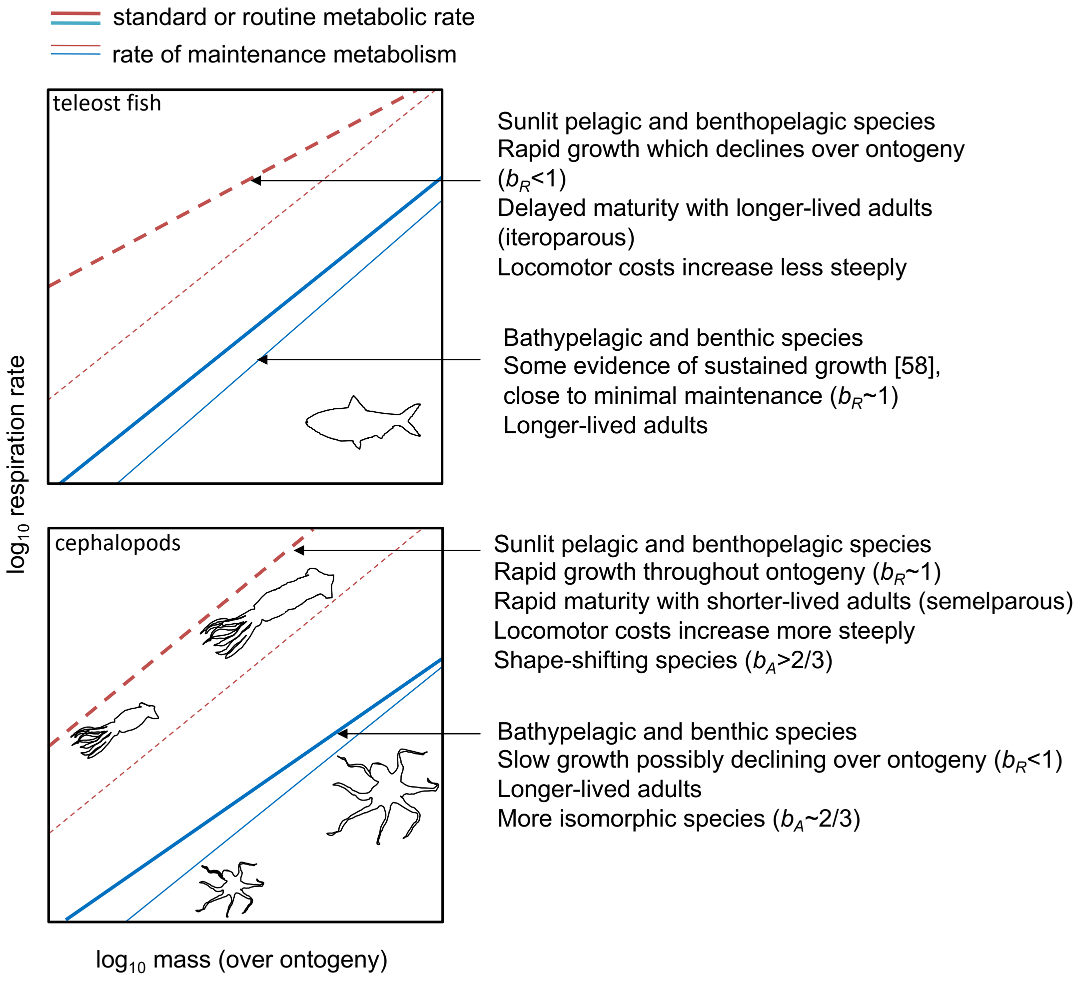


Figure 2.5. Comparisons of intraspecific body-mass scaling of respiration (metabolic) rates (R) among teleost fish and cephalopod species. **(a)** RMA regression between metabolic scaling exponent b_R and metabolic level as ln L_R for 89 fish species (without temperature correction) obtained from Killen *et al.*, (2010), [$r^2 = 0.18, p < 0.0001, b_R =$ 1.325 (95% CIs: 1.221, 1.429) – 0.145 (95% CIs: -0.173, -0.117) x ln L_R]. **(b)** RMA regression between b_R and ln L_R values for 23 cephalopod species (without temperature correction) obtained from our study [RMA regression, $r^2 = 0.194, p = 0.035, b_R = 0.617$ (95% CI: 0.525, 0.708) + 0.048 (95% CI: 0.029, 0.068)]. **(c)** Intraspecific relationships between ln R and wet mass (ln WM) for fish species, as previously compiled (Killen *et al.*, 2010). **(d)** Intraspecific relationships between ln R and ln WM for cephalopod species, as compiled in our study. For species with multiple b_R values based on multiple mass ranges, we took the average minimum and maximum masses as the mass range

shown here (refer to SI 2, *Calculation of mass range for Figure 2.5*). The thick black lines in panels (c) and (d) illustrate the approximate upper and lower boundaries for the scaling relationships between respiration rate and body mass of fish and cephalopods respectively. We predicted these boundaries by applying values obtained from the minimum and maximum ln L_R values obtained of the regression equations in panels (a) and (b), and their corresponding b_R values, to an animal of 21.65 g wet mass, which is the approximate mid-size value of the mass range reported.

We present two explanations for the contrasting relationships between b_R and L_R observed among fish and cephalopods. First, the MLBH predicts that at rest or during routine activity, as metabolic level increases across species, b_R should become increasingly influenced by surface- rather than volume-dependent processes. Increased influence of surface-dependent process would lead to a negative correlation between b_R and L_R among species in which surface area for resource or waste exchange (e.g. gills) typically scales sub-linearly with body mass, as is observed in teleost fish (Killen *et al.*, 2010). However, if the scaling of surface area for exchange of resources or wastes is not isomorphic, and high-energy species display steeper scaling of this surface area, a positive correlation between b_R and L_R may arise, as is observed in cephalopods. Body-shape shifting in cephalopods may allow b_A to increase in high L_R species, thus permitting the steeper scaling of whole-body metabolic demand resulting from ecologically favoured, elevated levels of sustained activity or growth, or both, to exert a greater influence on b_R, as predicted by the MLBH.

Second, an alternative or complementary explanation, for the contrasting metabolic scaling relationships between teleosts and cephalopods emerges from focusing only on the scaling of whole-body metabolic demands, specifically growth demands with body mass at routine levels. As overhead costs of growth contribute strongly to metabolic rate, even at resting levels (Parry, 1983; Rosenfeld *et al.*, 2015), a decrease in mass-specific growth demand with size may also contribute to lower b_R (the 'growth-scaling' hypothesis). In species such as teleost fish whose specific growth rate declines during ontogeny, fast-growing species will have a high proportion of metabolism determined by growth costs, and hence be predicted to have a low resting or routine metabolic scaling exponent, compared with animals with slower growth throughout ontogeny. However, if growth is rapid and sustained throughout ontogeny, as is observed in epipelagic cephalopod species (Jackson, 2004; Moltschaniwskyj, 2004), b_R should also be high, as we have observed. Many studies of diverse animals have also shown similar effects of exponential growth on b_R, as previously reviewed (Glazier, 2005, 2014a). We next discuss how life-history differences between cephalopods and teleosts at different habitat depths may, at least in part, explain the contrasting b_R and L_R correlations found (Figure 2.6).



Sunlit pelagic and benthopelagic species
Rapid growth which declines over ontogeny (b_R<1)
Delayed maturity with longer-lived adults (iteroparous)
Locomotor costs increase less steeply

Bathypelagic and benthic species
Some evidence of sustained growth [58], close to minimal maintenance (b_R~1)
Longer-lived adults

Sunlit pelagic and benthopelagic species
Rapid growth throughout ontogeny (b_R~1)
Rapid maturity with shorter-lived adults (semelparous)
Locomotor costs increase more steeply
Shape-shifting species (b_A>2/3)

Bathypelagic and benthic species
Slow growth possibly declining over ontogeny (b_R<1)
Longer-lived adults
More isomorphic species (b_A~2/3)

Figure 2.6. A proposed scheme to account for contrasting metabolic scaling across species of teleost fishes (upper panel) and cephalopods (lower panel). Red (dashed) lines depict metabolism in the sunlit pelagic and benthopelagic, and blue (solid) lines represent bathypelagic and benthic. Thick lines represent allometric relationships between standard or routine metabolic rates and body mass; thin lines represent maintenance metabolism (i.e. excluding overhead costs of growth and, for routine metabolism, excluding costs of locomotion). Although maintenance is assumed to scale approximately isometrically with body mass, contributions from growth overheads and locomotion may vary in amount and slope. In sunlit waters, with high predation risks favouring high locomotor activity and rapid growth, cephalopods have steeper metabolic scaling associated with steeper scaling of locomotor costs and exponential

growth, which is favoured when adult life is very short, and reproduction is semelparous. Body-shape shifting enables sustained cutaneous intake of resources, hence metabolism, in these active species. Although teleost fish also typically grow fast in sunlit water, their growth declines during ontogeny, resulting in lower metabolic demands of growth. This is likely associated with a longer adult life and typically iteroparous reproduction. Shallower scaling of locomotor costs and the low energetic costs of maintaining buoyancy due to swim bladders may also contribute to lower metabolic demands with size increase, contributing to their lower metabolic scaling exponent. At greater depths, growth in cephalopods is slower, and growth overheads contribute less to metabolic scaling. Shallower scaling exponents could also be associated with reduced shape-shifting.

Life history and energetic contrasts within well-lit waters

Metabolic level (L_R) is significantly affected by temperature and lifestyle (Figure 2.1a and 2.4a), and generally declines with increasing depth in many taxa (cephalopods (Seibel *et al.*, 1997); crustaceans (Ikeda, 1988); and teleost fish (Killen *et al.*, 2010)). Pelagic, benthopelagic and benthic cephalopods have significantly higher metabolic levels (ln L_R) than bathypelagic cephalopods. The similar metabolic levels (ln L_R) of pelagic and benthopelagic cephalopods may relate to the well-lit pelagic and neritic (near shore) environments that they inhabit.

For both cephalopods and teleosts in well-lit waters, visual predation and feeding interactions are likely important, and are associated with rapid locomotion (Seibel *et al.*, 1997; Seibel, Thuesen & Childress, 2000), rates of growth, and metabolism (or metabolic level). The relatively steep metabolic scaling observed in many of the most active pelagic cephalopod species is likely associated with exponential mass increases throughout ontogeny, including during adulthood (Forsythe

& Van Heukelem, 1987; Jackson & Choat, 1992; Moltschaniwskyj, 2004). Although a general growth model for cephalopods remains elusive (Jackson, 2004), many shallow water species commonly achieve exponential growth (Jackson, 2004; Moltschaniwskyj, 2004), which contrasts starkly with the systematic decline in mass-specific growth rate over ontogeny as seen in the von Bertalanffy growth trajectories of most fish species (Pauly, 1980). Hence, the growth-scaling hypothesis predicts that growth demands will affect metabolic scaling in cephalopods and teleost fish differently because of their different growth patterns (see Figure 2.6).

In epipelagic cephalopods, sustained rapid growth is likely related to semelparity and short lifespans (< 2 years) (O'Dor & Webber, 1986; Jackson, 2004; Glazier, 2005, 2006), whereas many fish species tend to have longer adult lifespans and are iteroparous (Winemiller *et al.*, 1992). For instance, the largest epipelagic cephalopod in our study, *Dosidicus gigas*, lives up to 2 years (Nigmatullin, Nesis & Arkhipkin, 2001). In stark contrast, relatively small clupeiformes (e.g. anchovies, herrings and sardines) mature at ~2 years old and live for a total of ~8 years, on average (Winemiller *et al.*, 1992). High predation in the pelagic environment may also favour continuously high levels of activity and thus locomotor energetic costs (O'Dor & Webber, 1986, 1991; Glazier, 2006) that scale steeply with body mass (e.g. $M^{0.8}$, O'Dor and Webber, 1986). Hence, growing in size results in active squids receiving smaller mass-specific savings in locomotor costs than do fish, whose locomotor costs scale less steeply as $M^{0.7}$ (White & Seymour, 2011). Consequently, squids may require high b_R and L_R to support high activity at all sizes, and to sustain near constant mass-specific growth rates throughout life (Glazier, 2006, 2014a). Higher resting or routine metabolic rates (and b_R) may reflect higher growth rates even if short-term experimental conditions during respiration measurements include starvation. Thus, in sunlit waters, differences in adult lifespan and reproductive intensity favouring different growth trajectories (exponential versus asymptotic), along with different scaling of locomotor costs, may influence the size-scaling of metabolic demands and account for contrasting metabolic scaling between cephalopods and teleost fish (Figure 2.6).

Moreover, we have shown that relatively steep metabolic scaling in the more active cephalopods is associated with enhanced body-shape shifting that permits greater surface-area enlargement for cutaneous resource uptake and waste elimination. Further, we argue that this interpretation of our findings is still consistent with the observed negligible contribution to respiration from the outer mantle surface in restrained adults of two squid species (Birk *et al.*, 2018), as those experiments did not account for how body shape-shifting would still increase the respiratory surface area directly in contact with seawater within the mantle, and potentially permit elongation of gills (Birk *et al.*, 2018). Lack of ventilation in the experimentally restrained adults may also reduce cutaneous respiration. Furthermore, it is unclear whether low cutaneous respiration would apply to active juveniles because juveniles often show more cutaneous respiration than adults in various aquatic animals (Graham, 1988; Rombough, 1998). Therefore, body-shape flattening or elongation during growth may enable species with more active lifestyles to overcome geometric constraints associated with isomorphic growth, and hence 2/3-power scaling of body-surface area and associated cutaneous and branchial material exchange rates. As a result, higher b_A (> 2/3) may permit higher b_R (> 2/3) (O'Dor & Hoar, 2000; Seibel, 2007; Rosa, Trueblood & Seibel, 2009; Hirst *et al.*, 2014).

Euclidean predictions of b_A using b_L ignore increases in surface convolutions or fractal dimension during ontogeny (e.g. convolutions from gill development), and can therefore underestimate surface-area increase (Okie, 2013; Hirst *et al.*, 2014; Glazier *et al.*, 2015). This under-prediction of surface-area enlargement may partially explain why most species in this study have b_R values above the upper limits of the b_A prediction envelope (Figure. 2.3). A better estimation of actual exchange surface areas, including the relative importance of gill versus cuticle exchange over ontogeny, would be beneficial for understanding b_R variation. Alternatively, under-prediction of b_R based on b_A predictions could also be due to the metabolic demands of growth (Glazier, 2006, 2014a; Glazier *et al.*, 2015) and possibly locomotion, which raises the question about the extent to which metabolic scaling is influenced by metabolic demand relative to resource supply (the growth-scaling hypothesis). Is shape change a response to high metabolic demands, or does shape change permit or drive the steep scaling of metabolic rates? As natural selection may favour a matching of resource supply capacity with demand, refuting either statement is likely to be difficult (Rosenfeld *et al.*, 2015). Nonetheless, understanding the importance of various factors influencing energy flow and assimilation in an organism will be crucial for improving our knowledge on how and why metabolic rate varies with size.

Metabolic scaling in the benthos and at greater depths

Bathypelagic cephalopods have significantly lower ln L_R values than species with other lifestyles (Figure 2.4a). Lower metabolic levels could result from reduced visual predation at greater depths (Seibel *et al.*, 1997), which could relax selection on maintaining high locomotor activity. This may also include a switch to sit-and-wait predatory behaviour, which has lower energetic costs (Seibel *et al.*, 1997; Seibel, 2007; Ikeda, 2016). A reduced requirement for high locomotor activity could also favour the use of buoyancy mechanisms (Seibel *et al.*, 2004), which include reduced amounts of metabolically active musculature, and accumulation of relatively high amounts of buoyancy-enhancing, low-density, metabolically inactive, ammonium or gelatinous body materials, as found in many bathypelagic cephalopod species (Voss, 1967; Seibel *et al.*, 1997, 2004). Although research on growth of deep-sea cephalopods is scarce, growth rates tend to be slower at greater depths (Semmens *et al.*, 2004), and mass-specific growth may decline with size in deeper-living and benthic species (Arkhipkin, 1997; Semmens *et al.*, 2004).

Benthic cephalopods have temperature-corrected ln L_R values that are significantly lower than those of pelagic species ($p = 0.005$). For benthic cephalopods, bottom structures may provide refuge from predation, while also enabling ambush foraging (Seibel *et al.*, 1997). Therefore, reduced active prey pursuit and predator avoidance in benthic octopods could decrease selection for high swimming speeds and the greater metabolic levels needed to sustain them. Hence, both benthic and deep-water species have lower predation risks and metabolic levels and are subsequently considered together in Figure 2.6.

As growth (Semmens *et al.*, 2004) and locomotion (Seibel *et al.*, 1997) tend to be reduced in deeper water and benthic-living species, supporting energy costs will also decrease. In contrast to cephalopods, metabolic scaling is steep in bathypelagic and benthic-living teleosts. Within the least active bathypelagic cephalopods and fish, cephalopods ($b_R = 0.76 \pm 0.04$) have significantly lower metabolic scaling exponents than do fish ($b_R = 0.94 \pm 0.04$; $t = -3.059$, $df = 9.596$, $p = 0.013$). This difference may relate to growth, as tentative evidence suggests that bathypelagic fish have steeper scaling exponents for growth with body mass in these habitats (Childress *et al.*, 1980) in comparison to cephalopods (see Figure 2.6 and Arkhipkin, 1997).

General relationship between metabolic scaling and metabolic level

Shape-shifting may have facilitated the significant positive relationship that we observed between b_R and L_R in cephalopods (Figure 2.2), as indicated by the positive relationship between b_R and $1/b_L$ (Figure 2.3). Hence, the more metabolically active squids had greater shape change during ontogeny, thus allowing them to maintain high relative surface area for exchange of resources and wastes. The positive relationship between b_R and $1/b_L$ parallels the significant relationship found in the phylum Mollusca (Hirst *et al.*, 2014). However, the molluscan relationship appeared to result largely from marked differences in the degree of shape shifting and metabolic scaling observed between cephalopod and pteropod species, whereas our analysis still finds a significant correlation between b_R and $1/b_L$ within a more complete cephalopod dataset (i.e. even when the strong influence of pteropods is excluded).

Our results provide some support for the MLBH, in that scaling of surface area for resources or wastes exchange correlates positively with routine $b_{R,}$ across species, which correlates positively with L_R. Shape-shifting, hence non-isometric scaling of inner-mantle body surface may allow for steeper b_R in the most active (high L_R) cephalopods. However, steeper ontogenetic scaling of growth in the most active species and its greater contribution to whole-body metabolism may also contribute to such non-negative associations between b_R and L_R, as proposed by the growth-scaling hypothesis.

Hence, we suggest that the observed positive correlation between b_R and L_R among cephalopod species occur because of coadaptive changes in several behavioural, life-history, morphological and metabolic traits that affect both resource supply and metabolic demand. Higher levels and steeper scaling of growth and locomotor activity in some (especially epipelagic) species likely involve sustained mass-specific metabolic demands throughout life, resulting in both higher metabolic levels (L_R) and steeper metabolic scaling (b_R). Steeper metabolic scaling could, in turn, be accommodated by steeper scaling of respiratory surface area (b_A) made possible by shape-shifting, which is supported by the correlation between b_R and b_A. As supply capacity and metabolic demand are likely coadjusted and thus convergent, metabolic scaling in cephalopods is likely a result of both resource demand and supply (Glazier, 2018b).

In conclusion, we present support for the importance of a meta-mechanistic approach to metabolic scaling (Glazier, 2014a, 2018a). In doing so, we have proposed an explanation for how and why cephalopods have metabolic scaling slopes that increase with increasing metabolic levels. We suggest that sustained metabolic demands of growth and potentially locomotion may explain near isometric metabolic rate scaling in those cephalopods with the highest metabolic levels. Such isometric scaling of metabolic rates may be supported by surface-area enlargement through shape shifting. The combination of body-shape shifting with costs and size-scaling of growth and locomotion, adapted to different mortality and energetic pressures, may explain the contrasting metabolic scaling of cephalopods and teleost fishes. Thus, even for similar-sized coexisting animals, differences in water depth, lifestyle, growth and body shape can cause striking differences in metabolic scaling.

Chapter 3

Insects and spiders have contrasting activity-dependence of metabolic scaling

Abstract

Metabolism fuels fundamental biological processes, and for reasons still keenly debated, commonly scales with body mass with an exponent (*b*) between 2/3 and 1. Between major terrestrial arthropod groups (insects and spiders), differences in the interspecific mass-scaling of metabolic rate and energy use remain unresolved. Resolving these differences between major taxonomic groups have important ecological implications, and may reveal phylogenetic limitations on energetics. We collated a new database on metabolic rates for non-flight activity (e.g. locomotion, calling) to compare active and resting energetics of insects (n_a = 94, n_r = 608, respectively) and spiders (n_a = 21, n_r = 111, respectively). After accounting for phylogeny and wing presence, we demonstrated that insects consume energy more rapidly than spiders. This difference is evident during activity among wingless animals, but is accentuated by including winged insects. Overhead costs of maintaining capacity for flight, higher mitochondrial densities in flight muscles, or increased metabolic scope for activity in winged insects could underlie this effect. Metabolic rates of large insects are approximately 10-fold and 4.5-fold that of similar-sized spiders during activity and at rest respectively. Both groups have similar *b* values at rest (0.795 and 0.724 respectively), but not during activity (1.047 and 0.673 respectively). The metabolic-level boundaries hypothesis (MLBH) prediction, that *b* values should approach 1 during activity, is supported by the data for insects, but not for spiders. This contrast may be associated with lower size-scaling of locomotor cost, or the cost of maintaining posture for locomotion, in spiders but not in insects. Appreciating the variation in metabolic scaling between these major

terrestrial arthropod taxa is important for understanding their contrasting energetic strategies.

Introduction

Metabolism reflects the collective biochemical processes that transform energy and material into biological structures and functions (Glazier, 2005). It therefore has profound physiological, ecological and evolutionary implications (Brown *et al.*, 2004), and is critical for understanding these processes (Humphries & McCann, 2014). Aerobic respiration is the main contributor of metabolic energy production in heterotrophs and is strongly linked to individual body size (Kleiber, 1932; Peters, 1983; Brown *et al.*, 2004; Glazier, 2005). The relationship between respiration rate (R) and body mass (M) is commonly described as a power function of the form $R = aM^b$ (Kleiber, 1932), where a is the mass-scaling coefficient, and b is the metabolic mass-scaling exponent. Observed variation in intra- and inter-specific b values have been associated with taxonomic affiliation, physiological state, developmental stage, body surface area and ecological factors such as temperature, habitat and mortality risk (DeLong *et al.*, 2010; Glazier, 2010, 2014a; Killen *et al.*, 2010; Tan *et al.*, 2019).

As metabolic rates are likely adaptive and under selection (Hudson, Isaac & Reuman, 2013), they may be strongly influenced by ecological factors (Killen *et al.*, 2010; Pequeno *et al.*, 2017; Tan *et al.*, 2019). The metabolic-level boundaries hypothesis (MLBH) (Glazier, 2005, 2010, 2014a) posits that ecological differences between organisms affect their metabolic level (i.e. the elevation of the metabolic scaling relationship), which in turn influences the scaling exponent b (Glazier, 2010, 2014a). The metabolic level (L) can be approximated as the mass-specific metabolic rate at the geometric midpoint of a respiration rate-body mass relationship (Glazier, 2009, 2010). The MLBH predicts that as L increases during physical activity, b should also increase. This is because the use of resource reserves or tolerance of waste accumulation should enable an individual to circumvent surface area-associated exchange-limits during maximum physical exertion. Consequently, metabolic rates are predicted to scale with tissue mass or volume, thus *b* values should approach 1.

Within terrestrial arthropods, the MLBH prediction that the value of *b* is higher during activity than at rest is supported by the interspecific mass-scaling of metabolic rates in insects during flight (Niven & Scharlemann, 2005; Glazier, 2010; but see Harrison, Klok & Waters, 2014). However, to our knowledge, no attempt has been made to evaluate this prediction during other non-flight activities in both insects and spiders. Spiders provide a contrasting arthropod taxon to test this prediction because they co-occur with insects, but are often considered to have lower metabolic rates than insects (Anderson, 1970). However, this claim remains contentious (Lighton & Fielden, 1995). Whereas lower metabolic rates and a lower scaling exponent have been reported for spiders in comparison to insects (Greenstone & Bennett, 1980; McCue *et al.*, 2016), others have reported that a single equation is sufficient to describe metabolic scaling of both terrestrial arthropod groups (Lighton & Fielden, 1995). Hence, the question of whether spiders and insects have similar metabolic rates and scaling exponents remains unresolved. Similarly, the question of how metabolic rate scales with body size across insect species also remains contentious (Chown *et al.*, 2007; Riveros & Enquist, 2011), and disagreements exist over whether metabolic rate scales with an exponent of 0.75, as predicted by the resource supply network model (West *et al.*, 1999). As insects represent over 60% of all extant arthropod species (Chown *et al.*, 2007), their metabolic scaling relationship has important implications for understanding energetics in terrestrial arthropods. Because arthropods are dominant organisms on Earth in terms of both abundance and biomass (McGavin, 2001), understanding arthropod metabolism can improve our mechanistic understanding of energy and nutrient flow through many ecosystems (Teal, 1962).

Previous comparisons between spiders and insects have tended to ignore phylogenetic relationships and focused predominantly on resting metabolic rates (Lighton & Fielden, 1995; McCue *et al.*, 2016). However, active non-flight metabolic rates (e.g. when walking, calling) may come closer to representing a wide suite of metabolically demanding activities in nature. Such activities are also an important component of fitness, as they could influence access to mates (Kasumovic & Seebacher, 2013; Friesen, Powers & Mason, 2017) and presumably reproductive success. Previously reported differences between insects and spiders also did not investigate the role of the presence of wings in relation to differences in metabolic rates, which may influence metabolic rates even during rest (Chown *et al.*, 2007). Furthermore, previous analysis of metabolic rates based on insect flight (Niven & Scharlemann, 2005) precluded comparisons between winged and wingless insects. Active, non-flight metabolic rates will therefore allow the inclusion of flightless insects to test how the presence of wings influence metabolic requirements during similar, non-flight activity within insects, and between insects and spiders.

To enable a rigorous comparison between insects and spiders, and to test the MLBH, we collated active metabolic rates for insects and spiders from the literature, and combined published datasets on resting metabolic rates for insects (Chown *et al.*, 2007) and spiders (McCue *et al.*, 2016). In addition, we compiled a supertree for insects and spiders based on a variety of available phylogenies. Unlike the previous chapter that investigated intraspecific mass-scaling relationship, we characterised interspecific body mass-scaling relationship here. Specifically, we test the hypotheses that (1) insects have significantly higher (non-flight) active and resting metabolic levels (defined as L_a and L_r respectively) than spiders, (2) winged insects have higher L_a and L_r than wingless insects, potentially caused by the overhead costs of maintaining flight musculature, and (3) resting metabolic rate scales more steeply with body mass in insects than spiders as was previously reported (McCue *et al.*, 2016), and active metabolic rate scales more steeply than resting metabolic rates in both groups as predicted by the MLBH. This study reveals that wingless and winged insects have a greater rate of energy use than spiders during rest and especially during activity: this finding has wide implications for understanding patterns in energy use in important invertebrate taxa.

Materials and methods

Data collection

We obtained species-specific active metabolic rates by searching the literature for measurements of respiration rates during non-flight activities. We carried out literature searches on Web of Science and Google Scholar using the search terms arthropod/insect/spider + active/running/calling/resting/standard + respiration/respiration rate*, and [<insect or spider order/infraorder>] + [activity type] + metabolic rate*/metabolism. We further identified related literature from reference lists of relevant papers. We identified data for resting metabolic rates from published data compilations of Chown *et al.*, (2007) and McCue *et al.*, (2016). We then obtained the original data from the primary publications whenever possible for this compilation according to own screening criteria (see below), and included more data from additional literature searches. We included only metabolic rates measured in adults to avoid variation in metabolic rates arising from differences in developmental stage (see electronic supplementary material, dataset S6.2).

We compiled active metabolic rates from a range of non-flight activities including locomotion, righting responses and sexual signalling (calling or chirping). We grouped righting responses with locomotion as no distinction was made between the two activities in the original study (Bartholomew & Casey, 1977). For locomotion, if metabolic rate at different running speeds were available, we selected the running speed at which measurement temperature was closest to 22 °C. This temperature is at the lower range of temperatures at which activity was measured, and was chosen to avoid inclusion of metabolic rates from thermally-stressed animals. If temperature was kept constant at different running speeds, we included respiration rate at the highest running speed. However, including data at the slowest running speed did not alter our conclusion (SI 3, Figure S3.1). We included resting metabolic rates where a resting state could be reasonably assumed from a cyclic gas exchange (as this is commonly indicative of a resting state) (Chown & Nicolson, 2004). However, as gas exchange information was not always available, animals observed to be motionless, or displaying minimal movements were also included. When multiple data for the same species were available, we reduced multiple measurements to a single data point by first selecting for flow-through methods, then for an experimental temperature closest to 22 °C, and lastly for any indication of real-time monitoring of activity. If data were available for both males and females of a species, we obtained a mean value from both sexes. We standardised metabolic rate measurements to $\mu l\ O_2\ ind^{-1}\ h^{-1}$ by an oxyjoule equivalent of $16 + (5.164 \times RQ)$, assuming that $RQ = 0.84$ (Lighton, Bartholomew & Feener, 1987) if specific oxy/carbon dioxide-joule equivalent was not stated. We standardised body mass to wet mass (mgWM) assuming that dry mass is 30% that of wet mass (Edney, 1977) unless the original study suggested a different conversion factor, which we followed. We also noted the presence or absence of wings for each insect species (referred to as wing status). However, it was not always possible to ascertain the functionality of wings, as winged insects may differ greatly in their capacity for flight. Although the classification of wing status as presence and absence is simplistic, such a classification may help to identify general metabolic differences associated with varying potential for flight. Including temperature in our Phylogenetic Generalized Least Squares (PGLS) regression for active metabolic rates did not significantly improve their fit to the data (Table 3.1, SI 3, Figures S3.2a and S3.2b). While temperature significantly influenced resting metabolic rates, temperature effects are unlikely to cause bias, as activation energies for mass-specific metabolic rates are similar for both insects (0.528 eV, 95% CI: 0.418, 0.637) and spiders (0.488 eV, 95% CI: 0.232, 0.743, SI 3.2, Figures S3.2c, S3.2d). We therefore did not standardise metabolic rates to a single temperature so as to avoid extrapolating the temperature dependence of metabolic rates. However, insects have a higher measurement temperature than spiders, (24.26 °C and 21.74 °C respectively, $t = 7.82$, $df = 170.35$, $p < 0.001$). Hence, to minimise the effect of temperature on differences between taxa, we also present comparisons for resting metabolic rates standardised to a common temperature of 22 °C (SI 3, Figure S3.3). We expressed the residuals of the Arrhenius plots (SI 3, Figure S3.2) relative to the predicted value at 22 °C (following Killen *et al.*, 2010) to obtain temperature-corrected metabolic rates.

Table 3.1. AICc model competition table comparing various PGLS regression models. Values in bold indicate the best fit models (ΔAICc < 2). k = number of parameters, AICc = bias adjusted Akaike Information Criteria, ΔAICc = AICc of model – AICc of best fit model, w_i = Akaike weight.

Model	**k**	**Log-likelihood**	**AICc**	**ΔAICc**	**w_i**
Active metabolic rates					
Insect					
body mass	2	-119.65	243.43	7.69	0.01
body mass + activity type	3	-119.64	245.55	9.81	0.003
body mass + wing status	**3**	**-115.45**	**237.16**	**1.42**	**0.229**
body mass + temperature	3	-118.22	242.71	6.97	0.014
body mass + wing status + activity type	4	-115.09	238.63	2.89	0.11
body mass + wing status + temperature[1]	**4**	**-113.64**	**235.74**	**0**	**0.467**
body mass + temperature + activity type	4	-118	244.46	8.72	0.006
body mass + temperature + wing status + activity type	5	-113.6	237.88	2.14	0.16
Spider					
body mass	**2**	**-20.99**	**46.65**	**1.07**	**0.288**
body mass + temperature[2]	**3**	**-19.08**	**45.58**	**0**	**0.494**
body mass + activity type	4	-19.48	49.47	3.89	0.071
body mass + temperature + activity type	5	-17	48	2.42	0.147
Resting metabolic rates					
Insect (exclude species without wing status)					
body mass	2	-561.06	1126.13	86.03	0
body mass + wing status	3	-558.31	1122.66	82.56	0
body mass + temperature	**3**	**-517.39**	**1040.82**	**0.72**	**0.41**
body mass + wing status + temperature[3]	**4**	**-516.01**	**1040.1**	**0**	**0.59**
Spider					
body mass	2	-71.64	147.38	13.86	0.001
body mass + temperature	**3**	**-63.65**	**133.52**	**0**	**0.999**

[1] Subsequent F-test found no significant temperature effect ($F_{1,90} = 3.05, p = 0.084$).
[2] Subsequent F-test found no significant temperature effect ($F_{1,18} = 3.59, p = 0.074$).
[3] Subsequent F-test found no significant wing status effect ($F_{1,605} = 2.91, p = 0.085$).

Data analyses

We undertook all statistical analyses using the statistical software R version 3.6.0 (R Core Team, 2019). We performed phylogenetic generalised least squares (PGLS) regressions (Grafen, 1989) to establish the relationship between $\log_{10}$ active or resting metabolic rate and $\log_{10}$ body mass for active and resting metabolic rates of both insects and spiders. The PGLS regressions were derived using the ‘caper’ package (Orme, 2013). We compiled a supertree for this analysis using information from 44 phylogenies obtained from published literature, academic theses and the NCBI taxonomy database via a phylogenetic tree generator ‘phyloT’ (https://phylot.biobyte.de/about.cgi) (refer to electronic SI 6.3, Figures S6.3.1-S6.3.9). Branch lengths were calculated following the method by Grafen (1989). We included wing status, and measurement temperature as an independent variable in the PGLS models, to assess its effect on the scaling exponent. The best fit model was selected as that with the lowest small-samples corrected AIC value (AICc) using the *AICc* function in the ‘MuMin’ package (Bartoń, 2019). If the difference between the lowest AICc and a model’s AICc (ΔAICc) was less than 2, a set of best fit models was considered instead of a single model. Moreover, we used *F*-tests to determine the significance ($p < 0.05$) of each parameter. For both insects and spiders, the inclusion of activity category did not significantly improve the model fit to the data on active (non-flight) metabolic rates (Table 3.1). This indicates that within arthropod groups, activity type does not influence how active (non-flight) metabolic rate scales with body size. To compare differences in metabolic rate between insects and spiders, and to test the MLBH predictions, we used metabolic level (L), calculated as the mass-specific respiration rate at the geometric midpoint of the metabolic rate-body mass relationship (Glazier, 2005, 2009). We used metabolic level per gram of body mass (L) as this allows comparisons among taxa (Glazier, 2009), whilst avoiding the autocorrelation between the intercept and the slope of the scaling relationship. This approach allows us to examine correlations between the values of b and L, and hence to evaluate the MLBH predictions. The geometric mid-point mass for active metabolic rate was similar in insects (104.4 mgWM) and spiders (437.9 mgWM), and comparison is unlikely biased by differences in body size. However, the geometric mid-point mass for resting metabolic level was an order of magnitude higher for spiders (284.1 mgWM) than insects (21.3 mgWM). Differences in L_r between the two groups are therefore contingent on the body mass compared, and increases with body mass.

Results

Active and resting metabolic levels

Insects have significantly higher active (L_a) and resting metabolic levels (L_r) than spiders (based on non-overlapping CIs), as we hypothesized (Figure 3.1). During non-flight activity, the value of L_a of insects (2.482 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 2.190, 2.814) is approximately 4-fold that of active spiders (0.604 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.601, 0.609). A comparison based only on metabolic rates during locomotion gave similar results (insects L_a: 2.482 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 2.213, 2.775, spiders L_a: 0.553 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.538, 0.569), which suggests that this difference is not the outcome of comparing different activity types (e.g. comparing calling insects with running spiders). Likewise, the L_r value for insects (0.631 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.220, 1.810) is also significantly higher, and is approximately 6-fold that of spiders (0.100 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.086, 0.115; Figure 3.1). However, this difference depends on body mass. In similar-sized animals, differences in L_r are observed only when body mass is above 80 mgWM.

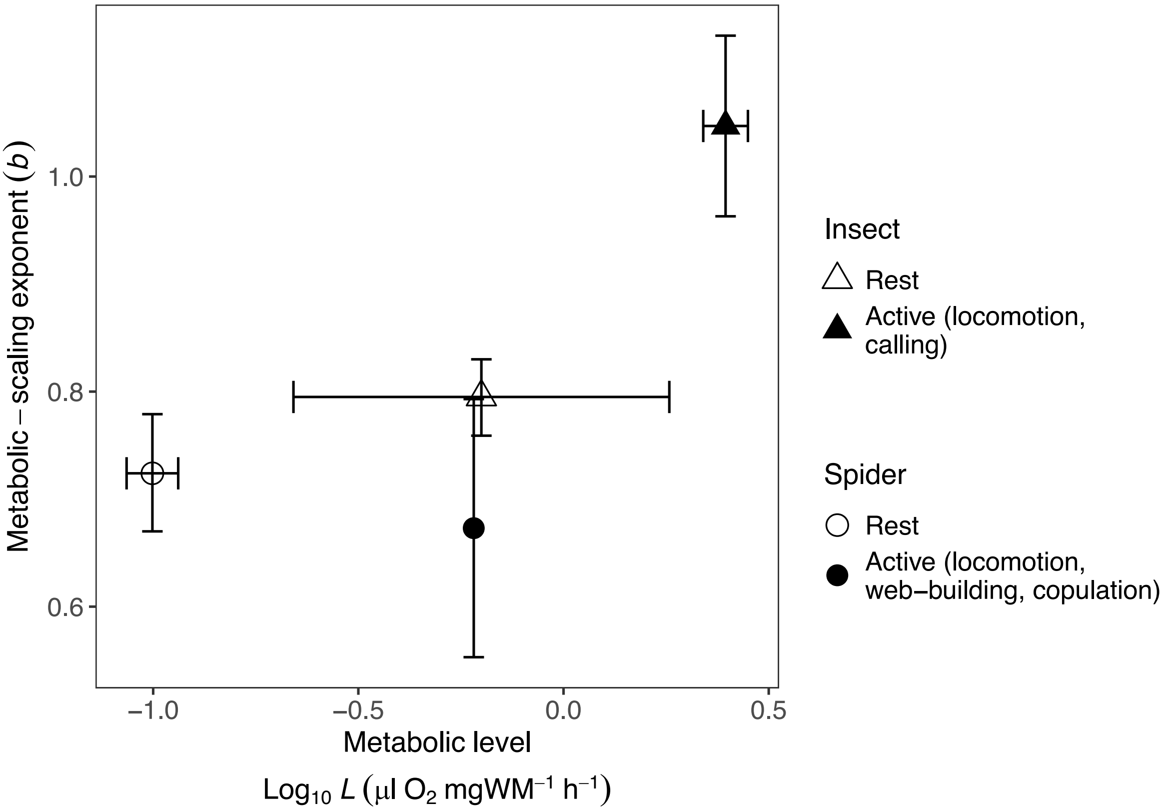


Figure 3.1. The $\log_{10}$-transformed metabolic level during non-flight activity and rest (L_a and L_r, respectively; µl O_2 mgWM^{-1} h^{-1}) and body-mass scaling exponents (b_a and b_r, respectively) obtained from interspecific PGLS regression for metabolic rates in insects and spiders. Metabolic rates are not temperature corrected. Error bars represent 95% confidence intervals of $\log_{10} L$ and b. During rest, b_r is not significantly different between insects and spiders, as indicated by the overlapping confidence intervals. However, insects have significantly higher L_r and L_a values than spiders. The b_a value is significantly higher than b_r in insects, but not in spiders. Error bars smaller than the symbols are not shown.

Higher metabolic level of insects is partially related to the presence or absence of wings (Figure 3.2). During non-flight activity, winged insects have a significantly higher L_a (3.054 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 2.668, 3.486) than wingless insects (1.749 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 1.697, 1.799; Figure 3.2a). Both winged and wingless insects have higher L_a than spiders. Unlike the differences in L_a, winged (1.062 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.529, 2.135) and wingless insects (0.408 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.148, 1.120) have similar values of L_r (Figure 3.2b). While insects have a higher resting metabolism (L_r) than spiders, the lower boundary of L_r in wingless insects approaches that of spiders (Figure 3.2b), and overlaps with that of spiders following temperature corrections (wingless insects: L_r = 0.351 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.118, 1.050, spiders: L_r = 0.101 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.087, 0.118). This difference in L_r is also contingent on body size. When comparing similar-sized animals, wingless insects (0.247 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.103, 0.595) have significantly higher L_r than spiders (0.090 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.079, 0.102) only at body sizes above 410 mgWM. In contrast, winged insects (1.589 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.737, 3.428) have higher L_r than even the smallest spider species (0.247 µl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.103, 0.595) considered (which has a mass of 3 mgWM).

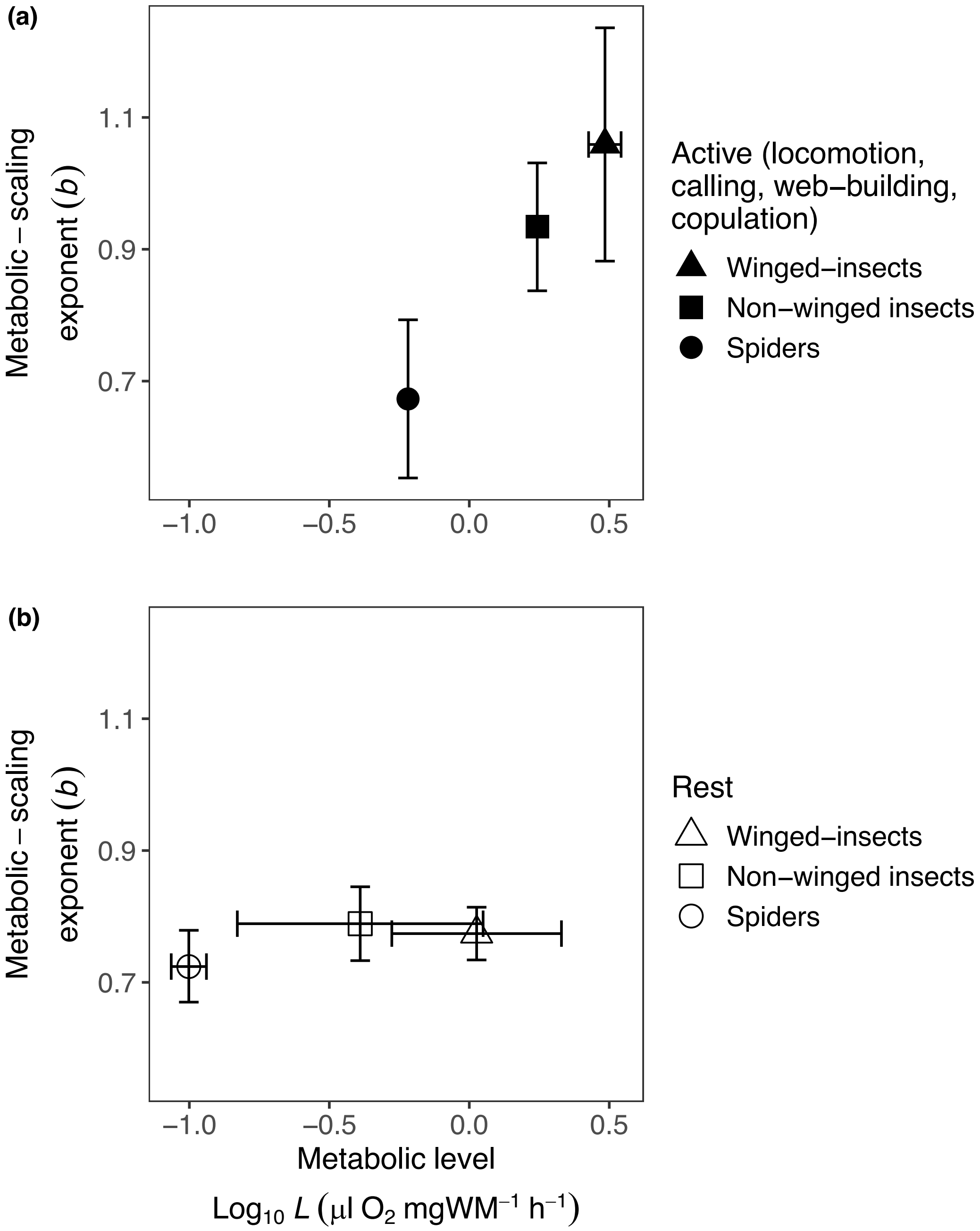


Figure 3.2. The $\log_{10}$-transformed metabolic level (L_a and L_r, μl O_2 mgWM^{-1} h^{-1}) and body mass scaling exponents (b_a and b_r, respectively) of the PGLS regression during **(a)** non-flight activity and **(b)** rest in insects and spiders. Insects are further divided based on wing status. Both winged and wingless insects have a significantly higher L_a and b_a than spiders. Winged insects also have a significantly higher L_a value than wingless insects, even during non-flight activity. During rest, b_r is not significantly different between winged insects, wingless insects and spiders. Unlike L_a, both winged and wingless insects have similar L_r values. However, the lower limit of L_r in wingless

insects is more similar to that in spiders. Error bars represent the 95% confidence intervals of $\log_{10} L$ and b, and error bars smaller than the symbols are not shown.

<u>Scaling of resting and active metabolic rates</u>

Active (non-flight) metabolic rates scale isometrically across insects ($F_{1, 92} = 611.9$, $r^2 = 0.869$, $P < 0.001$, $\log_{10}MR = 0.300 + 1.047 \times \log_{10}M$), as we hypothesized following the prediction of the MLBH (Figure 3.3a). However, active metabolic rate scaled sub-linearly across spiders with body mass to the power of approximately 2/3rds ($F_{1, 19} = 137.7$, $r^2 = 0.879$, $P < 0.001$, $\log_{10}MR = 0.645 + 0.673 \times \log_{10}M$, Figure 3.3b), which is in contrast to the MLBH prediction. Hence, insects ($b_a = 1.047$, 95% CI: 0.963, 1.131) have significantly higher b_a than spiders ($b_a = 0.673$, 95% CI: 0.553, 0.793). In contrast, b for resting metabolic rates in insects ($b_r = 0.795$, 95% CI: 0.759, 0.830) and spiders ($b_r = 0.724$, 95% CI: 0.670, 0.779) are not significantly different from one another. Differences in the values of b_a but not b_r, indicate that active metabolic rates increase more steeply with body size for insects than spiders (Figure 3.3). Larger insects (> 4000 mgWM) have active metabolic rates that are approximately 10-fold those of spiders of an equivalent body size. This contrast between the two taxonomic groups is less pronounced at rest, and resting metabolic rate in insects larger than 4000 mgWM is approximately 4.5 times that of similar-sized spiders. Correspondingly, the MLBH prediction that the value of b_a is higher than b_r is confirmed in insects, but not spiders.

The inclusion of wing status improved the PGLS model for resting and active metabolic rates, as determined by the lower AICc score, although ΔAICc < 2 (Table 3.1). The measure of phylogenetic correlation, λ (Pagel, 1999), for active metabolic rates in both insects and spiders is not statistically different from 0, indicating that the model residuals are not phylogenetically correlated. However, λ values of 0.89 and 0.39

for the scaling of resting metabolic rates in insects and spiders respectively, indicates strong phylogenetic correlation in the model residuals, especially in insects.

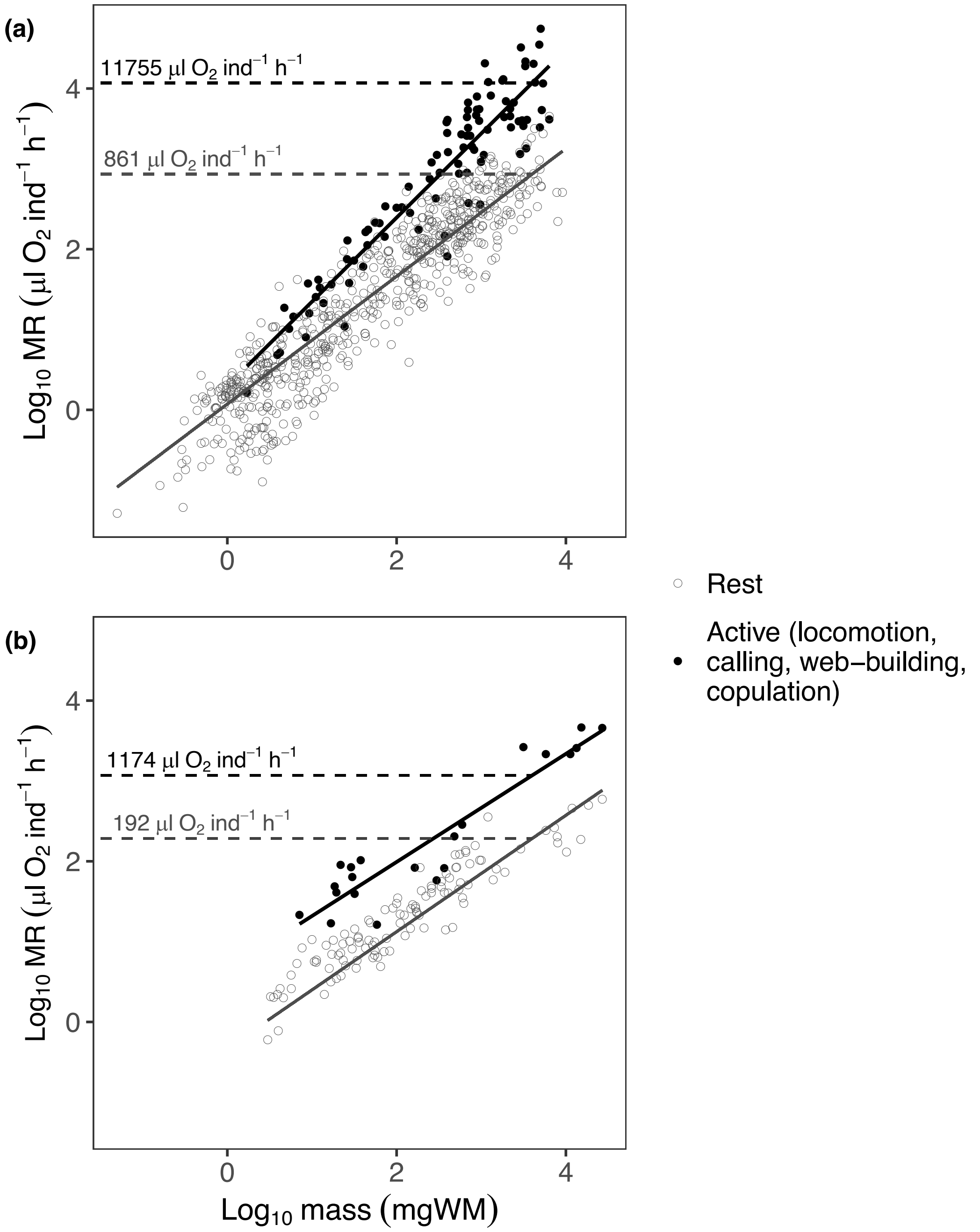


Figure 3.3. The interspecific scaling relationship of metabolic rates and body mass for **(a)** insects and **(b)** spiders at rest versus undergoing non-flight activity. Active metabolic rates scaled more steeply in insects than spiders. Within insects, active metabolic rates also scaled more steeply than resting metabolic rates. The scaling exponent of resting metabolic rates in insects is however not significantly different from

the scaling exponent of resting metabolic rates in spiders. Insects: the grey line indicates the scaling relationship for resting metabolic rate and the black line indicates the scaling relationship for active metabolic rate. Insect: $b_r = 0.795$ (95% CI: 0.759, 0.830); $b_a = 1.047$ (95% CI: 0.963, 1.131). Spiders: $b_r = 0.724$ (95%CI: 0.670, 0.779); $b_a = 0.673$ (95% CI: 0.553, 0.793). The black and grey dashed lines indicate the active and resting metabolic rate for a 4000 mgWM organism. Insects larger than 4000 mgWM have active metabolic rates that are approximately 10-times, and resting metabolic rates that are approximately 4.5-times that of spiders or similar size.

Discussion

Our findings have resolved several previously contested claims about differences in metabolic scaling between insects and spiders (Lighton & Fielden, 1995; McCue *et al.*, 2016). We showed that interspecifically, active metabolic rates scale near isometrically for insects ($b_a = 1.047$), but sub-linearly for spiders ($b_a = 0.673$, Figures 3.1 and 3.3), and that insects consume energy more rapidly than spiders both at rest and during activity. A higher scaling exponent during activity (b_a) than at rest (b_r) in insects is consistent with the MLBH, although this is not the case for spiders. This difference in b_a indicates that active metabolic rates of insects and spiders diverge with increasing body mass. Smaller insects (~600 mgWM) have active metabolic rates that are approximately 4.5 times higher than spiders. However, at larger body masses (above 4000 mgWM), active metabolic rates of insects are on average at least 10-times that of similar-sized spiders. Hence, metabolic rate differences are greatest between large insects and spiders during activity. In contrast, b_r is similar between the two taxa, and factorial metabolic rate differences during rest are relatively similar throughout the entire body mass range. Moreover, energetic differences suggest that different allometric equations are necessary for describing metabolic rates in insects and spiders (Greenstone & Bennett, 1980; McCue *et al.*, 2016).

Differences in metabolic level

Both winged and wingless insects have higher active non-flight (L_a) and resting metabolic level (L_r) than spiders (Figure 3.2). However, difference in L_r between wingless animals is size-dependent, highlighting that the wing status is an important component of energetic differences between insects and spiders. Moreover, while winged and wingless insects have similar L_r values, winged insects have higher values for L_a than wingless insects (Figure 3.2). This indicates that either the overhead costs of maintaining the capacity for flight is most pronounced during activity, or that winged insects have greater aerobic scope for non-flight activity than do wingless species. Higher metabolic costs could also be related to the maintenance of musculature associated with the presence of wings. Additionally, non-flight activity may also trigger activity in flight muscle, possibly because such muscles are directly associated with other activities such as running (Josephson, 2006). Higher overhead costs of maintaining capacity for flight could be partially caused by increased mitochondrial densities in flight muscles. Mitochondrial densities in the flight muscles of cockroach and bees (at 31-43% of muscle volume) (Stokes, 1987; Suarez *et al.*, 2000) are higher than in non-flight muscles of cockroach and crickets (at 5.5-6% and 3.5% of muscle volume respectively) (Stokes, 1987; Snelling *et al.*, 2011). In general, insect locomotory muscles appear to have higher mitochondrial densities than those of spiders, where mitochondrial densities were found to be approximately 0.1% of muscle volume (Linzen & Gallowitz, 1975). Hence, the locomotory muscles of insects, especially those muscles associated with flight, have a higher aerobic capacity than muscles in spiders.

In spiders, low aerobic capacity could underlie low L_a values, and reflect constraints in oxygen supply during activity. Such constraints may arise because the use of hydraulic pressure for locomotion necessitates increased prosoma pressure during locomotion (Anderson & Prestwich, 1975). As a result, haemolymph, and consequently, gas exchange between the opisthosoma (where the book lungs are located) and the prosoma (where oxygen is needed to sustain activity) may be restricted (Wilson & Bullock, 1973). Hence, the possible lack of circulation from the opisthosoma to the prosoma during vigorous exercise (Prestwich, 1988) could restrict the value of L_a relative to L_r. As low prosomal pressure is necessary for constant circulation and gas exchange, most spiders are severely fatigued to near inactivity after 1-2 min of maximum exertion (Prestwich, 1988) due to the accumulation of an O_2 debt (Schmitz, 2016). Therefore, it appears that spiders commonly rely on anaerobic metabolism for achieving peak muscular production. Hence, assessing metabolic rates from O_2 consumption may underestimate the energetic cost of activity in spiders. In contrast, the measurement of CO_2 production could overestimate aerobic metabolism as it qualitatively reflects the contribution of anaerobic metabolism to total activity cost (Cady, Delaney & Uetz, 2011). While comparison of L_a values obtained from measurements of CO_2 production does not alter our conclusion (spiders L_a: 0.873 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 0.837, 0.909, insects L_a: 2.482 μl O_2 mgWM^{-1} h^{-1}, 95% CI: 2.190, 2.814), we cannot rule out the possibility of underestimating L_a values in spiders. However, if respiratory structures are matched to functional demands (Weibel, Taylor & Hoppeler, 1991), such reliance on anaerobic metabolism nonetheless suggests that oxygen supply is matched to the low metabolic demands of spiders.

The use of hydrostatic pressure for appendage extension in spiders could also reduce L_r, as it would allow the maintenance of posture with a small number of active muscles (Anderson & Prestwich, 1975, 1982; Wilder, 2011). If a functional link between resting metabolic rates and active metabolic rates exists (Walton, 1993), lower L_r values in spiders could also result from a correlated response to low L_a (Reinhold, 1999). Spiders are exclusively predatory and mostly utilise a sit-and-wait strategy (McCue *et al.*, 2016). Over 90% of foraging time can be spent waiting (Samu, Szirányi & Kiss, 2003), which suggests a low-energy foraging strategy. As spiders rarely require prolonged activity for capturing prey (Schmitz, 2016), low resting metabolic rates could therefore arise due to their reduced requirement for sustained activity (Reinhold, 1999). Such low resting metabolic rates may be adaptive, and could partly underlie their tolerance to starvation (Overgaard & Wang, 2012). The extensively developed intestinal system of spiders, whose branches fill most of the opisthosoma, and are sometimes abundant in the prosoma (Foelix, 2011), may help support the ability to survive extended periods of starvation. Such an extensively developed midgut can conceivably induce space constraints within a spider. Hence, a potential trade-off may exist between increasing respiratory structures or musculature for increased power output, and investing in storage organs or structures. Since spiders largely digest prey externally and most of the energy for such external digestion is spent shortly after prey capture (Nespolo *et al.*, 2011), the extensive midgut, which also stores glycogen and lipids (Laino *et al.*, 2009; Overgaard & Wang, 2012), could have relatively low metabolic demands. As metabolic rates are influenced by the cost of maintaining muscular tissue (White & Kearney, 2013), such an extensive storage organ may reduce overall metabolic rates and hence L_r. In contrast, higher values of L_r and L_a of insects may reflect their generally more active lifestyle. In this case, increased mitochondrial density in insects may be favoured for increasing aerobic capacity for sustained activity, but consequently also increase L_r, especially in winged species (Reinhold, 1999). Hence, in addition to physiological and mechanical differences (i.e. muscle mitochondrial densities, use of hydrostatic pressure), differences in L_a and L_r between insects and spiders may also be strongly related to contrasts in their locomotor activity levels, including during foraging.

Differences in scaling exponent b

Our observation of isometric scaling of active non-flight metabolic rates in insects, is similar to previous findings for insects during flight ($b = 1.07$) (Niven & Scharlemann, 2005) and sexual signalling ($b = 1.18$) (Doubell *et al.*, 2017). Thus, active metabolic rates scale isometrically for insects during various activities. Using our database of resting metabolic rates that contained over 50% more insect species than previous studies (608, rather than 391) (Chown *et al.*, 2007), we found b_r to be significantly greater than 0.75 ($b_r = 0.795$, 95% CI: 0.759, 0.830), which is assumed by the Metabolic Theory of Ecology (MTE) (Brown *et al.*, 2004). This deviation in b_r from a value of 0.75 is not a consequence of phylogenetic nonindependence or wing status, as including wing status in the PGLS model did not affect b_r. Including Termitidae within the insect data has previously been shown to strongly affect the value of b_r obtained by some authors for smaller data sets (see Riveros & Enquist, 2011). But the exclusion of this family resulted in only a slightly lower b_r in this study (0.788, 95% CI: 0.752, 0.825), and the resultant value is still significantly greater than 0.75. Previous concerns about the data from the Termitidae were related to its accuracy, as these data were obtained from a single study that indirectly approximated O_2 consumption from CH_4 production (see Riveros & Enquist, 2011). However, we overcame this limitation by collating termite data from multiple sources that also included studies that directly measured O_2 use and CO_2 production (McComie & Dhanarajan, 1990; Wheeler *et al.*, 1996; Bignell *et al.*, 1997). Furthermore, phylogenetic correction should reduce the overall influence of a closely related phylogenetic group on b_r. Hence, the deviation in b_r from the prediction of the MTE is unlikely the result of a single outlier group – a proposal supported by the b_r value of insects obtained by OLS regression when Termitidae are excluded ($b_r = 0.789$, 95% CI: 0.766, 0.812).

The scaling exponent for metabolic rates of spiders at rest (0.724, 95% CI: 0.670, 0.779, Figure 3.3) is similar to that for insects (Lighton & Fielden, 1995), and to that previously reported for spiders (Greenstone & Bennett, 1980). However, OLS regressions without phylogenetic correction (McCue *et al.*, 2016) have found a smaller b_r value for spiders, likely due to the phylogenetic clustering of large spiders mainly from the infraorder Mygalomorphae. In our data, without phylogenetic correction, the scaling exponent for spiders ($b_r = 0.638$, 95% CI: 0.591, 0.685) is also significantly smaller than for insects ($b_r = 0.840$, 95% CI: 0.814, 0.865). Thus, phylogenetic corrections are important for interpreting metabolic scaling exponents in these two taxa.

The prediction of the MLBH - that b increases with activity, and should approach isometry at high activity levels - is confirmed in this study only for the insects. This prediction is partially based on the assumption that large animals with lower mass-specific metabolic rates or power production potentially utilise less of their power capacity than smaller animals, and would therefore be disadvantaged in comparison to smaller animals (Glazier, 2010). However, if energetic efficiencies (per W) increase with body mass, lower mass-specific metabolic rates need not reflect weaker performance capacity in large animals. Such efficiency gains could arise if the energetic cost of locomotion, or the cost of posture maintenance for locomotion, decreases with body size. The cost of posture maintenance for locomotion is reflected in the y-intercept obtained from the metabolic rate-locomotion speed relationship (Halsey, 2013). A comparison of this y-intercept suggests that the cost of maintaining posture decreases with body size for spiders but not insects (SI 3, Figure S3.4). Large spiders may therefore have lower energetic costs for posture maintenance than small spiders. In addition, body temperature of large spiders appear to be unaffected by activity

(Anderson & Prestwich, 1985), unlike large insects that tend to have elevated body temperature during activity (Bartholomew & Casey, 1977). Endogenous heat production suggests that large insects expend more energy during activity (Bartholomew & Casey, 1977), and could accentuate differences in the energetic costs of activity between large spiders and insects. Such differences could also be further intensified by the increased metabolic scope of winged insects for flight, which may also support the maintenance of high aerobic levels during non-flight activity.

Conclusions

Insects consume more energy than spiders during both rest and activity. In general, spiders may have relatively low energetic costs of foraging, and resting metabolic rates may constitute a major component of their daily energy requirements. Reducing active and resting metabolic rates may therefore increase starvation tolerance, and could correlate with longer lifespans or greater survival. In contrast, insects may have generally higher levels of locomotor activity than spiders. Hence, higher aerobic capacity for sustained activity through increased mitochondrial densities, likely increases both active and resting metabolic levels in insects, and is further accentuated by the potential for flight. We demonstrate that a single allometric equation for describing metabolic rates in insects and spiders is insufficient due to differences in metabolic levels, and the contrasting effect of activity on the scaling exponent. The energetic difference between insects and spiders is greatest during activity, and at large body sizes, as indicated by the different b_a values. We hypothesize that such differences are due to contrasting size-scaling of the cost of maintaining locomotory posture and/or of the cost of locomotion itself, which may be influenced by different maximum locomotor and energetic capacities. Future studies should distinguish between these possible mechanisms for metabolic scaling differences between these two diverse terrestrial arthropod groups.

Chapter 4

Body size and shape responses to warming and resource competition in protists

Abstract

Body size is a fundamental trait that impacts many aspects of species biology and ecology. It is in turn influenced by a suite of environmental factors, and is often inversely related to temperature. Although environmental conditions can also impact body shape, which could influence motility and the scaling of physiological rates, such responses are poorly understood and rarely quantified. We experimentally tested the independent and combined effects of environmental temperature, resource level and interspecific competition on the body shape and size responses of two model protist species. We also tested the degree to which these individual-level phenotypic responses are associated with population densities and species coexistence. Whereas the body size changes were mainly driven by environmental temperature, body shapes of both species were strongly influenced by resource competition. Low resource level favoured body shape elongation in both species, suggesting that relatively more elongate individuals with potentially higher swimming speed were advantaged in the environment with resource scarcity. However, competition had contrasting influence on the body shape of the two species. The weaker competitor, as indicated by decreased population densities during interspecific competition, exhibited relative body shape elongation, reflecting body shape responses at low resource levels. In contrast, the stronger competitor reduced relative elongation, reflecting body shape responses at high resource levels, which suggests that interspecific competition reduced resource scarcity. Body shape responses could thus be indicative of competitive abilities, where elongation may reflect weaker competitive abilities. Coexistence was observed in all treatments, likely because

body size of both species decreased similarly under warming. These findings suggest that both body shape and size should be considered in concert as they are interlinked and have important implications for ecological interactions and community dynamics.

Introduction

Climate change is a pressing global threat, which adversely impacts Earth's ecosystems and human wellbeing (Sheridan & Bickford, 2011; Ripple *et al.*, 2019). Climate change is associated with global mean temperature rise (IPCC, 2013, 2018) and an increased frequency of extreme temperature events (Easterling *et al.*, 2000; IPCC, 2012), which have profound impacts on biota (Vasseur *et al.*, 2014; Woodward *et al.*, 2016). These changes have already altered the distributional ranges and phenology of species (Parmesan & Yohe, 2003), which are widely recognised as two universal responses to global warming (Gardner *et al.*, 2011). More recently, body size reductions have emerged as a third universal ecological response to mean increase in global temperatures (Daufresne *et al.*, 2009; Gardner *et al.*, 2011; Sheridan & Bickford, 2011; Evans *et al.*, 2019), and also potentially extreme temperature events (Fischer, Klockmann & Reim, 2014). Such body size reductions could have profound ecological repercussions because body size is a fundamental trait (Peters, 1983) that underlies metabolic rates (Brown *et al.*, 2004), fecundity (Arendt, 2007), species interactions and community dynamics (Brose, Williams & Martinez, 2006b; Rall *et al.*, 2012). Hence, understanding and ultimately predicting the body size response of organisms to both long- and short-term warming is a crucial and globally important ecological challenge.

Body size is often reduced because most ectotherms mature at smaller body sizes when grown under warmer conditions (Atkinson, 1994; Atkinson *et al.*, 2003; Forster *et al.*, 2012). This inverse relationship between body size and temperature, the 'temperature-size rule' (TSR) is a phenotypically plastic response (Atkinson, 1994), and is one of the most common phenomena in biology (Angilletta *et al.*, 2004; Kingsolver & Huey, 2008). It is commonly observed in organisms as diverse as bacteria, protists, invertebrates, and ectothermic vertebrates (Atkinson *et al.*, 2003; Daufresne *et al.*, 2009; Forster *et al.*, 2012; Horne *et al.*, 2015). As body size influences various physiological rates (Hildrew *et al.*, 2007), temperature-induced body-size changes may disrupt biotic interactions (Sheridan & Bickford, 2011) and reduce species persistence (Sentis, Binzer & Boukal, 2017). Hence, individual responses could scale up to affect whole communities (Ohlberger, 2013).

Body shape is also an important functional trait that directly influences motility (Beveridge *et al.*, 2010; Gibert *et al.*, 2017), encounter rates and thus species interactions (Berger, 1980). Environmentally-induced body shape responses relating to resource availability and temperature have been observed in phytoplankton (Naselli-Flores & Barone, 2011), cyanobacteria (Jezberová & Komárková, 2007) and protists (Hammill, Petchey & Anholt, 2010; Gibert *et al.*, 2017). For instance, high resource levels have been found to increase protist body length (relative to other body axes), resulting in individuals being more elongate in shape (Gibert *et al.*, 2017). Such changes likely reduce drag and improve motility (Roberts, 1981; Vogel, 1994), which can increase prey encounters and hence resource uptake rates (Gibert *et al.*, 2017). The property of body shape being conserved as size increases is termed isomorphy. By contrast, non-isomorphic 'shape-shifters' (Hirst *et al.*, 2014) include species which become relatively more elongate (along the longest body axis, relative to other dimensions) or more flattened (more elongate in the two longest dimensions relative to the third) as they grow over ontogeny. Such body shape change also increase the scaling of surface area to body mass compared to that achieved in isomorphic species, as was discussed in chapter 2. Increased degrees of shape change over ontogeny have been related to increased body-mass scaling of metabolic rates in pelagic invertebrates that rely upon much of their body surface for exchange of oxygen (Hirst *et al.*, 2014; Glazier *et al.*, 2015; Tan *et al.*, 2019). This relationship between mass-scaling of surface area and of metabolic rate suggests that the relationship between body shape and size could underlie important physiological differences among species. However, as body shape responses to environmental change are rarely studied, it remains unclear whether and how body shape changes under the independent and combined effects of warming and resource availability.

The availability of food also influences body size and shape by providing the material and energy for maintenance, growth and reproduction (Sterner & Elser, 2002). In general, body size-at-stage increases at higher resource levels (Balčiūnas & Lawler, 1995; Ohlberger, 2013), but this effect often depends on the community context (Tabi, Petchey & Pennekamp, 2019). In particular, interspecific competition reduces resource availability (Fox, 2002), which can alter both body size and body shape. As higher temperatures increase metabolic demands (Brown *et al.*, 2004), warming can increase *per capita* competitive intensity (Jiang & Kulczycki, 2004; Lewington-Pearce *et al.*, 2019) and indirectly influence both body size and shape through reducing resource availability.

Here, we experimentally tested the independent and combined effects of temperature, resource availability and interspecific competition on the phenotypic changes in body size and shape of two bacterivorous protist species. Protists are ideally suited for controlled and replicated analyses of body size and shape responses to environmental change, as these traits are phenotypically plastic (Atkinson *et al.*, 2003; Gibert *et al.*, 2017) and responses can be observed after a few days of experimental manipulation (Atkinson *et al.*, 2003). Protists are commonly used as model organisms in population and community ecology (Altermatt *et al.*, 2015), and form an important compartment of natural food webs (Sherr & Sherr, 2002). Hence an improved understanding of individual responses, which are rarely considered (Tabi *et al.*, 2019), could be important for connecting individual-level responses to population-level responses in a multi-species context (Atkinson *et al.*, 2003).

We hypothesized that (1) individuals will be more elongate in shape at low resource level to improve swimming speed and enhance resource acquisition, and relative elongation will be greater at higher temperature and under interspecific competition due to temperature-enhanced resource demands; (2) a warmer environment will result in reduced body size as predicted by the TSR, and size reduction will be exacerbated at low resource level and under interspecific competition; (3) warming will strengthen interspecific competition as higher metabolic rates enhance energy demands and *per capita* consumption rates; (4) individuals more elongate in shape will achieve higher population densities due to increased encounter rates with bacterial resource, and hence increased use of available energy for growth and reproduction. Our results show that body-size changes may relate to whole-organism metabolic demand, while body-shape changes relate mainly to resource acquisition.

Materials and methods

Experimental design

To partition how experimental warming, resource level and interspecific competition influence body size and shape, we used two bacterivorous ciliate protozoa *Paramecium aurelia* and *Blepharisma japonicum*. These two species compete for similar bacterial resources (Clements *et al.*, 2013) and are morphologically distinct, which facilitates accurate identification. We obtained *P. aurelia* and *B. japonicum* as monocultures from Sciento (Manchester, UK). We maintained the single-species cultures at 21 °C in protozoa medium prepared by filtering 0.56 g L^{-1} of crushed protozoan pellets (Blades Biological Ltd., UK) in Volvic spring water through double-layered Rombouts no. 4 coffee filters (Hammill *et al.*, 2010, 2015). We maintained stock cultures in conical Erlenmeyer flasks containing 100 mL of autoclaved protozoan medium and a single wheat grain. While *B. japonicum* is able to form enlarged predatory morphs, they tend to occur at low nutrient levels (less than half the concentration used here) and were not observed in our experiment. Thus, we only considered competitive interactions in this study (Clements *et al.*, 2013).

For the competition experiment, we maintained populations of *P. aurelia* and *B. japonicum* in single-species cultures and compared their population dynamics with those in polycultures containing both species. We also factorially manipulated temperature (22 and 25 °C) and resource level (high, low), in order to investigate how body shape and size were influenced. The cultures were kept in Stuart orbital incubators (model SI500). Whereas 22 °C is a common maintenance temperature for protist cultures, 25 °C represents a modest warming of 3 °C. The two bacteria resource levels (SI 4, Figure S4.1) were established by adding different amounts of protozoan pellet into the protozoa medium (0.28 and 0.56 g L^{-1} spring water, Fox, 2002). Each of the 12 treatment combinations was replicated six times, resulting in 72 experimental microcosms in total. We initiated single-species treatments by seeding 100 individuals of either *P. aurelia* or *B. japonicum* in 4 mL of protozoa medium. At the same time, we initiated two-species treatments by seeding 50 individuals of each species in 4 mL of protozoa medium. Microcosms were maintained in the dark for 11 days resulting in approximately 20-30 generations, as generation times for the two species are on the scale of 2-3 generations per day. In all treatment combinations, both species grew to steady-state within 6 to 9 days (SI 4, Figure S4.2).

To monitor population growth, we collected samples daily during the first week of the experiment, and then every two days. At each sampling event, we subsampled 0.1 mL of medium and fixed it with a final concentration of 0.5% Lugol's media (Sherr & Sherr, 1993). We pipetted this subsample along the edge of a Bogorov counting chamber, and counted all the individuals present under a Nikon SMZ1500 dissecting microscope to estimate population densities. After each sampling, we replaced the volume sampled with 0.1 mL of fresh medium. We also added 0.2 mL and 0.5 mL of fresh media on day 4 and day 7 respectively to control for any resource depletion and evaporative water loss, which maintained bacterial resources (SI 4, Figure S4.1).

To measure the body size and shape of protists at the end of the experiment, we fixed 0.5 mL of subsample from each microcosm with a final concentration of 0.5% Lugol's media. We then photographed an average of ten individuals of each species from each microcosm under a Leica DMIL inverse microscope at 40x magnification. Lugol's media has been found to have minimal effect on *P. aurelia* cell width (Hammill *et al.*, 2010), and has also been previously used for measuring *Blepharisma* spp. size (Chapman, 2016). We measured body length and width using ImageJ analysis software (Schneider, Rasband & Eliceiri, 2012), calculating body size as biovolume from the length and width measurements assuming a standard geometric shape of a prolate spheroid (DeLong & Vasseur, 2012; Forster, Hirst & Esteban, 2013; Chapman, 2016). We then obtained the mean body length, width and estimated size (biovolume) of each species for each replicate derived from 9-11 photographed individuals. We refer to cell size and shape as body size and shape, as it is understood more broadly in the context of the TSR (Adams *et al.*, 2013).

Statistical analyses

To compare phenotypic responses of both species across all treatment combinations, we applied a factorial ANOVA with the trait of interest (*P. aurelia* or *B. japonicum* length:width ratio, body size, and population density) as the response variable, and temperature, resources level and the interspecific competition, and their interactions as the explanatory variables. For *P. aurelia*, we used the model residuals obtained from the regression of length:width ratio against body size as the response variable, because the length:width ratio was negatively correlated with body size ($F_{1, 46} = 12.41$, $p = 0.001$, r^2

= 0.213, SI 4, Figure S4.3), and larger individuals were less elongate in shape. We used linear regression to test for a relationship between population density and body size, and between population density and length:width ratio (*B. japonicum*), or the residuals obtained from the length:width ratio-body size regression (*P. aurelia*), as length:width ratio is correlated with body size for *P. aurelia*, but not *B. japonicum.* All statistical analyses were performed in R statistical software version 3.6.0 (R Core Team, 2019). We detected unequal variances in the body size data of both species, but using heteroscedasticity-consistent errors with the 'white.adjust' argument in the *Anova* function in the package *car* (Fox & Weisberg, 2019) did not alter our results. We therefore report the results of our factorial ANOVA. We also quantified the temperature-size response from the formula $(\exp^{(\text{slope})}-1)*100$ = % change in body size per °C (Forster *et al.*, 2012), where the slope was derived from the regression of [natural log] ln (individual body size) against temperature.

Results

Body shape

The residuals of the length:width ratio obtained from the linear regression of length:width ratio against body size of *P. aurelia* were significantly influenced by resources and competition (i.e. presence of *B. japonicum*, $p < 0.001$, Table 1, Figure 1). At low resource level, length:width ratios were higher than predicted based on body size, indicating that individuals are relatively more elongate in form when resources are scarce, as we hypothesized. However, in contrast to our hypothesis, interspecific competition reduced the residuals of the length:width ratio (Figure 1a), which indicates individuals with less elongate body shape. In *B. japonicum*, experimental warming significantly reduced the length:width ratio ($p = 0.002$, Table 4.1, Figure 1b). Competition and resources interactively influenced the length:width ratio of *B.*

japonicum, with competition and low resource level increasing the length:width ratio (p = 0.017, Table 4.1, Figure 1b). As we hypothesized, interspecific competition favoured relative elongation in the shape of *B. japonicum*, and high resource level appeared to decrease relative shape elongation, but only without interspecific competition.

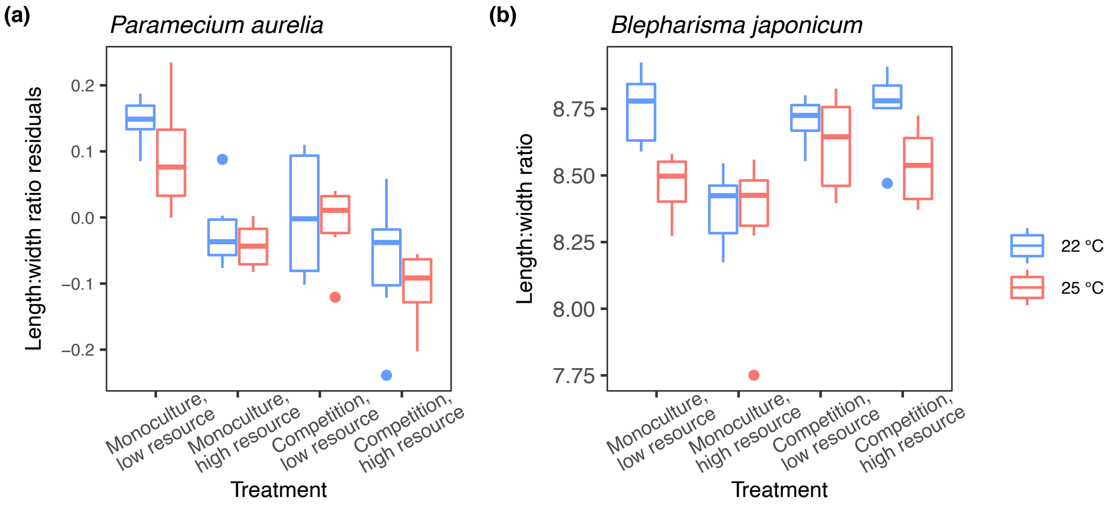


Figure 4.1. Body shape responses (measured as the length:width ratios), indicating the elongation of *Paramecium aurelia* and *Blepharisma japonicum* across all experimental treatment combinations. **(a)** Length:width ratio of *P. aurelia* is based on the model residuals of the regression of length:width ratio against body size. Resource and competition significantly influenced the body shape of *P. aurelia*. At low resource level, body shape is relatively more elongated than predicted based on body size, while competition reduces the extent of elongation. **(b)** In *B. japonicum*, experimental warming reduces the length:width ratio, indicating less elongated body shape. Competition and resource supply interactively influenced body shape, and high resource level reduced relative elongation, but only in the absence of competition.

Table 4.1. Summary statistics from the analysis of variance (ANOVA) testing the independent and interactive effects of experimental warming, competition, and resources on body shape, body size and population density of both model species. Body shape was approximated as length:width ratio or the model residuals obtained from the regression of length:width ratio against body size.

Response / Treatment	***Paramecium aurelia***			***Blepharisma japonicum***		
	d.f.	F	p	**d.f.**	F	p
Body shape						
Competition	1, 40	18.34	<0.001	1, 40	12.78	<0.001
Resource	1, 40	32.05	<0.001	1, 40	7.89	<0.001
Temperature	1, 40	2.47	0.124	1, 40	11.29	0.002
C × R	1, 40	2.67	0.110	1, 40	6.23	0.017
C × T	1, 40	0.07	0.787	1, 40	0.04	0.847
R × T	1, 40	<0.01	0.979	1, 40	0.25	0.617
C × R × T	1, 40	0.49	0.487	1, 40	3.50	0.069
Body size						
Competition	1, 40	1.52	0.224	1, 40	2.79	0.103
Resource	1, 40	0.96	0.334	1, 40	50.11	<0.001
Temperature	1, 40	17.90	<0.001	1, 40	17.38	<0.001
C × R	1, 40	4.89	0.033	1, 40	2.56	0.117
C × T	1, 40	0.91	0.347	1, 40	2.69	0.109
R × T	1, 40	0.17	0.684	1, 40	0.70	0.406
C × R × T	1, 40	3.71	0.061	1, 40	0.40	0.533
Population density						
Competition	1, 40	8.67	0.005	1, 40	19.41	<0.001
Resource	1, 40	10.56	0.002	1, 40	0.90	0.348
Temperature	1, 40	4.01	0.052	1, 40	0.72	0.402
C × R	1, 40	10.29	0.003	1, 40	2.45	0.125
C × T	1, 40	0.58	0.451	1, 40	0.84	0.364
R × T	1, 40	0.03	0.871	1, 40	0.14	0.706
C × R × T	1, 40	0.23	0.637	1, 40	0.99	0.326

Body size

As we hypothesized and in agreement with the TSR, body size of both *P. aurelia* ($p < 0.001$, Table 4.1, Figure 4.2a) and *B. japonicum* ($p < 0.001$, Table 4.1, Figure 4.2b) significantly declined with experimental warming. Interspecific competition and resource level interactively influenced *P. aurelia* body size ($p = 0.033$, Table 4.1). Their body size decreased under interspecific competition, but only at low resource level (Figure 4.2a). Low resource level also reduced the body size of *B. japonicum* ($p < 0.001$, Table 4.1, Figure 4.2b), but interspecific competition did not influence the effect of resources on body size ($p = 0.117$, Table 4.1, Figure 4.2b). Contrary to our hypothesis, the effect of experimental warming on body size was not exacerbated by low resource level in both species ($p = 0684$, *P. aurelia*; $p = 0.406$, *B. japonicum*; Table 4.1, Figures 4.2a and b respectively).

Population density

Resource level and competition interactively influenced the population density of *P. aurelia* ($p = 0.003$, Table 4.1, Figure 4.2c). Interspecific competition had a positive effect on the population density of *P. aurelia*, but only at low resource level. In contrast, the population density of *B. japonicum* was negatively affected by interspecific competition, but not by resource level ($p < 0.001$, Table 4.1, Figure 4.2d). However, temperature did not alter population densities of *P. aurelia* ($p = 0.052$) or *B. japonicum* ($p = 0.402$, Table 4.1, Figure 4.2c and d), and coexistence was observed under all experimental conditions (SI 4, Figure S4.2). Hence, contrary to our hypothesis, warming did not affect the strength of interspecific competition in any observable way in our experiments.

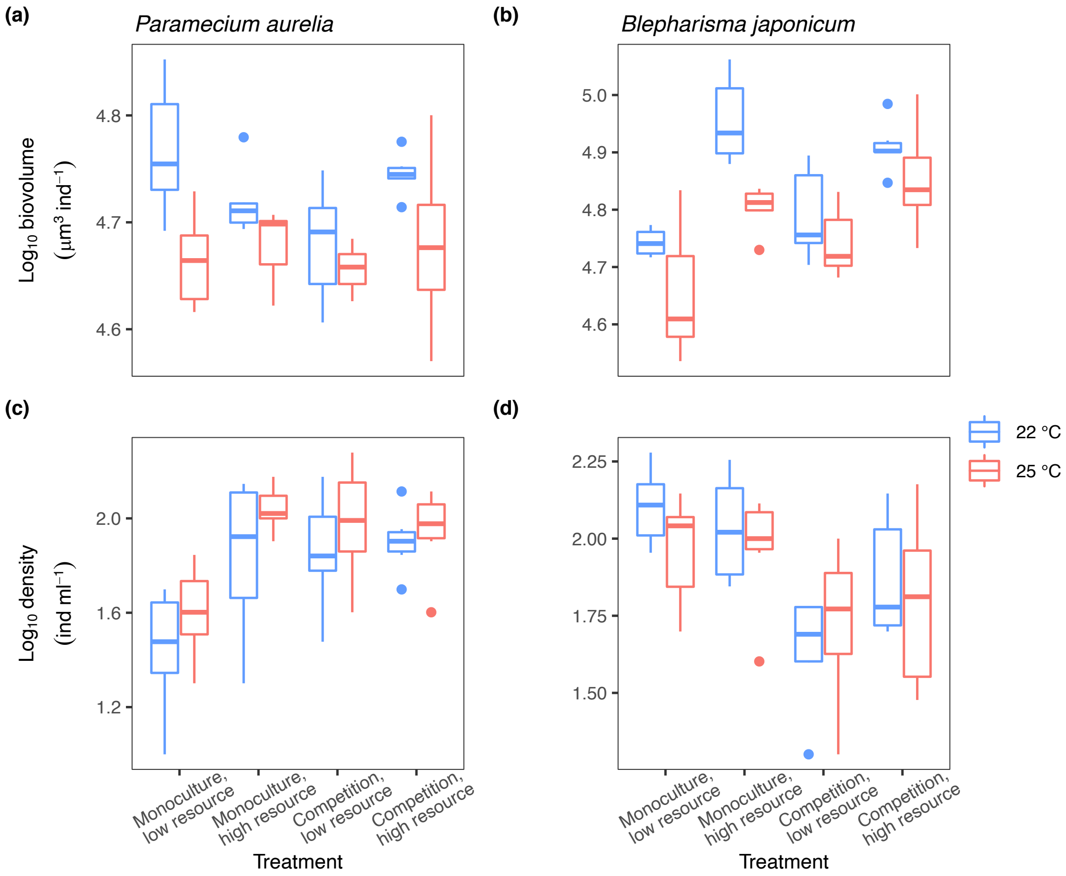


Figure 4.2. The mean body size and population density of two model species across all experimental treatment combinations. **(a)** Experimental warming reduces *P. aurelia* body size. Interspecific competition reduces the body size of *P. aurelia*, but only at low resource level. **(b)** Experimental warming and low resource level significantly reduce *B. japonicum* body size. **(c)** *P. aurelia* population density is significantly affected by the interactive effect of resource and competition. Interspecific competition increases the population density of *P. aurelia*, but only under low resource level. **(d)** *B. japonicum* population density is negatively affected by interspecific resource competition.

There is an indication that the population density of *P. aurelia* was negatively related to body size, although the effect was marginally non-significant ($F_{1,46} = 3.99$, $p = 0.052$, Figure 4.3a). In contrast to our hypothesis, a negative relationship between population density of *P. aurelia* and the residuals of the length:width ratio to body size regression ($F_{1,46} = 8.17$, $p = 0.006$, $r^2 = 0.151$, Figure 4.3c) indicates that *P. aurelia* population density is higher when body shape is relatively less elongated than predicted based on body size. We found no relationship between the population density of *B. japonicum* and either body size ($F_{1,46} = 0.10$, $p = 0.740$, Figure 4.3b) or length:width ratio ($F_{1,46} = 0.69$, $p = 0.412$, Figure 4.3d).

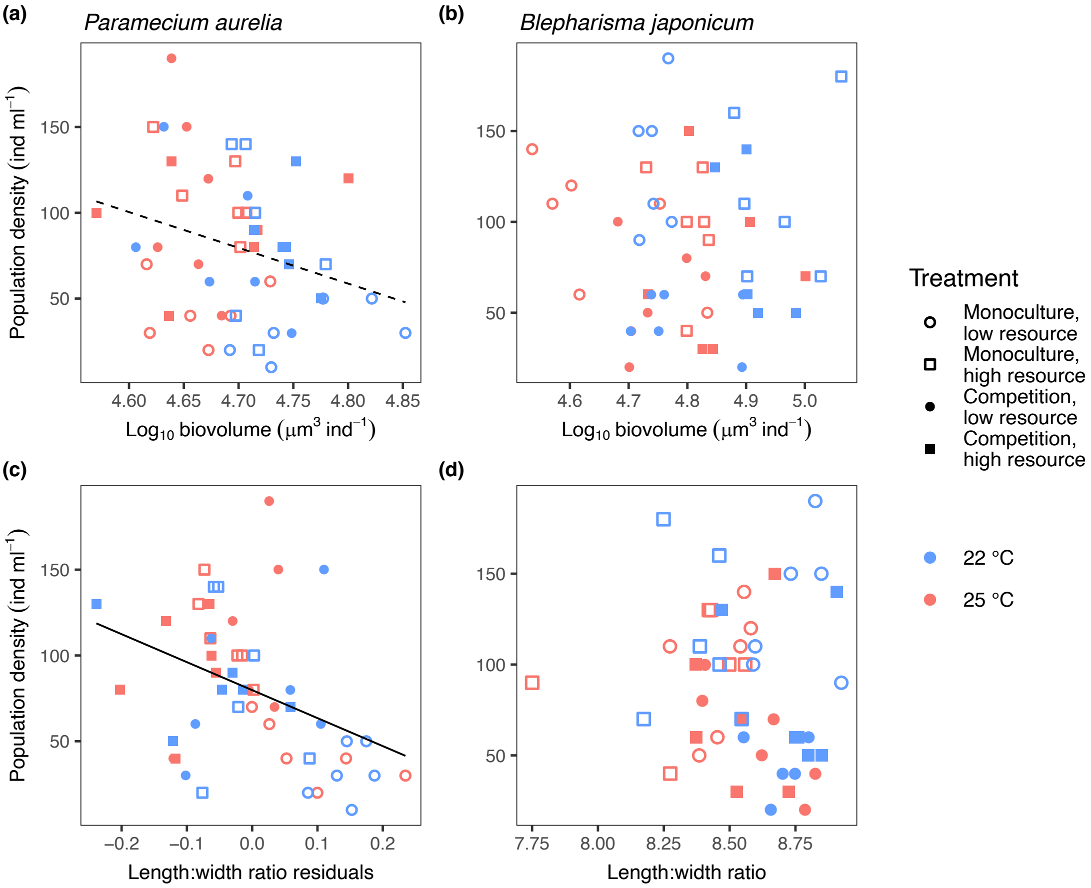


Figure 4.3. The relationship between population density and body size or between population density and length:width ratio. **(a)** There is an indication that population density of *Paramecium aurelia* is negatively related to mean body. **(b)** There is no relationship between population density of *Blepharisma japonicum* and mean body size. **(c)** Population density of *P. aurelia* is negatively related to the length:width ratio residuals from the regression of length:width ratio against body size, indicating that population densities are higher when individuals are less elongated. **(d)** In contrast,

there is no relationship between length:width ratio and population density of *B. japonicum*.

Discussion

Understanding how the environment modulates functional traits can improve our knowledge of how environmental changes affect biotic interactions, species coexistence and community dynamics (McGill *et al.*, 2006). Such knowledge is increasingly important in the face of growing pressures from increasing magnitude and frequency of extreme temperature events (IPCC, 2012; Oliver *et al.*, 2018). Our simple factorial experiment showed that the model protist species have divergent body shape responses to interspecific resource competition (Figure 4.1 and 4.4), but similar body size responses to warming (Figure 4.2a, b and 4.4). This suggests that body shape is closely linked to competitive abilities, while body size responses are primarily driven by temperature, and possibly metabolic demands. Non-isomorphic species such as *P. aurelia*, which changes body shape with body size, may be better competitors than isomorphic species, such as *B. japonicum*, where body shape does not change with body size. Size decrease coupled with relative shape elongation may improve resource acquisition, reduce time to reproduction, and could bring competitive advantages at low resource levels. However, similar body size reductions of both species under experimental warming could compensate for the temperature-enhanced metabolic demands and maintain *per capita* competitive intensity.

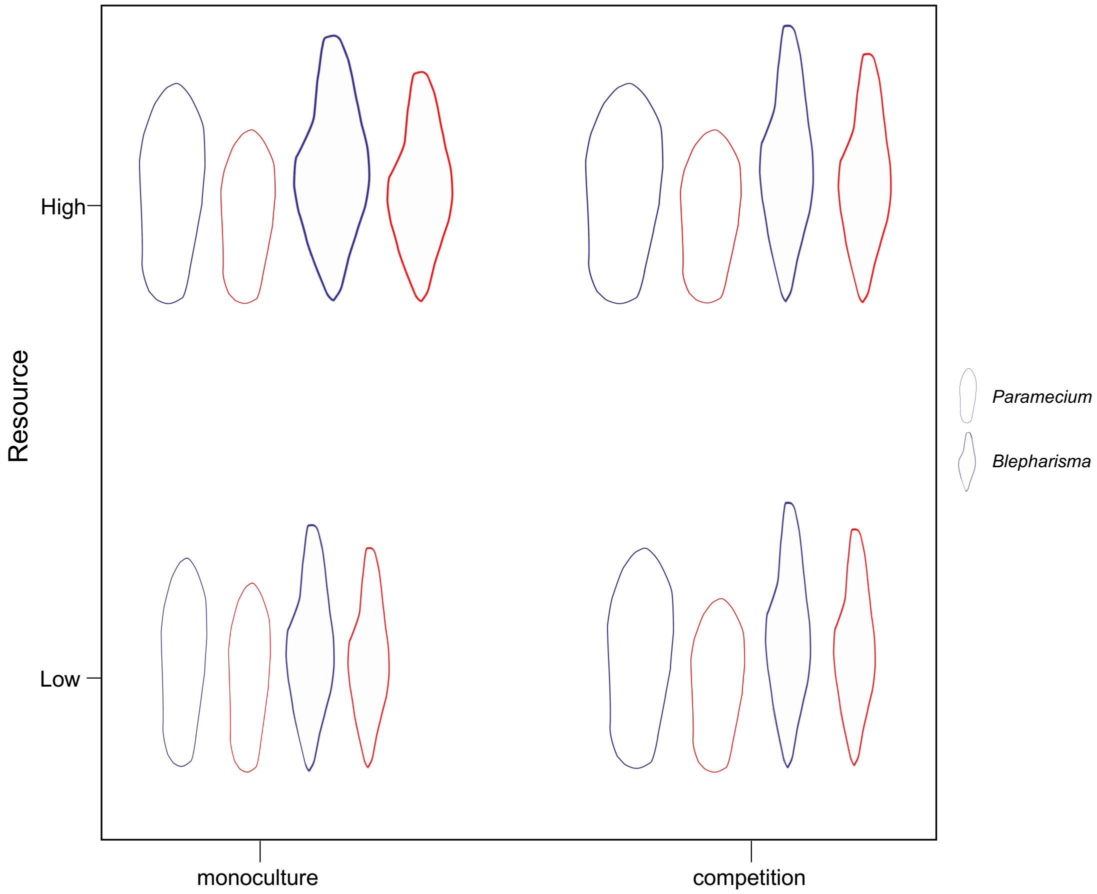


Figure 4.4. A schematic representation of how body shape and size respond to the combination of increased temperature (red symbols: 25 °C, blue symbols: 22 °C), resource level and interspecific competition. At low resource level, relatively more elongate individuals appear to be favoured, while elongation decreases at high resource level. Experimental warming also reduces relative elongation in *B. japonicum* but not in *P. aurelia*. In *P. aurelia*, interspecific competition reduced relative elongation, and body shape response is similar to that observed at high resource level. In contrast, competition favoured relative elongation in *B. japonicum*, resulting in a similar response as that observed at low resource level. This suggests that *P. aurelia* is the superior competitor, which is likely more efficient in extracting shared resources than *B. japonicum*. As a result, interspecific competition results in *P. aurelia* body shape that is similar to that found in high resource monocultures. This is further corroborated by a positive competition effect on the population density of *P. aurelia* but a negative

competition effect on the population density of *B. japonicum* (Figures 4.2c and d). As hypothesized, experimental warming significantly reduced the body size of both species.

Low resource levels appear to favour relatively elongate-shaped individuals (Figure 4.1 and 4.4), as we hypothesized. However, contrary to our hypothesis, this response is not enhanced by experimental warming. Changes in the length:width ratio indicate that for both species, body length and width do not grow proportionally under different environmental conditions (Gibert *et al.*, 2017). The likely benefits of body-shape elongation (greater length:width ratios) may include enhanced swimming speed and resource acquisition, and/or reduced energetic costs of motility (Roberts, 1981; Banerji *et al.*, 2015; Gibert *et al.*, 2017; Pennekamp *et al.*, 2019), and are associated with resource scarcity.

Interspecific competition led to shape elongation of *B. japonicum*, reflecting response observed in single-species treatments with low level of resources. This suggests that the presence of *P. aurelia* reduced resources available to *B. japonicum*. In contrast, competition reduced shape elongation in *P. aurelia*, and the resulting body shape was similar to that observed at high resource level (Figure 4.1a). The contrasting body-shape response to competition may therefore suggest that *P. aurelia* was a better competitor than *B. japonicum* under the tested experimental conditions. This suggestion is further supported by the positive effect on the population density of *P. aurelia* when in competition (Figure 4.2c), but the negative effect on *B. japonicum* population density at both resource levels when in competition (Figure 4.2d). Interspecific competition also significantly decreased the population biovolume density of *B. japonicum* (SI 4, Figure S4.4), but not total assemblage biovolume density (SI 4, Figure S4.5). This further implies that *P. aurelia* was better at acquiring resources and negatively affected *B.*

japonicum. The positive competition effect on *P.* a*urelia* is observed only at low resource level, likely because resource competition is more intense under these conditions. At high resource level, *P. aurelia* is unable to extract more resource from the environment during competition than in monocultures, as is evident from similar population densities in high resource monocultures and polycultures (Figure 4.2c), although they still negatively affected *B. japonicum* population densities (Figure 4.2d).

Contrasting influence of resources on body shape was found in another protist species, *P. bursaria*, where individual shape is relatively more elongate at high levels of bacterial resources (Gibert *et al.*, 2017). Shape elongation at high resource levels contrasts with our observations. However, as swimming speed may display a hump-shaped response in relation to resources level (Johansson & Leonardsson, 1998), increased resources could positively or negatively influence swimming speed, depending on the specific resource range being tested. When resources are scarce, energy gains should increase with swimming speed as resource level increases (Werner & Anholt, 1993). However, at intermediate to high resource levels, low travel distances between prey could potentially decrease the benefits of higher swimming speed (Johansson & Leonardsson, 1998), or reduced locomotion costs, resulting in relatively less elongate individuals. Observations on *P. bursaria* could therefore be partly related to the lower resource levels provided by Gibert *et al.*, (2017), whose high resource treatment was 40% that of our low resource level. Additionally, *P. bursaria* is a mixotrophic protist, and body shape elongation may also be favoured, in order to enhance zoochlorellae exposure to light (Naselli-Flores & Barone, 2011). Hence, the observed body shape response could be influenced by a space trade-off between bacterial and light acquisition (Gibert *et al.*, 2017).

In agreement with the temperature-size rule (Atkinson *et al.*, 2003; Forster *et al.*, 2012) and our hypothesis, body size of both species declines as temperature increases

(Figures 4.2a and b). Contrary to our hypothesis, body size decrease was not exacerbated by low resource level or interspecific competition. While low resource level also reduces *B. japonicum* body size, this effect is not mediated by temperature or interspecific competition. As interspecific competition leads to body-shape elongation (Figure 4.1b), selection for longer (SI 4, Figure S4.6) individuals with higher swimming velocities may be traded off against body-size decrease. Such a trade-off may limit the extent of body-size reductions under interspecific competition, which may therefore negatively influence *B. japonicum* population density at carrying capacity (Figure 4.2d).

Body size declined by 4.37% $°C^{-1}$ (95% CIs: 2.18%, 6.50%) in *P. aurelia* and 6.49% $°C^{-1}$ (95% CIs: 1.93%, 10.83%) in *B. japonicum*. Body size declines are similar in both species and are not significantly different from a size reduction of 1.7% $°C^{-1}$ (95% CIs: 1.1%, 2.4%) calculated across a range of protist species (Forster *et al.*, 2012). As heterotrophic organisms require essential resources such as food and oxygen, body size decline could result from greater temperature-dependence of resource demand than of resource supply. The supply of oxygen depends on the concentration and diffusion rate of dissolved oxygen, and is relatively insensitive to temperature (Woods, 1999; Atkinson *et al.*, 2003). In contrast, metabolic rate, which influences resource demands generally increases more strongly with temperature (Woods, 1999; Brown *et al.*, 2004). Hence, a dichotomy between oxygen supply and demand could arise at higher temperatures. Smaller body size could therefore be favoured to increase surface area for resource uptake in relation to mass, or to compensate for increased oxygen demands at higher temperatures (Atkinson *et al.*, 2003).

Body size is a major determinant of metabolic rate, with smaller individuals in general having lower absolute metabolic rates (Brown *et al.*, 2004; DeLong *et al.*, 2010). Applying an interspecific mass-scaling exponent of metabolic rate of 0.902 and an activation energy of 0.61 eV (DeLong *et al.*, 2010), we can estimate the metabolic demands of the protists in our various treatments. Estimated metabolic rates per individual were higher at 25 °C despite smaller body sizes in all treatments but one (*B. japonicum* in high resource monocultures, SI 4, Figure S4.7). This suggests that size reduction could have occurred to compensate in part for the increased metabolic demands at 25 °C. However, temperature-enhanced food demands could also strengthen food limitations, and could likewise play a role in influencing body size (DeLong, 2012; DeLong *et al.*, 2017). Higher metabolic rates and lower water viscosity at 25 °C also likely enhanced swimming speed (Beveridge *et al.*, 2010), which could improve resource capture, and the use of available energy for growth, potentially increasing carrying capacity (Gibert *et al.*, 2017). However, warming was not associated with detectable increases in population densities (Figures 4.3a and b), which suggests that improved resource capture is counteracted by increased energetic costs of growth (Barneche, Jahn & Seebacher, 2019), or the cost of maintenance at higher temperatures. Hence, higher *per capita* resource demand likely limit additional energetic allocation for growth and reproduction. We also found no indication that elongation, and hence higher swimming speed, increased population density (Figures 4.3c and d), unlike a previous study (Gibert *et al.*, 2017). In contrast, population density was negatively correlated with *P. aurelia* body elongation (Figure 4.3c). As *P. aurelia* was relatively more elongated at low resource level, this negative relationship could be due to low resource level rather than due to elongation alone.

Experimental warming did not alter competitive interactions, and both species were able to coexist across all treatment combinations. *P. aurelia* was a stronger competitor than *B. japonicum* at both temperature levels, as indicated by its higher population densities (Figures 4.2c and d) and higher population biovolume densities (SI 4, Figure S4.4) than *B. japonicum* in polycultures. This is in contrast to previous studies that observed competitive exclusion at higher temperatures (Jiang & Kulczycki, 2004;

Jiang & Morin, 2004; Englund *et al.*, 2011), which is likely due to temperature-enhanced metabolic demands that increased *per capita* competition (Jiang & Morin, 2004). As competition strength depends on physiological and resource acquisition traits (Jiang & Morin, 2004; Lewington-Pearce *et al.*, 2019), similar body size reductions at higher temperature could have maintained *per capita* competition strength (Sheridan & Bickford, 2011). This could underlie species coexistence at both temperature levels in this study. However, as environmental changes will likely alter resource level, understanding how body shape changes modulate body size response among different interacting species could be important for predicting the impact of warming on competitive protistan communities.

This study shows that resource competition influenced body shape, while body size is predominantly influenced by temperature. Body-shape responses of both species to resource level were similar, but this was not the case for competition (Figures 4.1 and 4.4). Our results suggest that changes in body shape may be indicative of the competitive ability of a species. Superior competitors that are more efficient in extracting resources should reduce their body elongation, mirroring body shape responses at high resource levels. In contrast, body size responses may be universally driven by the temperature-dependence of metabolic demands. Similarity in body size reductions across different competitors could maintain the constant strength of competitive interactions under warming. However, body shape responses such as relative elongation may influence or constrain body size response to environmental changes. It could therefore be valuable to investigate the applicability of these findings to protists and microzooplankton in general. Such knowledge will be crucial for assessing the importance of the relationship between body shape and body size, and body shape response to competition as a functional trait for predicting competitive hierarchies and the extent of temperature-size responses. To this end, a deeper understanding of how body shape and size change across environmental gradients could potentially improve the trait-based forecasting of warming impacts on natural ecosystems.

Chapter 5

Regional diversity provides limited insurance to montane-lake communities influenced by heatwaves

Abstract

Climate change is associated with increased frequency and magnitude of extreme weather events, such as heatwaves. Although short-term heatwaves can cause local species extinctions, habitat connectivity and dispersal from regional species pools are predicted to provide insurance against these negative effects and facilitate community recovery. Using a mesocosm experiment, we tested the impacts of heatwaves on the size structure of montane-lake communities, and whether the changes in organism abundance, biomass and body size are reduced by introduction of planktonic species from 15 regional lakes. While the experimental heatwave reduced the elevations of the size spectra, the introduction of zooplankton species from regional pool weakened this negative effect. The zooplankton introductions potentially increased ecosystem productivity, as indicated by a weaker decline in phytoplankton biomass. This positive effect of zooplankton introduction persisted after the heatwave, likely as a result of greater copepod recovery that can enhance nutrient regeneration. However, the size spectra steepened after the heatwave, likely due to decreases in the abundance of larger organisms, which were not influenced by regional diversity. The heatwave also decreased total community biomass, and plankton introduction reduced this negative effect. Total biomass of all experimental communities recovered after the heatwave, achieving similar biomass levels as initial conditions. This recovery was not influenced by plankton introduction, indicating that the ability to accumulate biomass is relatively robust to short-term thermal stress. These findings suggest that connectivity to the

regional biodiversity could partially mitigate some heatwaves impacts on the community size spectra.

Introduction

Climate change and the associated rise in mean global temperatures (IPCC, 2013) has significantly impacted natural ecosystems. Global warming has been shown to have shifted species distributions and phenology (Parmesan & Yohe, 2003), and reduced the body sizes of individuals and communities (Daufresne *et al.*, 2009; Gardner *et al.*, 2011; Morán *et al.*, 2015; Evans *et al.*, 2019). Climate change is also predicted to increase the frequency and magnitude of extreme temperature events such as heatwaves (Easterling *et al.*, 2000; IPCC, 2012). Heatwaves can cause physiological stress in organisms (Parmesan, Root & Willig, 2000), potentially resulting in stronger, and more immediate consequences to biota than increases in mean global temperatures (Thompson *et al.*, 2013; Vasseur *et al.*, 2014; Woodward *et al.*, 2016). For instance, heatwaves can push organisms quickly beyond their thermal optima (Vasseur *et al.*, 2014), preclude evolutionary adaptations and potentially cause local extinctions (Gutschick & BassiriRad, 2003). Heatwaves have been already implicated in the abrupt reduction of local species abundances (Martínez, Cárdenas & Pinto, 2003), shifts in community composition (Wernberg *et al.*, 2016; Filbee-Dexter & Wernberg, 2018), and could also potentially alter the size structure of ecological communities (Heneghan *et al.*, 2019).

Ecological communities are usually composed of many organisms with small body sizes and few organisms with large body sizes. This negative relationship between abundance and body size is described by a power function, $N \propto M^{b_{ss}}$ (Sheldon *et al.*, 1972; Peters, 1983; White *et al.*, 2007), where N is the abundance, M is body mass and b_{ss} denotes the abundance-body mass scaling exponent. This relationship is commonly referred to as the 'abundance-size spectrum' (Heneghan *et al.*, 2019), or the 'size spectrum' (Kerr & Dickie, 2001). As body size relates to a suite of biological rates including metabolism (Peters, 1983; Brown *et al.*, 2004), growth (Hirst & Forster, 2013; Kiørboe & Hirst, 2014), feeding rates and trophic interactions (Brose *et al.*, 2006a;

Hildrew *et al.*, 2007; Rall *et al.*, 2012), the abundance-body mass relationship provides an important link between individuals and the whole community. Changes in b_{ss} indicate both an altered energy flow through the food web (Jennings & Blanchard, 2004; Woodward *et al.*, 2005; White *et al.*, 2007) and shifts in the distribution of biomass across different food web compartments. Hence, there is a continued interest in understanding the factors that govern the value of b_{ss} (White *et al.*, 2007; Reuman *et al.*, 2008; Perkins *et al.*, 2018; Peralta-Maraver *et al.*, 2019).

Changes to b_{ss} values have been associated with climate warming (Yvon-Durocher *et al.*, 2011; Dossena *et al.*, 2012). In freshwater pelagic communities, experimental warming may steepen b_{ss} (Yvon-Durocher *et al.*, 2011), presumably because higher temperatures favour smaller organisms (Daufresne *et al.*, 2009; Sheridan & Bickford, 2011). More negative (steeper) b_{ss} values suggest that warming reduces the efficiency of energy transfer through the food webs (Jennings & Blanchard, 2004; Heneghan *et al.*, 2019), as more small organisms are needed to support fewer large consumers. This also indicates that warming disproportionately increases the allocation of resources to small species, likely because small, rapidly reproducing species are superior competitors for limiting resources and competitively exclude larger species (Litchman *et al.*, 2009). Such changes can result in shifts towards smaller prey organisms (Daufresne *et al.*, 2009), and could also favour smaller consumers with a grazing preference for such prey (Rice, Dam & Stewart, 2015). This can potentially alter top-down trophic cascades common in freshwater communities (Rossberg *et al.*, 2019). However, warming can also reduce b_{ss} values if nutrient supply increases with temperature, indicating interactive effects between nutrient supply and environmental warming (O'Gorman *et al.*, 2017). These findings highlight the complex impacts of the long-term increase in mean environmental temperatures (Yvon-Durocher *et al.*, 2011; Woodward *et al.*, 2016; O'Gorman *et al.*, 2017). Short-term heatwaves may cause similar changes to b_{ss} values as increases in long-term mean temperature, and could also be governed by rapid changes in community composition (Wernberg *et al.*, 2016; Filbee-Dexter & Wernberg, 2018). However, such predications have not been evaluated before. Heatwaves can increase metabolic rates of individuals (Gillooly, Brown & West, 2001), and higher biomass-specific respiration rates could reduce the amount of biomass that is supported per unit energy (Yvon-Durocher *et al.*, 2012). This could reduce the elevation (i_m) of the size spectrum, which is represented by the abundance at the geometric midpoint of the abundance-body size relationship, and reflects the carrying capacity (Shin & Cury, 2004), or productivity of the ecosystem (Daan *et al.*, 2005; Shin *et al.*, 2005; Guiet, Poggiale & Maury, 2016). However, as heatwave impacts are rarely studied, very little is known about the effects of transient thermal stress on the size spectra of ecological communities.

Changes in community composition can result from local extinctions and colonisation of individuals from the regional species pool (Leibold *et al.*, 2004). As colonisation depends on species dispersal from the regional species pool, connectivity to other habitats that improves species exchanges among habitats, can enhance local biodiversity, and influence local community compositions (Leibold *et al.*, 2004; Leibold & Chase, 2018). Moreover, connectivity to a diverse regional species pool that facilitates species dispersal may counteract local species loss during disturbance, and buffer community responses during environmental changes (Loreau *et al.*, 2003; Thompson & Shurin, 2012; Thompson, Rayfield & Gonzalez, 2017). Connectivity to a diverse regional species pool may therefore provide spatial insurance (Loreau *et al.*, 2003; Thompson *et al.*, 2017) by introducing stress-tolerant species that could compensate for species that went locally extinct and buffer community responses to abiotic stress. Hence, regional biodiversity and dispersal-mediated supply of species could reduce the impacts of heatwaves on local community biomass, community size structure and energy transfers. Such potential buffering effects of regional biodiversity has been described as the spatial insurance hypothesis (Loreau *et al.*, 2003).

Increased frequency and severity of heatwaves under the future climate (Coffel, Horton & Sherbinin, 2018; Frölicher, Fischer & Gruber, 2018; Stillman, 2019) could decrease habitat connectivity due to increased water loss that reduces habitat availability (Bond, Lake & Arthington, 2008). This future scenario calls for a better understanding of how colonisation from regional species pools may buffer ecological communities against heatwaves. However, the combined effects of heatwave and connectivity to a diverse regional species pool on the community size structure have not been investigated before. We address this critical knowledge gap by investigating the effects of experimental heatwave on the size structure of montane plankton communities in outdoor mesocosms. We tested the specific hypotheses that (1) the heatwave would both reduce the elevation (i_m) of the community size spectrum, and (2) steepen the slope of the community size spectrum (b_{ss}) due to reduction in abundance of large individuals and an increase in abundance of small individuals; (3) the heatwave would also reduce total community biomass, as higher mass-specific respiration would reduce the amount of biomass that can be supported; and (4) changes in i_m, b_{ss} and community biomass during the heatwave would be buffered by connectivity to the regional species pool, reducing the impact of environmental stress.

Materials and methods

Experimental design

We tested these hypotheses in an outdoor mesocosm experiment between June and August 2018 at WasserCluster Lunz (WCL) Biological Station, Lunz am See, Austria. We established 40 land-based mesocosms (height: 810 mm, inner diameter: 770 mm, Figure 5.1) from modified food safe PE containers (ARICON Kunstoffwerk, GmbH, Solingen, Germany). We insulated the mesocosms with mineral wool to reduce the thermal impact of rapid changes in air temperature to achieve smaller temperature fluctuations, similar to natural temperature variation in larger lakes. We covered the mesocosms with 250 µm-mesh net lids to minimise natural dispersal of invertebrates that could also introduce microbes into the mesocosms. The net lids allowed air exchange, and only minimally shaded the mesocosms. We filled mesocosms with 300 L of water from the nearby, oligotrophic, montane, Lake Lunz (N 47°85’ E 15°05’, 608 m.a.s.l) using a centrifugal pump. The water was filtered through a coarse sieve (500 µm) to exclude dirt and fish larvae from the mesocosm. Cladocerans were absent in the mesocosms, likely because the pumping negatively affected mesozooplankton. We therefore introduced *Daphnia longispina* to achieve cladoceran densities of 3 ind L^{-1} in the mesocosms, which is the mean summer density of *Daphnia* in the regional montane lakes (Horváth *et al.*, 2017). Additionally, we allowed 12 days for local species sorting and community establishment before the application of experimental treatments. We washed and cleaned the removable inner wall and bottom plates of the mesocosms every two weeks to minimise the growth of benthic algae and their impact upon the planktonic system.

Figure 5.1. The experiment was performed in outdoor experimental mesocosms at the WasserCluster Lunz (WCL) Biological Station, Lunz am See, Austria.

To investigate the combined impacts of heatwave and connectivity on the community size structure, we manipulated connectivity as two factors and heatwave as a single factor. Specifically, we simulated dispersal from the regional species pool as microbial dispersal and zooplankton dispersal. We crossed microbial dispersal (M- or M+), zooplankton dispersal (Z- or Z+) and heat treatments (H- or H+) in a full-factorial design with 5 replicates for each treatment combination. This resulted in a total of eight treatment combinations and 40 mesocosms, including a non-dispersal control treatment. We collected the dispersal microorganisms and zooplankton from 15 regional lakes (SI 5, Table S5.1) that included several lowland habitats with known heat-tolerant plankton species. To obtain the microbial inoculum, we screened this pooled community with a 30 µm-mesh to remove the large organisms. The zooplankton inoculum was obtained by screening the pooled community with a 100-μm sampling net and collecting organisms retained in the net. For microbial dispersal, we introduced 3 L of pooled microbial inoculum to the M+Z- and M+Z+ mesocosms, which represented 1% of total mesocosm volume. We also introduced 3 cladoceran species (106 individuals) and 4 copepod species (412 individuals) pooled from the regional lakes into M-Z+ and M+Z+ mesocosms (see SI 5, Table S5.2 for species list). Zooplankton inoculum density was standardised to 20 L, which was the volume sampled for the microbial inoculum. We then applied the heatwave treatment (H-/H+) three days after the simulated dispersal to a total of 20 mesocosms. We established the experimental heatwave using submersible aquarium heaters (Kratina *et al.* 2012) and maintained these elevated temperatures for 14 days. Four days of heating was required to achieve an offset of +5 °C in the heated mesocosms compared to the ambient temperature treatment. The experimental heatwave was then achieved by maintaining this +5 °C offset for seven days. Following the heatwave, we removed the submersible aquarium heaters, and temperature returned to ambient temperature levels (in unheated mesocosms) after three days. Mean water temperature was 25.9 °C (± 0.03 s.e., $n = 20$) in the heated treatments during the heatwave. Due to unseasonably cold weather that coincided with the period of the experimental heatwave, we also increased water temperatures in the unheated mesocosms to 21.0 °C (± 0.08 s.e., $n = 20$) with submersible aquarium heaters to maintain the 5°C temperature difference between the temperature treatments. To prevent vertical temperature gradients, an airlift was used to constantly mix the water column with a compressor connected to a PVC tube hanging in the middle of the water column.

While mesocosms represent simplified freshwater ecosystems, they support realistic levels of biocomplexity, and provide a compromise between the complexity of the natural ecosystems and the control of laboratory studies (Kratina *et al.*, 2012;

Stewart *et al.*, 2013). As mesocosm experiments allow the isolation of temperature and dispersal effects on replicated communities, it remains a powerful approach for investigating community response to climate change despite its limitations (Stewart *et al.*, 2013).

Sampling and sample processing

We sampled all mesocosms three times over the experimental duration: (1) two days after the introduction of the regional inoculum, but before the heatwave was applied (t_0), (2) at the end of the experimental heatwave when the heaters were turned off (t_h), and (3) 16 days after the heatwave (t_{ah}), which was 27 days after t_0. To avoid unintentional dispersal, we collected water samples through taps (inner diameter: 100 mm) fitted on the side of the mesocosms. We increased airflow prior to each sampling event to ensure the plankton were well mixed. We collected zooplankton samples by filtering 20 L of water sample through a 50-µm-mesh and preserved the organisms collected on the mesh in absolute ethanol. We collected phytoplankton samples from 3 L of water samples and preserved them with Lugol's iodine solution (Sherr & Sherr, 1993). We replaced the sampled volume with sterile-filtered (0.2 µm), chlorine-free tap water, and added phosphorus to maintain a final concentration of approximately 15 µg L^{-1} P in each mesocosm.

We determined the body size and densities of phytoplankton and crustacean zooplankton species and taxa to construct the community size spectra. On average, there were 21 phytoplankton taxa, and four dominant zooplankton taxa (*Daphnia*, *Bosmina*, Cyclopoida and Calanoida) per mesocosm (Dataset S6.5). We counted phytoplankton with a Leica DMI3000 B inverted microscope following Utermöhl (1958). At least 400 sedimentation units (e.g. filaments, colonies, or single cells) were counted, following Lund *et al.* (1958). We estimated biovolume and wet weight from characteristic geometrical shapes of individual taxon (Hillebrand *et al.*, 1999) using the mean cell size of at least 30 individuals for the dominant taxa. We converted phytoplankton wet mass to carbon mass applying a conversion factor of 0.14 (Rocha & Duncan, 1985; Vadstein *et al.*, 1988). To obtain zooplankton body size, we measured the length of the first 20 individuals of each of the dominant species encountered in our samples with a Zeiss Stemi 2000-C dissecting microscope. For cladocerans we measured the length from the top of the head to the base of the caudal spine. For copepods we measured the tip of the cephalothorax to the base of the furcas (Bottrell *et al.*, 1976). We subsequently converted length measurements to dry mass following the length-weight relationships reported in McCauley (1984). For zooplankton species that were present at low densities, we used mean body size measurements obtained from replicates of the treatment combination where they reached higher densities. When a species occurred only at low densities in a particular treatment combination, mean body size was obtained from all the individuals measured. For copepod nauplii we used published mean body length of 194.54 μm, based on the measured geometric mean length of *C. abyssorum* nauplii (Ludovisi *et al.*, 2008). We converted zooplankton dry mass to carbon mass applying a conversion factor of 0.4 (Reiss & Schmid-Araya, 2008). Due to time constraints, these measurements were limited to three replicates for each treatment combination. Consequently, two replicates were removed from each experimental treatment combination, and 24 replicates were included in the analyses.

Statistical analysis

We constructed the size spectra for the entire phytoplankton and crustacean zooplankton community by dividing the total range of mean species carbon mass (μgC) into 10 logarithmic bins of equal widths. We then regressed the $\log_{10}$ (abundance L^{-1} + 1) of each bin against the body sizes at the geometric mid-point of each size bins (Reuman *et* *al.*, 2008; Yvon-Durocher *et al.*, 2011). Using ordinary least squares regression, we obtained the intercept and slope (b_{ss}) of the community size spectra (Dataset S6.6). We calculated i_m as the abundance at the geometric midpoint of the size spectrum (Heneghan *et al.*, 2019), to avoid correlation between the slope and the intercept (Daan *et al.*, 2005). Community size spectra are predominantly determined by the biomass of different-sized species, rather than the variations in size structure within species (Gaedke, 1992; Shephard *et al.*, 2012). Hence, differences in species size should approximate size structure, and represent a reasonable approach for understanding consequences of interspecific size variation on the community size structure.

To determine the impacts of heatwave and connectivity on the community size spectrum, we first obtained changes in the elevation (i_m) and slope (b_{ss}) of the community size spectrum (Δi_m or $\Delta\ b_{ss}$) in each mesocosm during the heatwave (t_h) or after the heatwave (t_{ah}) relative to that before the heatwave (t_0). We used changes in the response variables over the experimental duration rather than absolute values to compensate for initial differences between replicates. Negative Δi_m or Δb_{ss} values therefore indicate that these variables decreased during or after the heatwave relative to time t_0. As changes in i_m can also be influenced by differences in the geometric mid-point mass of the size spectra, we repeated the analysis by comparing the elevation at a fixed mass, determined as the mean geometric midpoint mass of all size distributions (SI 5, Figure S5.1). To determine the effects of heatwave on the abundance of small and large individuals, defined as abundance in the smallest and largest size bin respectively, we calculated changes in the abundance (Δabundance) of individuals at time t_h relative to t_0, and at time t_{ah} relative to t_0. We also calculated changes in community biomass (Δbiomass) between these time points. Negative Δabundance and Δbiomass values therefore indicate decreases in abundance of small or large individuals, and decreases in community biomass at time t_h or t_{ah}, respectively. We used a factorial ANOVA with

Δi_m, Δb_{ss}, Δabundance and Δbiomass as the response variables, and heatwave treatment, microbial dispersal, zooplankton dispersal, and their interactions as the independent variables to test for treatment effects. We carried out all statistical analyses in R 3.6.0 (R Core Team, 2019).

Results

The size spectra covered a body mass range of 6.5 orders of magnitude and ranged from 10^{-6} to 10^{0} µgC. In general, the community size spectra were well characterised by a linear model (mean $r^2 = 0.70 \pm 0.01$ s.e., Figure 5.2). The elevation of the size spectra (i_m) ranged from 53 to 1042 ind L^{-1} (mean = 207 ind L^{-1}) across the sampling time points. Mean i_m (113 ind L^{-1}) during the heatwave (t_h) was lower than mean i_m (175 ind L^{-1}) before the heatwave (t_0), a decrease to 65% ($\Delta i_m = -0.189$) of the midpoint abundance before the heatwave. As we hypothesized, the heatwave decreased i_m, but the decrease was reduced by zooplankton dispersal (H × Z, $p = 0.046$, Table 5.1, Figure 5.3a). However, this difference is not significant when the midpoint mass was standardised to the mean midpoint mass of all size distributions (SI 5, Figure S5.1a). The interactive heatwave and zooplankton dispersal effect persisted after the heatwave (H × Z, $p = 0.018$, Table 5.1, Figure 5.3b), and was also significant based on the elevation obtained at the mean geometric midpoint mass of all size spectra (SI 5, Figure S5.1b). While i_m increased in general after the heatwave ($i_m = 446$ ind L^{-1}), and mean midpoint abundance increased 2.5 fold ($\Delta i_m = 0.404 \pm 0.052$ s.e.), this increase was enhanced by zooplankton dispersal in the heated mesocosms ($\Delta i_m = 0.503 \pm 0127$ s.e. with zooplankton dispersal; $\Delta i_m = 0.243 \pm 0.062$ s.e. without zooplankton dispersal, Figure 5.3b).

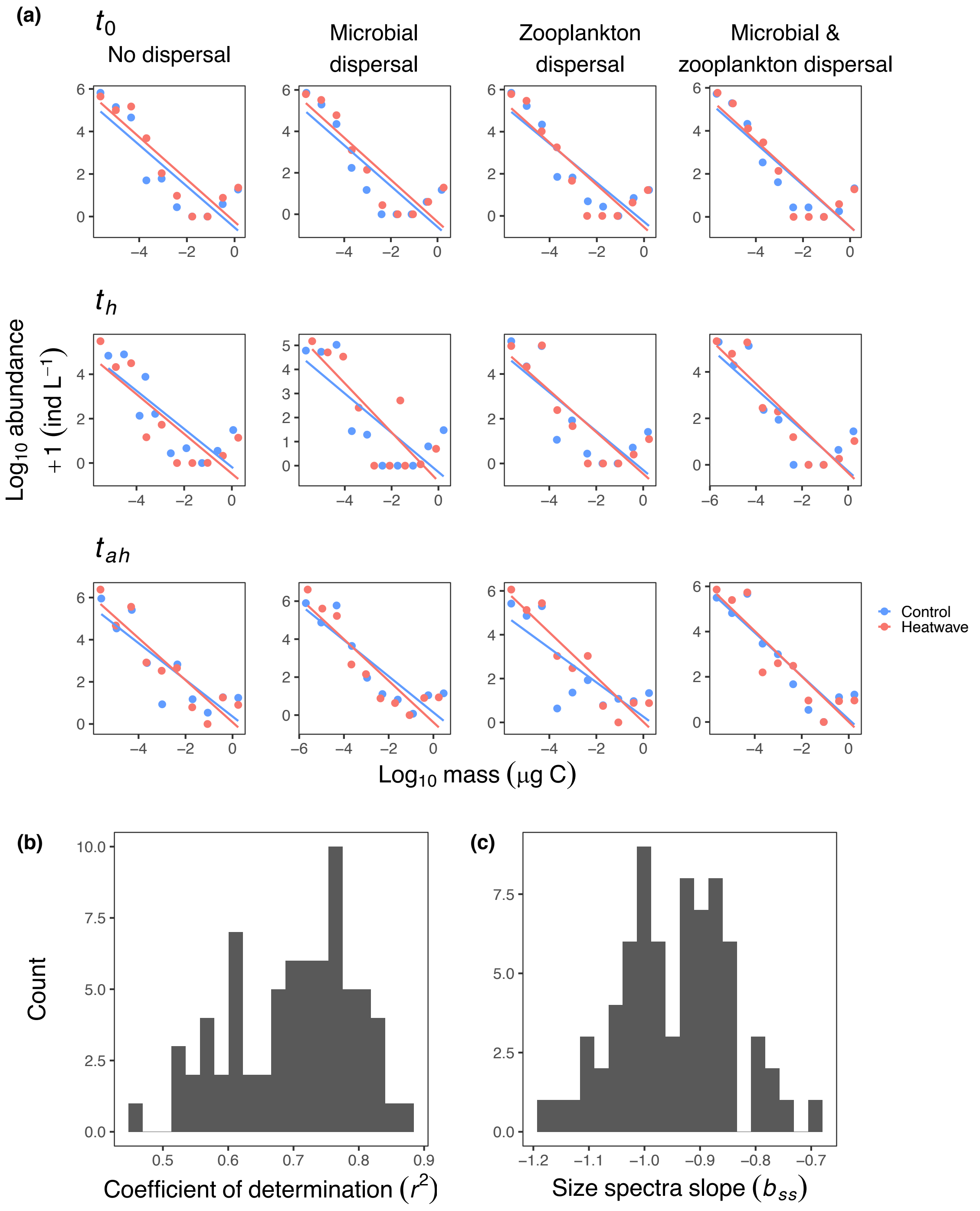


Figure 5.2. (a) The size spectra across the three sampling time points and dispersal treatments based on the mean abundance and geometric median mass of the size bins obtained from the three replicates of each treatment combination. **(b)** The distribution of the coefficient of determination (r^2) for the OLS regression between abundance in each size bin against the geometric median mass of the size class. **(c)** The distribution of the slopes of the community size spectra (b_{ss}).

Table 5.1. Summary statistics from the analysis of variance (ANOVA) testing the independent and interactive effects of heatwave, microbial dispersal and zooplankton dispersal on changes in the midpoint abundance (Δi_m) and slope of the community size spectra (Δb_{ss}) during the heatwave (t_h), and after the heatwave (t_{ah}) in relation to before the heatwave (t_0).

	heatwave (t_h) - before heatwave (t_0)			**after heatwave (t_{ah}) - before heatwave (t_0)**		
Response / treatment	**d.f.**	***F***	***p***	**d.f.**	***F***	***p***
$\Delta \boldsymbol{i_m}$						
Heatwave	1,16	3.06	0.099	1,16	0.44	0.518
Microbial dispersal	1,16	1.12	0.306	1,16	0.23	0.637
Zooplankton dispersal	1,16	2.64	0.124	1,16	0.02	0.885
H × M	1,16	1.16	0.297	1,16	2.58	0.128
H × Z	1,16	4.68	0.046	1,16	6.91	0.018
M × Z	1,16	0.02	0.880	1,16	0.73	0.405
H × M × Z	1,16	0.48	0.499	1,16	0.93	0.348
$\Delta \boldsymbol{b_{ss}}$						
Heatwave	1,16	0.42	0.525	1,16	4.43	0.052
Microbial dispersal	1,16	0.01	0.940	1,16	2.08	0.168
Zooplankton dispersal	1,16	0.96	0.343	1,16	0.18	0.674
H × M	1,16	0.31	0.586	1,16	0.52	0.481
H × Z	1,16	0.14	0.712	1,16	0.04	0.841
M × Z	1,16	0.02	0.881	1,16	0.01	0.930
H × M × Z	1,16	<0.00	0.989	1,16	0.71	0.411

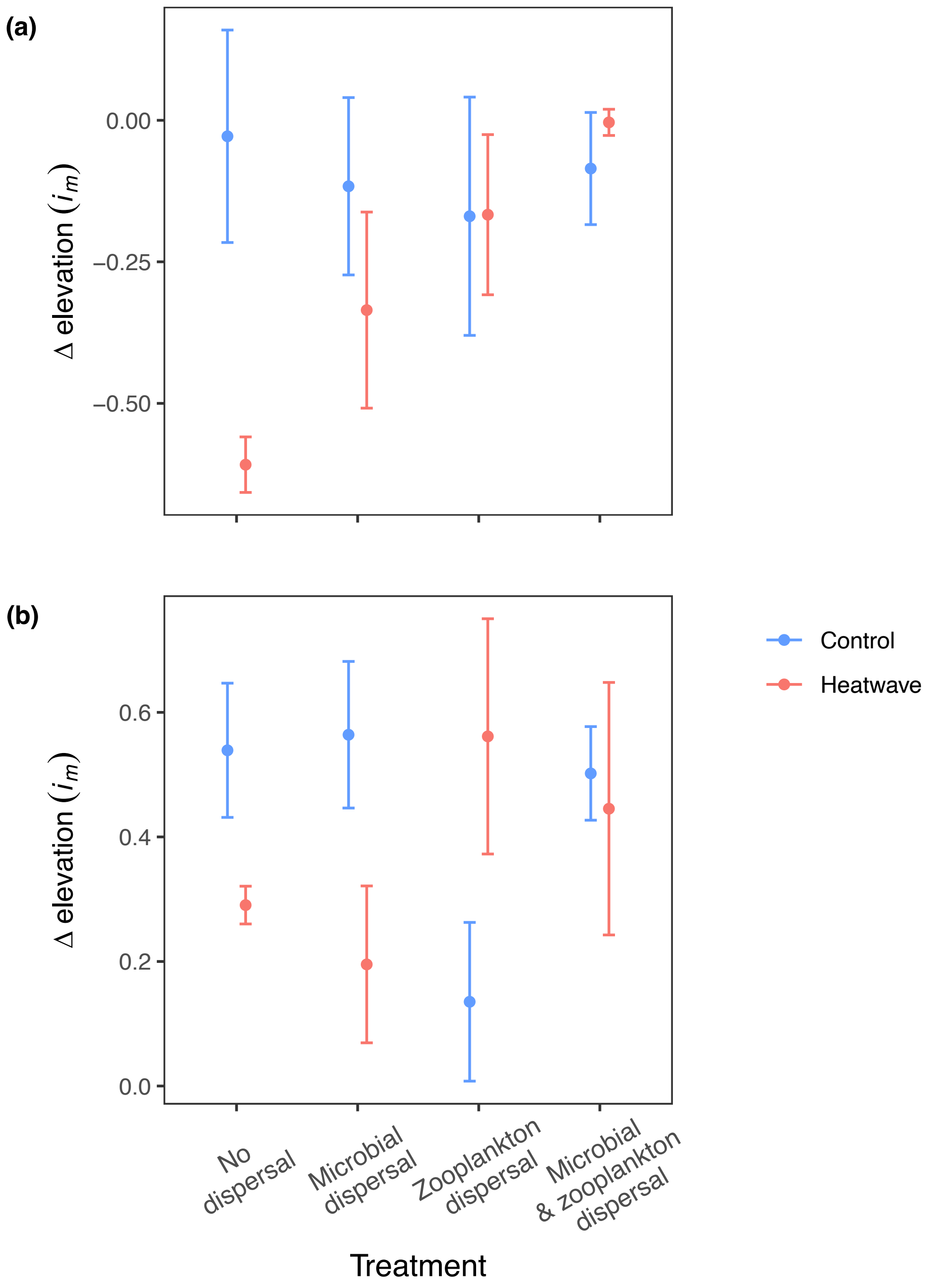


Figure 5.3. (a) The heatwave and zooplankton dispersal interactively influenced changes in the elevation of the size spectra (Δi_m) during the heatwave, although this effect was not significant when the mean midpoint mass of all size spectra was used (SI 5, Figure S5.1a). **(b)** The heatwave and zooplankton dispersal also interactively influenced Δi_m after the heatwave, and zooplankton dispersal increased i_m, as indicated by higher positive Δi_m values in the heated mesocosms. This effect was also significant when the mean geometric midpoint mass of all size spectra was used to obtain the

elevation (SI 5, Figure S5.1b). The points represent the mean Δi_m of each treatment combination, and the error bars indicate the standard error. All Δi_m during and after the heatwave are expressed relative to initial conditions.

The slope of the community size spectra (b_{ss}) ranged from -1.185 to -0.700 (mean = -0.946 ± 0.012, Figure 5.2c). In general, the b_{ss} value was higher (i.e. a shallower slope) at time t_h (-0.892 ± 0.018) than at time t_0 (-0.984 ± 0.015), and changes in b_{ss} (Δb_{ss}) between time t_h and t_0 were generally positive ($\Delta b_{ss} > 0$, Figure 5.4a). In contrast to our hypothesis, the heatwave did not steepen b_{ss}, which would have been indicated by a negative heatwave influence on Δb_{ss} ($p = 0.525$, Table 5.1, Figure 5.4a). However, after the heatwave, mean b_{ss} of the heated mesocosms (-1.030 ± 0.028) was steeper than in the unheated mesocosms (-0.891 ± 0.028). Relative to t_0, Δb_{ss} value in heated mesocosms ($\Delta b_{ss} = -0.026 \pm 0.037$ s.e.) was negative, and lower than that in unheated mesocosms (0.072 ± 0.024 s.e.), which suggests that b_{ss} steepened after the heatwave. However, this difference was marginally non-significant ($p = 0.052$, Table 5.1, Figure 5.4b).

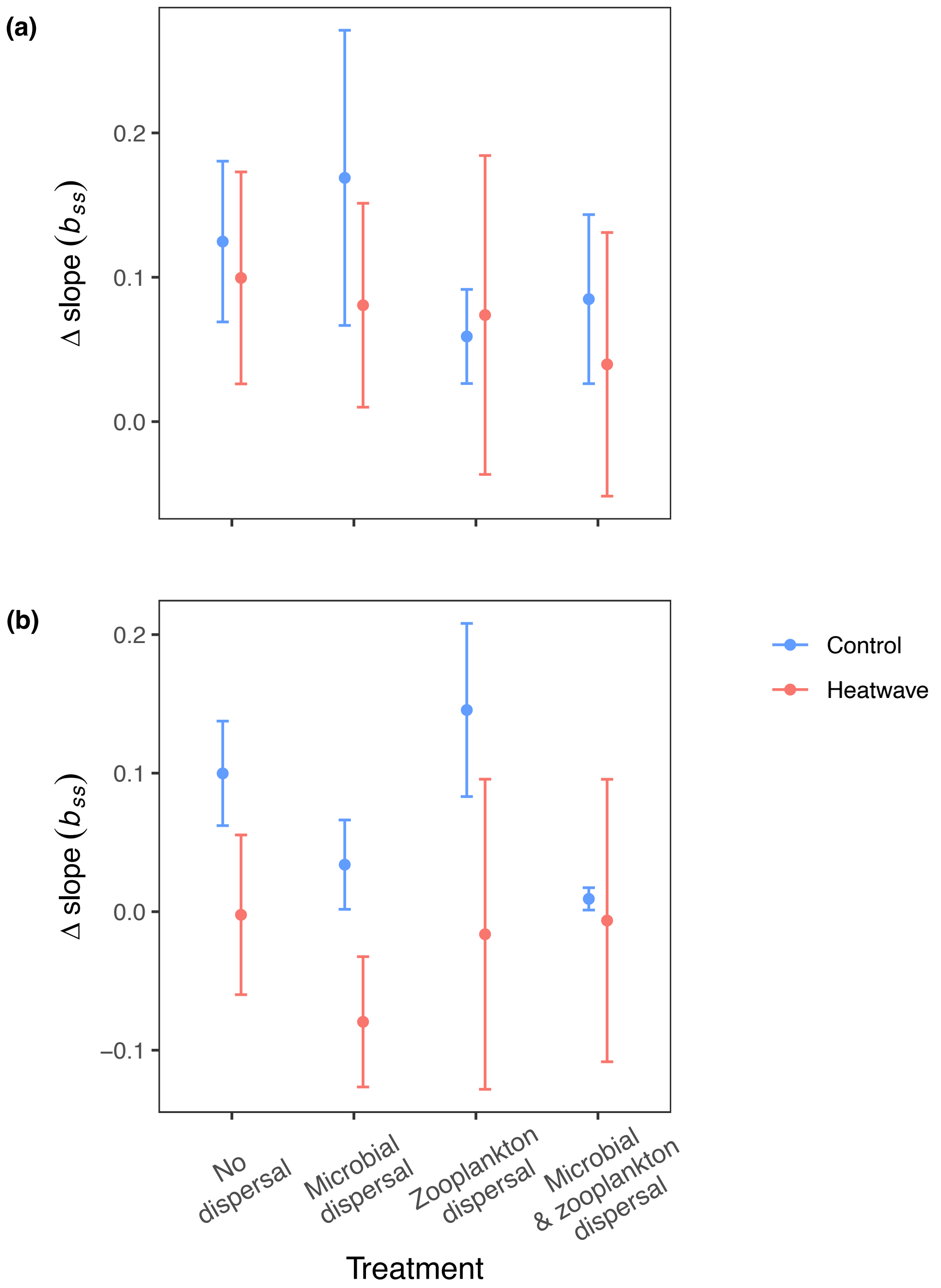


Figure 5.4. (a) The heatwave did not influence changes to the slopes of the community size spectra (Δb_{ss}) during the heatwave. **(b)** However, more negative Δb_{ss} values in heated than unheated mesocosms after the heatwave indicate that the heatwave steepened the slopes of the community size spectra. In contrast, b_{ss} values tend to be positive in unheated mesocosms after the heatwave, which indicates shallower community size spectra slopes. All Δb_{ss} during and after the heatwave are expressed relative to initial conditions.

In contrast to our hypothesis, the heatwave did not influence changes in the abundance (Δabundance) of small individuals at time t_h relative to t_0 ($p = 0.494$, Table 5.2, Figure 5.5a), or after the heatwave relative to t_0 ($p = 0.140$, Table 5.1, Figure 5.5b). However, the heatwave resulted in a decrease in the abundance of large individuals, both during ($p < 0.001$, Table 5.2, Figure 5.5c) and after the heatwave ($p = 0.001$, Table 5.2, Figure 5.4d), as we hypothesized.

Table 5.2. Summary statistics from the analysis of variance (ANOVA) for the independent effects of heatwave, microbial dispersal and zooplankton dispersal on changes in the abundance of small and large organisms, and biomass during the heatwave (t_h), and after the heatwave (t_{ah}) in relation to before the heatwave (t_0).

	heatwave (t_h) - before heatwave (t_0)			**after heatwave (t_{ah}) - before heatwave (t_0)**		
Response / treatment	**d.f.**	***F***	***p***	**d.f.**	***F***	***p***
Δabundance (small organisms)						
Heat	1,16	0.49	0.494	1,16	2.41	0.140
Microbial dispersal	1,16	0.32	0.582	1,16	0.46	0.509
Zooplankton dispersal	1,16	0.16	0.697	1,16	2.12	0.165
H × M	1,16	0.21	0.655	1,16	1.56	0.230
H × Z	1,16	0.33	0.571	1,16	0.45	0.511
M × Z	1,16	0.43	0.523	1,16	0.13	0.728
H × M × Z	1,16	<0.00	0.952	1,16	1.17	0.296
Δabundance (large organisms)						
Heat	1,16	41.01	<0.001	1,16	16.7	0.001
Microbial dispersal	1,16	0.64	0.436	1,16	0.47	0.503
Zooplankton dispersal	1,16	0.01	0.943	1,16	0.63	0.440
H × M	1,16	0.61	0.447	1,16	1.64	0.218
H × Z	1,16	3.68	0.073	1,16	0.22	0.645
M × Z	1,16	0.18	0.677	1,16	1.31	0.270
H × M × Z	1,16	0.25	0.624	1,16	0.28	0.605
Δbiomass						
Heat	1,16	19.43	<0.001	1,16	0.25	0.624
Microbial dispersal	1,16	0.13	0.724	1,16	0.64	0.435
Zooplankton dispersal	1,16	0.46	0.507	1,16	1.75	0.205
H × M	1,16	0.24	0.628	1,16	0.88	0.362
H × Z	1,16	3.22	0.092	1,16	1.91	0.186
M × Z	1,16	5.71	0.029	1,16	0.89	0.361
H × M × Z	1,16	0.13	0.720	1,16	0.01	0.913

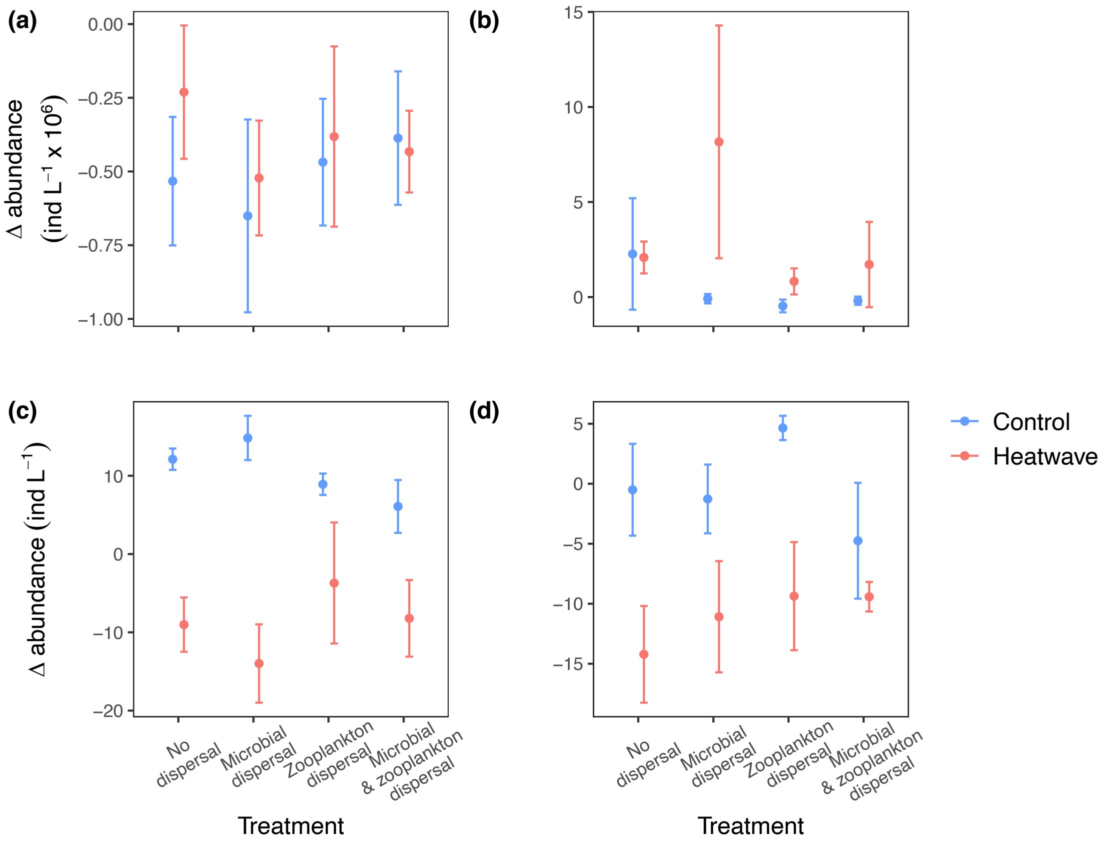


Figure 5.5. Experimental treatments did not influence changes in the abundance (Δabundance) of small individuals **(a)** during the heatwave or **(b)** after the heatwave. However, the heatwave significantly increased the degree to which the abundance of large individuals decrease both **(c)** during the heatwave, and **(d)** after the heatwave, as indicated by negative Δabundance values in the heated mesocosms. All Δabundance during and after the heatwave are expressed relative to initial conditions.

In agreement with our hypothesis, the heatwave also significantly reduced community biomass, as assessed by greater decreases in biomass (Δbiomass) between time t_h and t_0 ($p < 0.001$, Figure 5.6a) in heated mesocosms. Between time t_0 and t_h, the Δbiomass value was negative in heated mesocosms (Δbiomass = -22.238 μgC L^{-1} ± 5.151 s.e.), but positive in unheated mesocosms (Δbiomass = 3.481 μgC L^{-1} ± 3.682 s.e.). Microbial and zooplankton dispersal also interactively influenced Δbiomass (M × Z, $p = 0.029$, Table 5.2, Figure 5.6a). Microbial or zooplankton dispersal weakened the degree to which biomass decreased in heated mesocosms, although the buffering effect was lower when both microbial and zooplankton dispersal occurred. Biomass increased after the heatwave relative to t_0 (Figure 5.6b), and biomass changes (Δbiomass = 12.338 μgC L^{-1} ± 5.565) were not affected by the heatwave, microbial dispersal or zooplankton dispersal (Table 5.2).

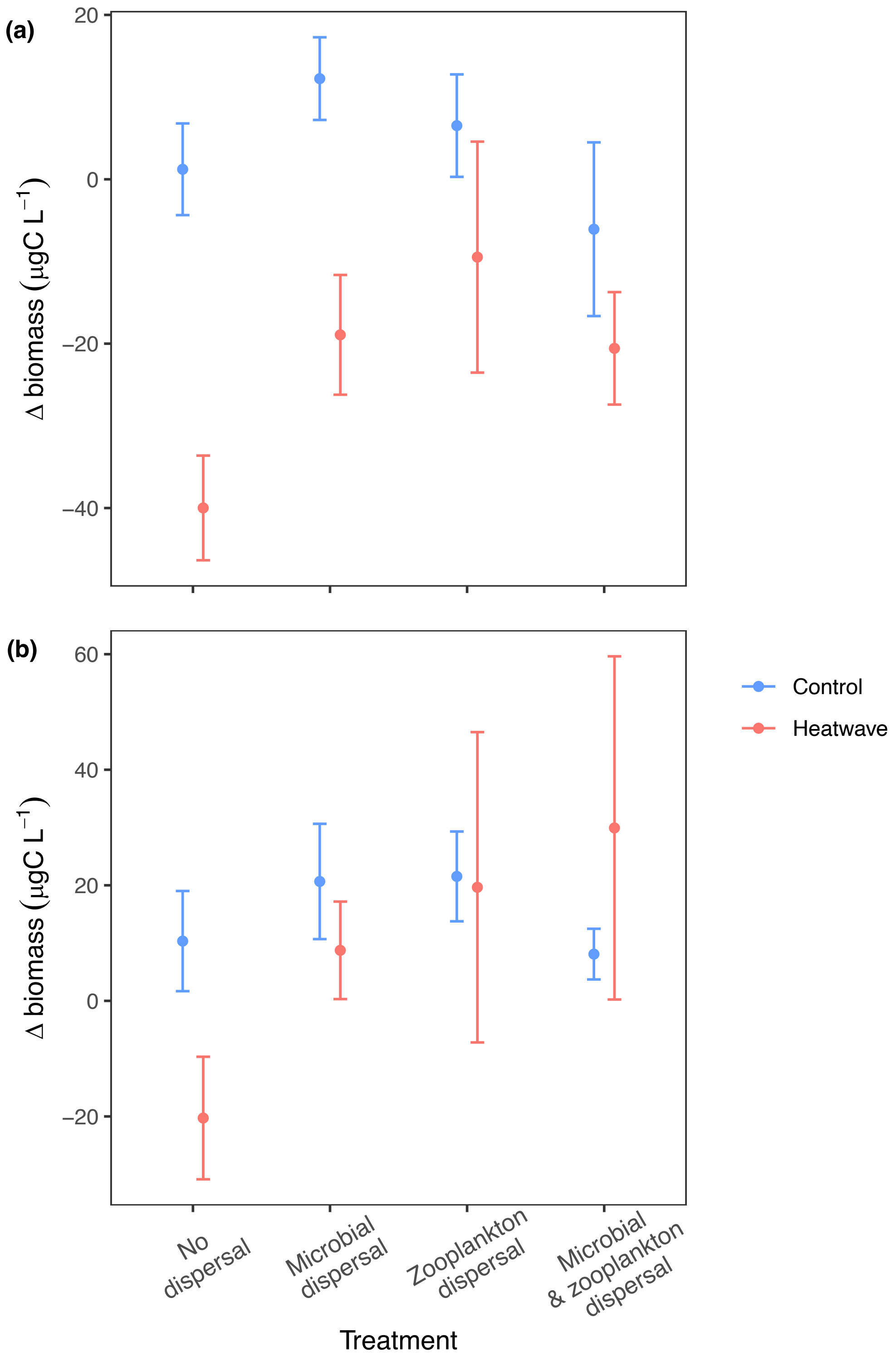


Figure 5.6. The effects of experimental treatments on changes in total community biomass (Δbiomass) during and after the heatwave expressed as differences relative to initial conditions. **(a)** The heatwave increased the degree to which biomass decreases in the heated mesocosms during the heatwave, resulting in more negative Δbiomass values. Microbial and zooplankton dispersal interactively influenced Δbiomass, and

reduced the degree to which biomass decrease during the heatwave, as indicated by lower Δbiomass in mesocosms where dispersal occurred. However, dispersal effects were not additive, and simultaneous microbial and zooplankton dispersal did not further lower Δbiomass. **(b)** Biomass recovered after the heatwave, and was not influenced by heatwave or species dispersal, as indicated by Δbiomass values that are either positive or close to 0, reflecting similar community biomass as initial conditions.

Discussion

Extreme, transient weather events, such as heatwaves, are forecasted to increase in frequency and intensity under future climate change (IPCC, 2012; Oliver *et al.*, 2018; Stillman, 2019), and understanding how communities respond to such events is critical for predicting climate change impacts. Our experimental findings suggest that zooplankton dispersal modulated the effects of heatwave on the elevation (i_m) of the community size spectra. In contrast, changes to the slope of the size spectra (b_{ss}) observed after the heatwave were not affected by dispersal, and indicate that changes in community size structure occurred with a time-lag. Community biomass decreased during the heatwave, and this biomass reduction was dampened by microbial and zooplankton dispersal in heated mesocosms. However, community biomass recovered after the heatwave in all treatment combinations, indicating that total biomass of mountain lake communities could be relatively robust to short-term heatwave events. These findings show that connectivity to the regional species pool may reduce the negative impacts of extreme weather events.

Community size structure

Zooplankton dispersal increased the degree of elevation of the size spectra (i_m) of experimental communities after the heatwave (Figure 5.3b, SI 5, Figure S5.1b), as indicated by increased Δi_m values. This suggests that zooplankton dispersal reduced the impact of heatwave on the elevation of the size spectra as hypothesized (Heneghan *et al.*, 2019). As comparisons of i_m depends on the midpoint mass, the significant results obtained in both analyses suggest that changes to i_m caused by the experimental treatments are robust. Hence, higher connectivity to the regional zooplankton species pool could mitigate the changes to carrying capacity or productivity (Shin & Cury, 2004; Daan *et al.*, 2005; Guiet *et al.*, 2016) after heatwaves.

The buffering effects of zooplankton dispersal could arise because it enhanced the recovery of copepod abundance, but not cladoceran abundance after the heatwave, as indicated by Δabundance ≥ 0 (SI 5, Figure S5.2). Recovery of copepod abundance, but not cladoceran abundance could indirectly increase phytoplankton production, which could have enhanced the elevations of the size spectra. This indirect effect could occur if zooplankton communities improved nutrient regeneration. As cladocerans commonly sequester more limiting nutrients than copepods (Sommer & Sommer, 2006), greater recovery of copepod abundance could increase available nutrients for compensatory growth of phytoplankton (Sommer & Sommer, 2006). Zooplankton communities less dominated by cladocerans could therefore increase phytoplankton biomass and productivity (Bergquist & Carpenter, 1986). Furthermore, copepods alleviate grazing pressure of small phytoplankton by predation of protozoa consumers (Burns & Schallenberg, 1996; Tiselius *et al.*, 2016), in contrast to cladocerans that do not alleviate grazing pressure of phytoplankton by other planktonic grazers (Sommer & Sommer, 2006). Hence, an increased abundance of copepods could also reduce overall grazing pressure on phytoplankton communities. Lower grazing pressure, and increased nutrient regeneration could therefore increase phytoplankton productivity, or the carrying capacity in these communities, increasing the elevation of the community size spectra. This ‘rescue’ effect highlights that copepod recovery can be hindered in isolated ecosystems, and indicates that coloniser identity influences buffering effects.

While the slope of the community size spectra (Δb_{ss}) did not change during the heatwave itself (Figure 5.4a), the slopes were steeper after the heatwaves in heated (b_{ss} = -1.030) versus unheated (b_{ss} = -0.891) mesocosms. This trend suggests that the heatwaves altered the community size structure, although such changes occurred with a time-lag of 16 days. Steeper size spectra after the heatwaves were caused by reduced abundance of large individuals in heated mesocosms (Figures 5.5a, b), while the abundances of small individuals were unaffected (Figure 5.5c, d). This also suggests lower trophic efficiencies (O’Gorman *et al.*, 2012; Heneghan *et al.*, 2019) after the heatwaves, as a similar number of small individuals are needed to support fewer large consumers.

Steeper slope of the size spectra was also observed under long-term experimental warming, but this was caused by an increased relative abundance of small organisms (Yvon-Durocher *et al.*, 2011). Hence, while both long-term warming and a transient heatwave steepened the slope of the community size spectra, the same patterns appear to be driven by different underlying mechanisms. The stronger effect of heatwaves on large organisms suggests that they may be more sensitive to short-term thermal stress than smaller organisms. This could occur because food limitations for zooplankton grazers tend to be more severe at high temperatures (Moore, Folt & Stemberger, 1996), likely because temperature increases metabolic demands (Gillooly *et al.*, 2001) and grazing rates (Peter & Sommer, 2012). Furthermore, rapid temperature increases could also push large species beyond their thermal optimal (Vasseur *et al.*, 2014), and constrain their capacity to meet respiratory demands (Pörtner, Bock & Mark, 2017). Within species, short-term warming could also selectively reduce the abundance of large individuals, if smaller individuals are more heat-tolerant (Craddock, 1976; Peck *et al.*, 2009). Increased mortality early in ontogeny due to generally less thermal resistant juvenile stages (Pandori & Sorte, 2019) could also limit the recruitment of large individuals post-heatwave. Hence, increased food limitation and increased zooplankton mortality likely underlie steeper community size spectra under the influence of short-term heatwaves.

Community biomass

As we hypothesized, the heatwave significantly reduced the total community biomass (Figure 5.6a). However, microbial and zooplankton dispersal affected community biomass changes during the heatwave. Both microbial and zooplankton dispersal weakened the degree to which the biomass decreased in heated mesocosms, but this buffering effect was not additive (Figure 5.6a). Zooplankton dispersal, but not microbial dispersal, positively influenced phytoplankton biomass (SI 5, Figure S5.3a), and weakened the reduction in community biomass during the heatwave. The effect of zooplankton dispersal on phytoplankton biomass suggests that zooplankton dispersal could have a greater buffering effect on total community biomass, and could therefore mask effects of accompanying microbial dispersal. Positive zooplankton dispersal effects may be due to improved nutrient regeneration, which could increase phytoplankton biomass and productivity (Bergquist & Carpenter, 1986). However, zooplankton dispersal did not buffer changes in zooplankton biomass during or after the heatwave (SI 5, Figure S5.3c and d), likely because a 16-day period, which corresponds to a single generation in cladocerans (Bottrell, 1975), may not be sufficient to detect differences in zooplankton biomass recovery. Community biomass recovered to levels similar to initial condition 16 days after the heatwave, and was unaffected by connectivity (Figure 5.6b), which suggests that biomass recovery in our montane lake is relatively robust to short-term temperature increase.

Conclusions

Connectivity, as simulated by the introduction of species from a regional pool in this study, could be crucial for buffering montane lake communities against heatwave effects. Zooplankton dispersal improved the recovery of copepod abundance after the heatwave (SI 5, Figure S5.2d), and facilitated increase in phytoplankton biomass (SI 5, Figure S5.3a). Better copepod recovery resulting in communities less dominated by cladocerans may underlie higher productivity and enhanced carrying capacity, as reflected by a greater elevation of the size spectra. Dispersal also weakened the degree to which biomass decreased during the heatwave, but had no effect on the slope of the size spectra, suggesting a limited buffering capacity of increased connectivity.

Mesocosms can support diverse multitrophic food webs with community structure similar to those in natural ecosystems (Brown *et al.*, 2011; Kratina *et al.*, 2012). If our findings are representative natural ecosystems, copepod recovery from heatwaves in relatively isolated montane lakes might be hampered by low rates of dispersal from the regional species pool. These findings also indicate that larger organisms could be disproportionately affected during transient heatwaves, and underlie changes in the community size structure. Given the prominence of top-down cascades in freshwater communities (Rossberg *et al.*, 2019), such changes could alter ecosystem structure and functions such as nutrient cycling. However, the buffering effects of connectivity warrants further investigation, given its potential to mediate community responses to extreme events, as indicated by this study. Conserving habitat connectivity and regional diversity could therefore be an important part of management strategies mitigating against the negative effects of extreme climatic events.

Chapter 6

General conclusions

In this thesis, I have explored how ecological circumstances could influence the mass-scaling of metabolic rates in a diverse range of taxa that reside in contrasting environments. My findings test and challenge several important ecological theories, including the prominent metabolic theory of ecology (Brown *et al.*, 2004) and the metabolic-level boundaries hypothesis (Glazier, 2005, 2010, 2014a), and highlight factors besides body mass that influence metabolic rates. I presented alternate views on the role that ecological context (Glazier, 2006; Pequeno *et al.*, 2017), and body shape change (Hirst *et al.*, 2014; Glazier *et al.*, 2015; Tan *et al.*, 2019) may have on rates of energy use in organisms. In doing so, I highlighted the importance of appreciating both mass-related resource demand and surface area-related resource supply capacity, for understanding variations in the mass-scaling of metabolic rates. Hence, the current focus on internal and external constraints of resource supply should extend to also incorporate the energetic pressures associated with different life history strategies and environments. Such considerations could be key for understanding the mechanisms underlying metabolic scaling relationships, and advancing the development of metabolic theories.

The abiotic and biotic environment, which includes temperature, food availability and competition, can alter metabolic demands and resource availability, and therefore influence body size and body shape. I studied the effects of these environmental factors on body size and shape in heterotrophic protists. Contrasting body shape responses of the two species under resource competition indicate that body shape changes could relate to competitive ability, consistent with the adaptive value and function of body shape for resource acquisition (Grover, 1989; Naselli-Flores, Padisák & Albay, 2007; Naselli-Flores & Barone, 2011). Body shape likely influences food acquisition through altering the energetic cost, or speed of locomotion (Roberts, 1981; Pennekamp *et al.*, 2019). Unlike body shape response, similar magnitude of body size decrease under warming likely maintained *per capita* competitive intensities, which potentially facilitates species coexistence during environmental change.

Environmental conditions structure entire communities, because species abundance is constrained by the resources available in the environment, and by the energetic demands of the individual organisms within the community. Such energetic demands are strongly related to body size, which also influences organism abundance. Hence, environmental warming and short-term heatwaves can alter the community size structure, as warming tends to decrease body size. Body size changes can also influence organism abundance, and are potentially modified by temperature effects on individual resource demand and resource availability. To understand how short-term heatwaves alter body size distributions in communities, I studied the impacts of an experimental heatwave on the size structure of planktonic communities. My findings indicate that the heatwave decreased the elevation, and potentially steepened the slope of the relationship between organism abundance and body size. The heatwave negatively influenced the abundance of large organisms, and is congruent with the paradigm that larger organisms are disproportionately affected by warming (Daufresne *et al.*, 2009; Forster *et al.*, 2012). Hence, warming potentially has serious implications for top-down trophic cascades that are common in freshwater communities (Rossberg *et al.*, 2019). However, introduction of species from the regional species pool reduced the negative heatwave effect on the elevation, but not changes to the slope of the relationship between organism abundance and body size. This effect of species introduction supports the theory that habitat connectivity can reduce the impact of stress events (Loreau *et al.*, 2003; Leibold *et al.*, 2004). However, empirical observations that dispersal had limited capacity to maintain food web size structure under acidification, suggests that dispersal could have limited importance for maintaining biomass structure in relation to some types of environmental stress (Limberger *et al.*, 2019).

In this thesis I applied a combination of approaches, including meta-analyses, laboratory microcosm, and outdoor mesocosm experiments, to determine and quantify variation in the size-scaling of metabolic rates, body shape and body size response, as well as community size structure responses to environmental factors. Understanding environmental influence on the interplay between body size, body shape, and energetic and resource demands is essential for improving the knowledge concerning the consequences of climate warming on individuals to ecosystems.

Metabolic rate-body size relationship

In chapter 2, I investigated the effects of body shape on intraspecific metabolic-scaling relationships in cephalopods. The correlation between intraspecific metabolic scaling exponent (*b*) and the degree of body shape elongation or flattening in pelagic cephalopods, suggests that body shape changes can underlie variations in the size-scaling of metabolic rates. Relative body shape flattening or elongation during ontogeny increases the body size-scaling of body surface area, and is related to steeper scaling of metabolic rates. I also explored the prediction of the metabolic-level boundaries hypothesis (MLBH), that *b* is correlated with metabolic level (i.e. elevation of the metabolic rate-body size relationship). Unlike teleost fish, which exhibit a negative relationship between intraspecific *b* value and metabolic level, *b* positively covaries with metabolic level in cephalopods. The metabolic scaling differences between these taxa could relate to divergent size scaling of growth demands related to different life history strategies. Specifically, near-isometric scaling of metabolic rates in the most active cephalopod species could be influenced by near-exponential growth throughout ontogeny, likely related to semelparity. Active teleost fish, by contrast, tend to exhibit iteroparity, and exhibit rapid growth that declines over ontogeny. We hypothesize that iteroparity is potentially associated with reduced routine metabolism, which facilitates maintaining a metabolic safety margin to promote greater adult longevity. Itero- and semelparity may therefore be correlates of adult longevity, and the metabolic safety margin required to achieve longevity under energetic challenges. In addition, the contrasting scaling relationships between these two coexisting marine taxa could in part relate to differences in the size-scaling of locomotion costs; locomotion cost scales less steeply with body size in teleost fish than in cephalopods. Hence, teleost fish obtain greater energetic savings in locomotion with size increase than cephalopods. The contrasting relationship between *b* and metabolic level could therefore reflect divergent scaling of growth and locomotion demands with body size in cephalopods and teleost fish. This striking difference demonstrates the importance of life history strategies, growth and body shape in understanding variation in metabolic scaling, whilst also demonstrating that metabolic scaling exponents should not be assumed to be invariant and fixed values (of 3/4 or 2/3rds for example). Future research should therefore investigate how the size-scaling of growth demand influences metabolic scaling and body shape, and establish how growth demand relates to life history strategies and environmental factors. Ideally a wide range of taxa should be utilised to explore such aspects. Such knowledge can improve the understanding of metabolic scaling relationships as a potential trait that responds to physical and ecological constraints.

In chapter 3, I compared the interspecific relationship between body size and metabolic rate in insects and spiders, two major terrestrial arthropod groups. I showed that insects have greater respiration rates than spiders both at rest and during activity. Crucially, the two taxonomic groups exhibit different size-scaling of active (non-flight) metabolic rate. As a result, energetic differences between insects and spiders during non-flight activity increase with body size; large insects have active metabolic rates that are approximately 10-fold greater than those of similar-sized spiders. The possession of wings underlies part of this difference, which suggests higher overhead costs associated with maintaining capacity for flight in winged versus wingless insects and spiders. Such costs could also be related to higher mitochondrial densities in flight muscles, and potentially increased metabolic scope for activity in winged insects. Spiders, by contrast, have low aerobic capacity, and commonly rely on anaerobic metabolism for maximum activity. This contrast suggests that differences relating to the need for sustained activity associated with ecological strategies in the two taxonomic groups influences aerobic capacity. Active metabolic rates in spiders scaled with an exponent of 2/3. As active metabolic rates reflect the size-scaling of oxygen supply capacity during high metabolic demand, allometric scaling indicates that aerobic capacity per unit body mass is lower in large spiders. Since active metabolic rates potentially have direct influence on performance capacity, efficiency gains due to shallower size-scaling of locomotion cost in spiders may allow the maintenance of locomotory performance across the size range, despite lower aerobic capacity per unit body mass in large spiders. Hence, contrasting size-scaling of active metabolic rates could be underlined by different size-scaling of locomotion cost, or the cost of maintaining locomotory posture, which may be influenced by different maximum locomotor and energetic capacities. Further studies should therefore further explore these possible mechanisms to better understand the influence of various metabolic demands on the size-scaling of metabolic rates between insects and spiders.

These analyses of intra- and inter-specific body mass-scaling of metabolic rate lead to the conclusion that rather than assuming an average tendency for metabolic-scaling, understanding variations in the size-scaling of metabolic rates can provide insights into the physiology and ecology of organisms. Identifying the factors that influence the scaling of metabolic demands and resource supply capacity with body size, and integrating such knowledge with energetic and mortality pressures associated with life history strategies and the environment could be key for advancing metabolic theories and developing new theories that can better explain and predict the patterns seen in nature.

Temperature-size rule in competitors

In chapter 4, I tested the influence of biotic and abiotic environment on body size and body shape of two heterotrophic protist species. As many characteristics of an organism scale with body size, understanding body size responses to environmental temperature is crucial for predicting how warming impacts individuals, populations and communities. Both protist species reduced their body size when reared in the warmer environment, as predicted by the temperature-size rule (Atkinson, 1994; Atkinson *et al.*, 2003). As warming enhances metabolic resource demands, but has a weaker influence on the rate of resource (e.g. oxygen) supply, resource demand is predicted to increase more rapidly than resource supply (Verberk *et al.*, 2011). Hence, the observed body size reduction may in part occur to compensate for increased metabolic demands under warming. However, body shape response was influenced by the availability of resources, and could be indicative of competitive abilities. Body shape responses may therefore be useful for predicting competitive hierarchies in protistan communities, aiding the ability to forecast community assembly under environmental warming.

Unlike the general pattern observed in body size responses to environmental temperature, a broad understanding of how internal and external factors influence body shape (and in turn body surfaces associated with resource supply) is still lacking. I showed that body shape strongly responds to resource availability in protists, but further research is needed to extend the generality of these findings to other taxa. While the relationship between body shape and body size differed between the model competitors, both species exhibited similar body size decrease in response to warming. As body shape changes influence surface area and potentially resource uptake, non-isomorphic and isomorphic species could exhibit different magnitude of size reduction with warming in response to changing metabolic demands. Body size response to warming should therefore be quantified in future studies for taxa with different body shape-body size relationships (i.e. isomorphic versus non-isomorphic species), especially in those species dependent upon large portions of their external body surface for the exchange of materials. Such data could help us to unravel the mechanisms underpinning variation in body size responses to warming.

Heatwaves and community size structure

In chapter 5, I experimentally tested how a short-term heatwave impacts the size structure of freshwater communities, and how these changes are buffered by introduction of species from the regional species pool. Ecological communities are commonly composed of many small organisms and few organisms with large body size (Sheldon *et al.*, 1972; White *et al.*, 2007; Heneghan *et al.*, 2019). Organism abundance decreases with their body size, and this relationship is often characterised by a power law. On log-log axes (i.e. log-transformed abundance vs. log-transformed body mass), this relationship is commonly linear, and the slope of this relationship describes how strongly organism abundance decreases with body size. The slope of the abundance-body size relationship steepened after the heatwave, and this effect was driven by reduced biomass and abundance of larger consumers in warmer conditions. This suggests that the heatwave decreased the efficiency of energy transfer through the community, indicating reduced trophic efficiency and increased food limitation during short-term temperature increase. Alternatively, or additionally, the heatwave could have increased mortality of large zooplankton taxa by constraining their capacity to meet respiratory demands (Pörtner *et al.*, 2017). Zooplankton mortality could reduce nutrient regeneration, and potentially reduce both zooplankton and phytoplankton biomass in low-nutrient ecosystems (Getzlaff & Oschlies, 2017). Positive effects of zooplankton introduction on phytoplankton biomass and the elevation of the community size structure suggest that heat-tolerant colonisers from warmer habitats could mitigate community changes caused by the heatwave (Loreau *et al.*, 2003; Thompson & Shurin, 2012). This buffering effect of zooplankton introduction indicates that isolated, montane lakes could be more vulnerable to short-term climatic events due to low rates of natural species dispersal.

Because metabolism is related to both body size and species-identity, future research should explore how changes in community composition influence energy use in the community. Understanding how metabolism scales with body size and temperature could be critical for predicting how changes in community composition alter the diverse energetic pathways underlying the community size structure (Guiet *et al.*, 2016). Such knowledge is critical for understanding how climate change might alter the structure and function of ecosystems. An increased connectivity to the regional biodiversity pool reduced the impacts of a heatwave on planktonic communities. Hence, future research should also identify ways to improve connectivity between habitats, which underlies the potential buffering effect of regional biodiversity on local ecosystems.

Summary

My findings show that variation in the body-mass scaling of metabolic rates can relate to taxonomic affiliation, physiological state, body shape and environmental influence, reinforcing other recent findings on these topics (Glazier, 2006; DeLong *et al.*, 2010; Killen *et al.*, 2010; Ohlberger *et al.*, 2012; Carey, Sigwart & Richards, 2013; Hirst *et* *al.*, 2014; Glazier *et al.*, 2015; Tan *et al.*, 2019). Furthermore, such findings counter the idea that mass-scaling of metabolic rate is fixed on a central value (e.g. 3/4-power law; Brown *et al.*, 2004). I further demonstrate that comparing active metabolic rates can reveal differences between taxonomic groups that are not observed when comparing rates at rest. Differences in interspecific scaling of active metabolism between major taxonomic groups are linked to the size-scaling of resource supply during high demand, which reflects contrasts in energetic demands associated with differences in modes of locomotion and feeding between the groups. My findings also support the commonly observed temperature-size rule (Atkinson, 1994; Atkinson *et al.*, 2003; Forster *et al.*, 2012; Arendt, 2015), and that warming tends to reduce body size at various levels of biological organisation from cells and organisms to communities (Daufresne *et al.*, 2009; Gardner *et al.*, 2011; Forster *et al.*, 2012; Ohlberger, 2013; Morán *et al.*, 2015; Evans *et al.*, 2019). A decrease in body size has profound impacts on species via altering their vital and physiological rates (e.g. rates of metabolism, growth, grazing and mortality), and on species interactions (Sentis *et al.*, 2017). I showed that body shape of model protist species is influenced by resource availability and that contrasting body shape response under resource competition is potentially indicative of competitive abilities. Body shape response is therefore likely important for understanding competitive interactions, at least in protists. Body size changes also have consequences that can extend to higher levels of biological organisation. At the community level, I showed that heatwaves alter the abundance-body size relationship, including reduction in the number of large consumers that can be supported by smaller prey. Such changes could be mitigated by the influx of potentially heat-tolerant zooplankton individuals and species from warmer habitats, thereby highlighting the possible significance of connectivity to regional biodiversity pools to buffer against disturbance effects (Thompson & Shurin, 2012; Leibold, Chase & Ernest, 2017; Limberger *et al.*, 2019;

Dib *et al.*, 2019). Consequently, montane and alpine lake communities, which are relatively isolated from the regional species pools, and also experience higher rates of warming (Pepin *et al.*, 2015) are likely highly vulnerable to disturbance, and should be prioritised by future research.

A climate emergency has been recently declared by scientists (Ripple *et al.*, 2019). While most discussions relate to the rising average global temperatures, the frequency and magnitude of heatwaves are also predicted to increase (Oliver *et al.*, 2018; Stillman, 2019). Consequently, heatwaves such as those that broke temperature records in June 2019, as reported by the World Meteorological Organization, are likely to occur more frequently. Hence, it has never been more pressing to understand the impacts of warming on organism body size and shape, rates of energy use, and the interdependence and wider implications of these factors. Appreciating the variation in metabolic rate-body size relationships, and the potential factors underlying this variation could improve our mechanistic understanding of the consequence of climate warming on populations, communities and ecosystems. Crucially, this will also help us identify species and communities most vulnerable to climate change, and potentially aid in informing mitigation and conservation efforts to deal with future climatic challenges.

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Supplementary information

Supplementary information Chapter 2 (SI 2)

The mass range for b_R

The body-mass range influences the accuracy of scaling regressions and therefore scaling exponents (b_R); generally the variability of b_R decreases as the mass range increases (Bokma, 2004; White & Seymour, 2005; Moses *et al.*, 2008). As most of the reduction in the standard deviation of b_R occurs when the mass range exceeds 1 order of magnitude (White & Seymour, 2005), b_R values were omitted when mass ranges covered less than one order of magnitude or were not reported following Hirst, Glazier & Atkinson, (2014). This additional screening step removed two species, and representation of one family. The relationship between ln L and b_R [$r^2 = 0.218$, $p = 0.033$, $n = 21$, $b_R = 0.621$ (95% CI: 0.526, 0.716) + 0.049 (95% CI: 0.028, 0.069) x ln L_R)], or between b_R and $1/b_L$ [$r^2 = 0.542$, $p = 0.015$, $b_R = 0.215$ (95% CI: -0.123, 0.552) + 1.650 (95% CI: 0.740, 2.561) x $1/b_L$)] are not materially affected, suggesting that the patterns found were robust. In general, the additional screening step improved the r^2 values of the relationships.

Lifestyle categorisation

Following Killen, Atkinson & Glazier, (2010), cephalopod species were categorised into pelagic, benthopelagic, benthic or bathypelagic lifestyles. Pelagic species live in the water column and often feed near the surface. Benthopelagic species occupy shelf waters, feeding, living near the bottom, but rarely rest on the bottom. Depending on depth, they are also associated with mid- and surface waters. Benthic species live on the bottom and are in direct contact with substrates. Bathypelagic species are deep-living species at depths of approximately 1000-5000 m. Data and species description used to classify the lifestyle of each species were obtained from relevant literature (Dataset S6.1, Table S2).

Phylogenetic tree construction

The phylogenetic tree (Figure S2.1) was compiled following published phylogenies (Lindgren *et al.*, 2005, 2012; Wakabayashi *et al.*, 2012; Ibáñez *et al.*, 2018; Sanchez *et al.*, 2018) based on molecular data. Branch length was calculated following (Grafen, 1989).

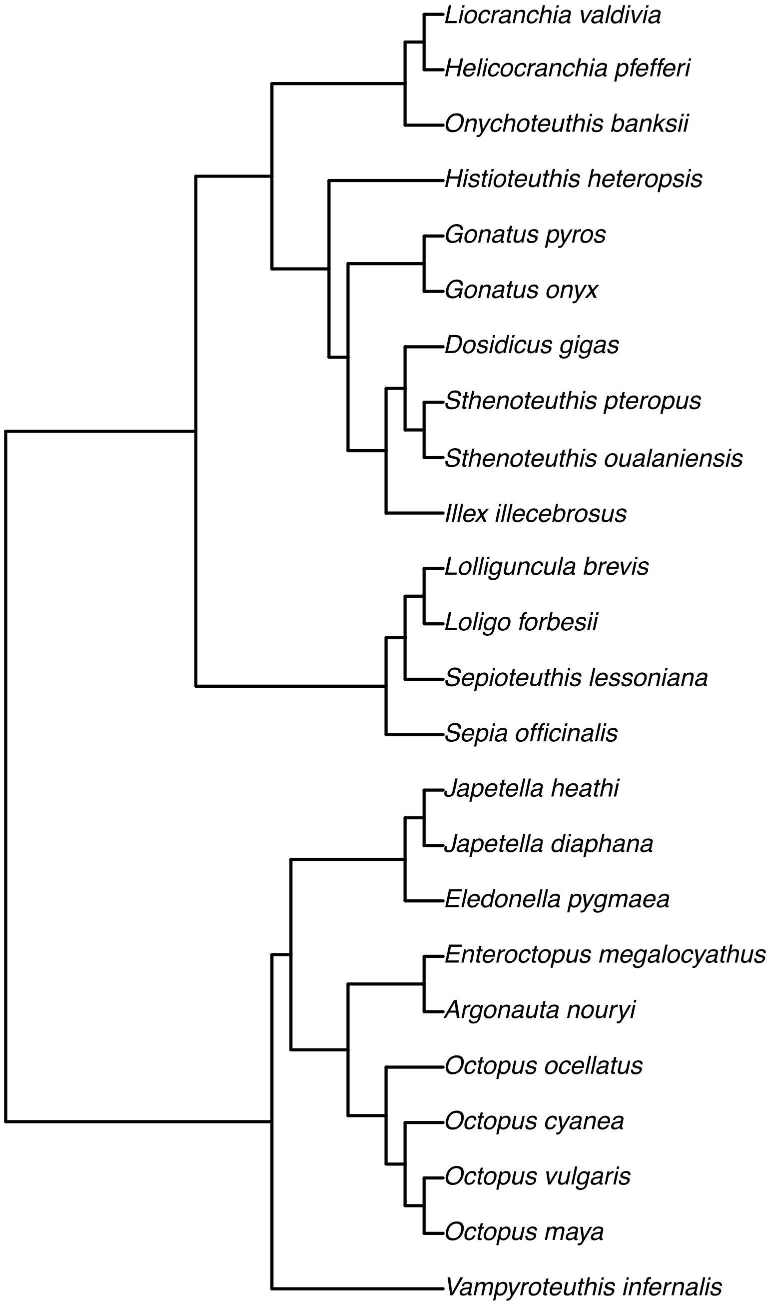

Figure S2.1. Phylogenetic tree of cephalopod species in our data set.

Calculation of mass ranges for Figure 2.5

As the mass range of metabolic rate-body mass relationships differ between studies for species with more than one b_R value, the minimum and maximum mass plotted in Figure 2.5 was based on the average minimum and maximum masses of multiple mass ranges. For instance, minimum mass of *Sepia officinalis* based on six metabolic rate-body mass relationships ranged from 0.1 – 15.00 g. The arithmetic mean of the six minimum mass values was then used as the minimum mass for the metabolic rate-body mass relationship for *Sepia officinalis* in Figure 2.5.

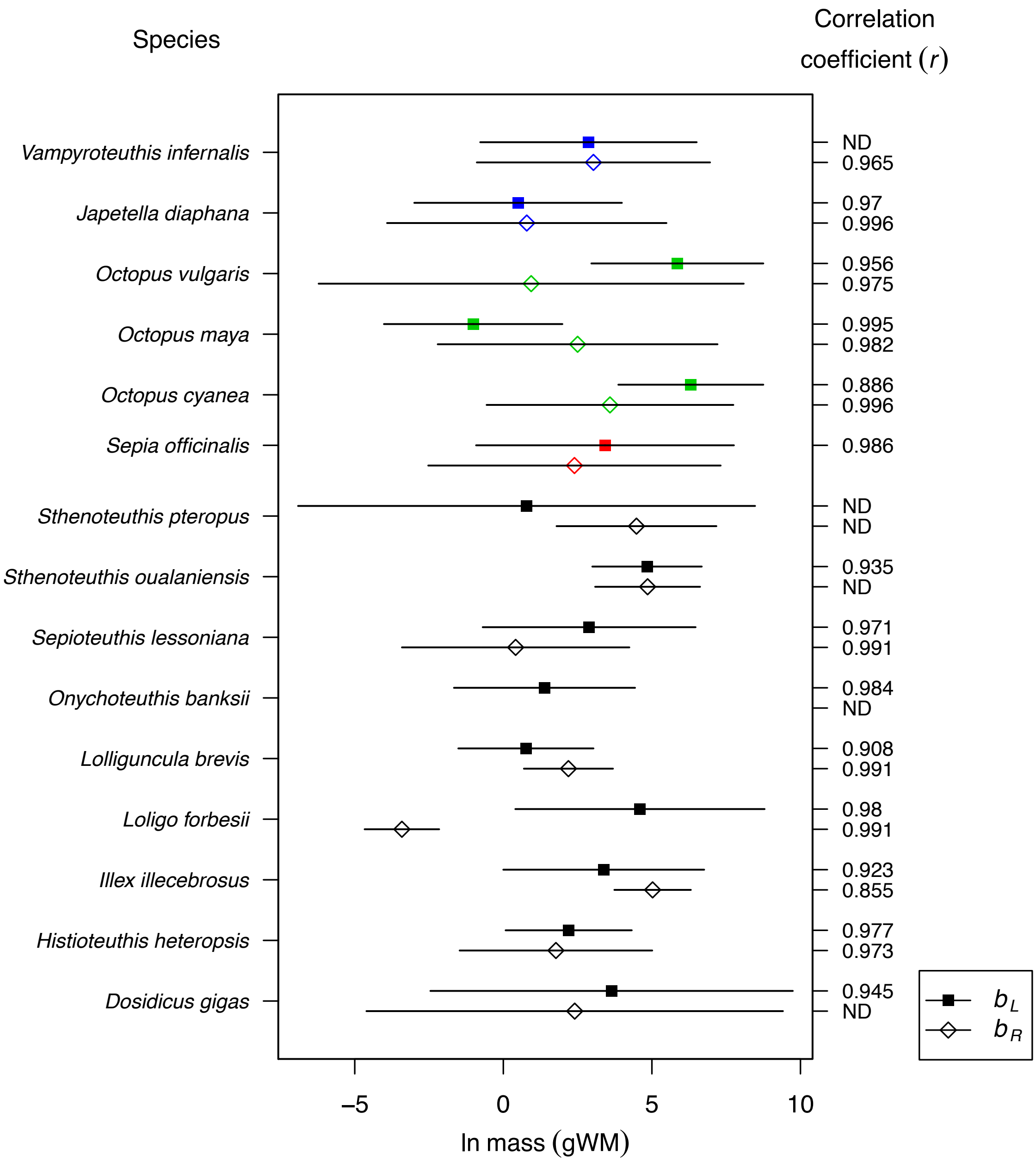

Figure S2.2. The range of logged wet masses for each mass-length and respiration-mass regression from which b_L and b_R values were derived. The correlation coefficients (r) of each regression are shown on the right-hand axis. In cases where multiple regressions were available and regression exponents were derived as arithmetic means, the mass range reflected is based on the mean minimum and maximum mass of all the regressions. The different lifestyles are represented by black (pelagic), red (benthopelagic), green (benthic) and blue (bathypelagic) symbols. The mass range for the respiration-mass relationship of *Onychoteuthis banksii* was not reported.

Supplementary information Chapter 3 (SI 3)

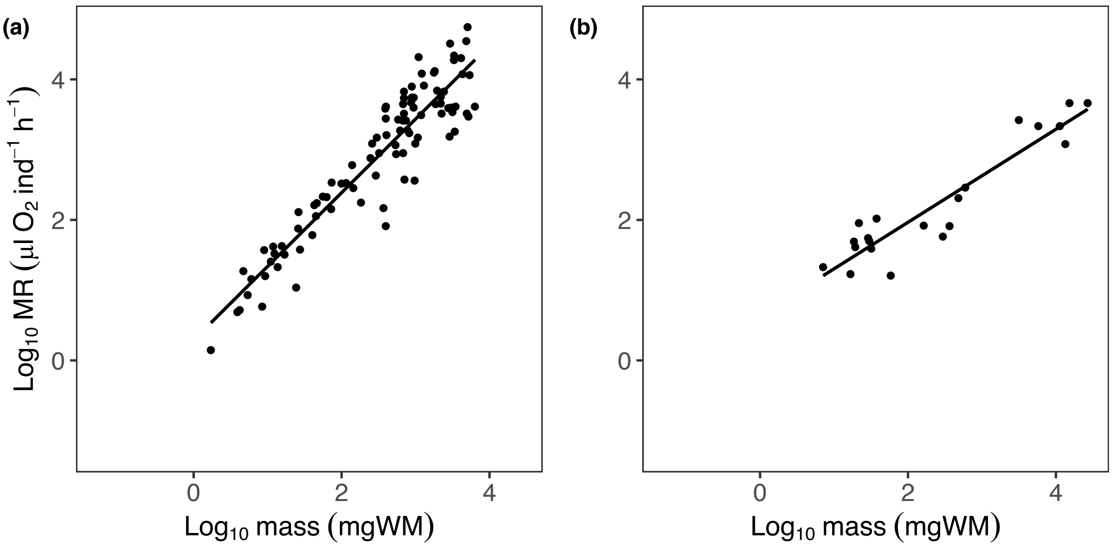

Figure S3.1. The interspecific scaling relationship of metabolic rates and body mass in **(a)** insects and **(b)** spiders undergoing non-flight activity based on minimum running speed. Active metabolic rates scaled more steeply in insects than spider (insect b_r = 1.048, 95% CI: 0.963, 1.134; spiders b_r = 0.662, 95% CI: 0.542, 0.782), and the results are similar with that obtained from using maximum running speed.

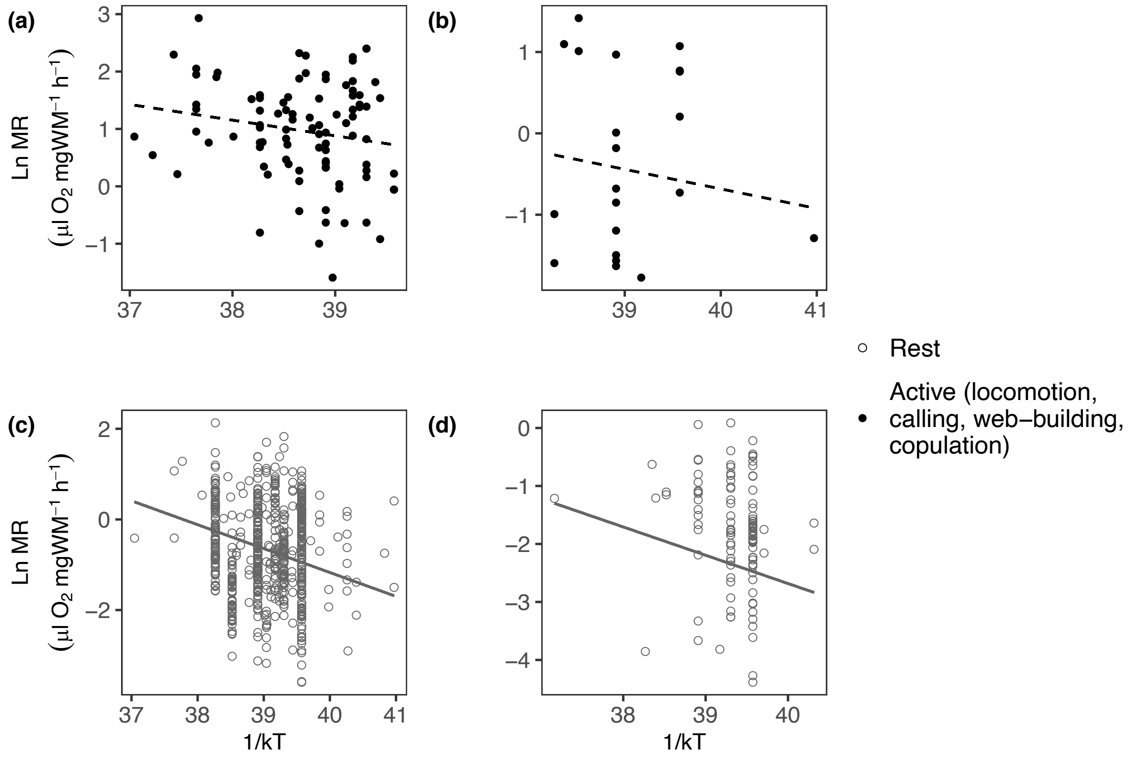

Figure S3.2. Arrhenius plots illustrating the relationship between temperature expressed as $1/kT$ and ln-transformed mass-specific metabolic rates. T is temperature in Kelvin and k is the Boltzman constant (8.62×10^{-5} eV K^{-1}). **(a)** Active mass-specific metabolic rates of insects are not correlated with temperature ($F_{1,\,92} = 3.05$, $p = 0.084$). **(b)** Likewise, active mass-specific metabolic rates of spiders are not correlated with temperature ($F_{1,\,19} = 0.39$, $p = 0.537$). **(c)** In contrast, resting mass-specific metabolic rates are positively correlated with temperature in insects ($F_{1,\,635} = 89.75$, $r^2 = 0.124$, $p < 0.001$, slope = -0.528, 95% CI: -0.637, -0.418) and **(d)** spiders ($F_{1,\,109} = 14.31$, $r^2 = 0.116$, $p < 0.001$, slope = -0.488, 95% CI: -0.743, -0.232). The residuals of the Arrhenius plot are expressed relative to the fitted equation value at 22 °C to obtain metabolic rates corrected to a common temperature of 22 °C. Insect resting metabolic rate data presented here includes species with undefined wing status. These species were subsequently excluded from other analyses.

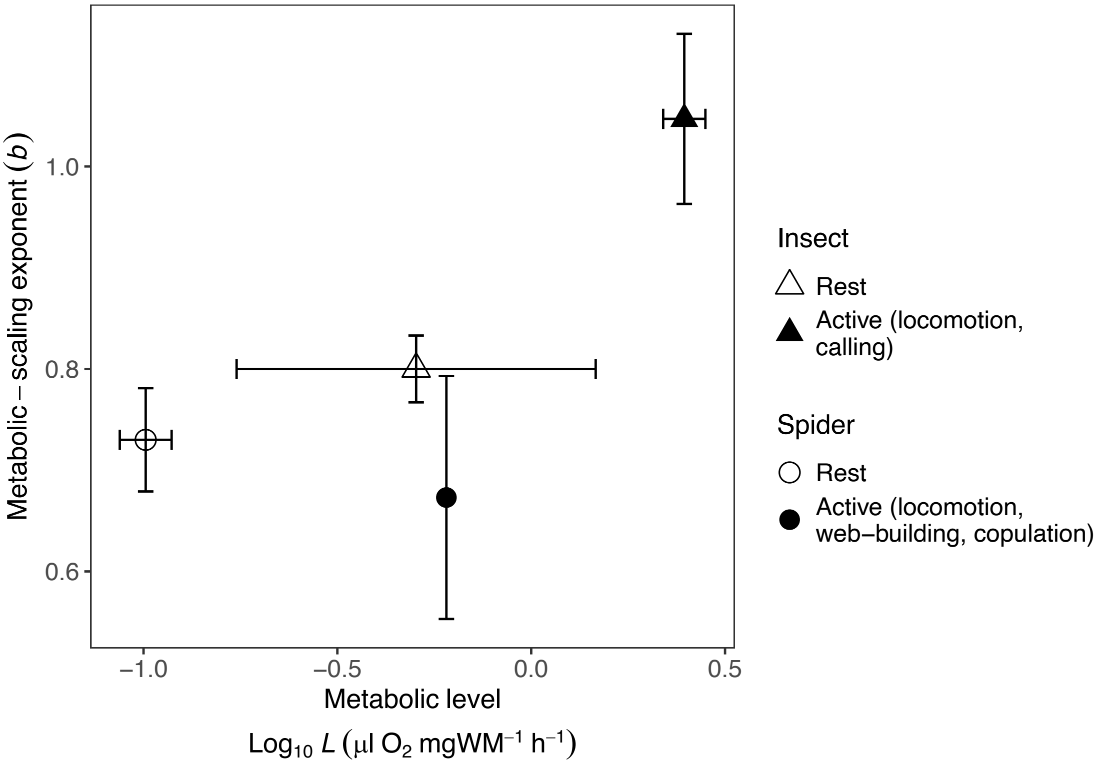

Figure S3.3. The $\log_{10}$-transformed metabolic level (L_a and L_r, μl O_2 mgWM^{-1} h^{-1}) and slope (b_a and b_r) of the PGLS regression for active and resting metabolic rates in insects and spiders. Resting metabolic rates are temperature corrected. The error bars represent the 95% confidence intervals of $\log_{10} L$ and the slope. The slopes of insects and spiders are not significantly different during rest, as indicated by the overlapping confidence intervals. However, insects have significantly higher L_a and L_r than spiders. The slope (b_r) also increased with activity in insects but not spiders. Within insects, b_a is significantly higher than b_r. These results are similar as our comparisons without temperature corrections.

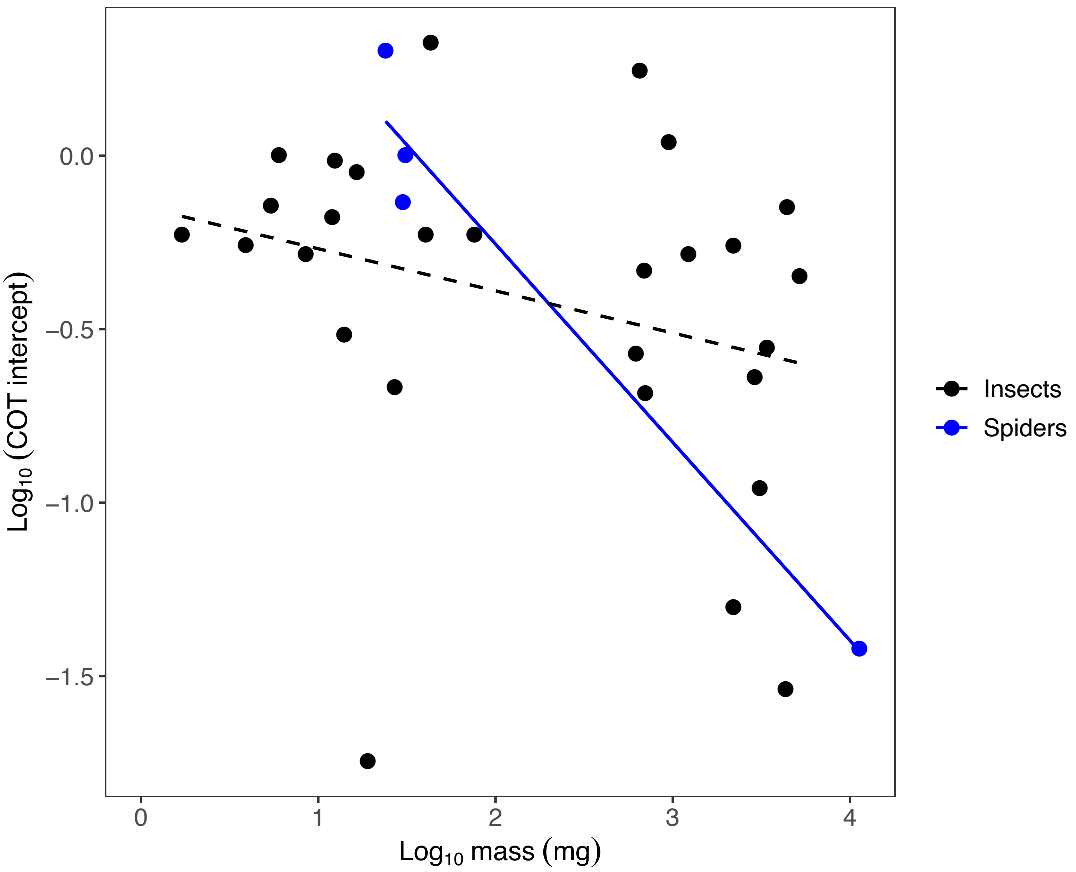

Figure S3.4. The *y*-intercept of the linear relationship between metabolic rate and locomotion speed, also referred to as the cost of transport (COT) intercept against $\log_{10}$ body mass. The *y*-intercept is associated with the energetic costs of maintenance and includes energetic requirements for maintaining posture related to locomotion (Halsey, 2013). The negative relationship between the *y*-intercept and body mass suggests that spiders gain efficiency with size increase ($F_{1,2} = 44.81$, $r^2 = 0.957$, $P = 0.022$, intercept $= 0.888 - 0.571 \times \log_{10}M$). A similar relationship is not observed in the insects ($F_{1,26} = 2.35$, $r^2 = 0.083$, $P = 0.137$). Data were obtained from the data compilation of White *et al.* (2016).

Supplementary information Chapter 4 (SI 4)

Bacterial densities

To enumerate bacterial densities, we fixed 200 µl of sample at the start, on day seven, and at the end of the experiment with 2% formaldehyde (v/v). Bacterial densities were then enumerated with a flow cytometer by staining 100 µl of fixed samples with SYBR Green for 10 min following Gasol & Morán, (2015).

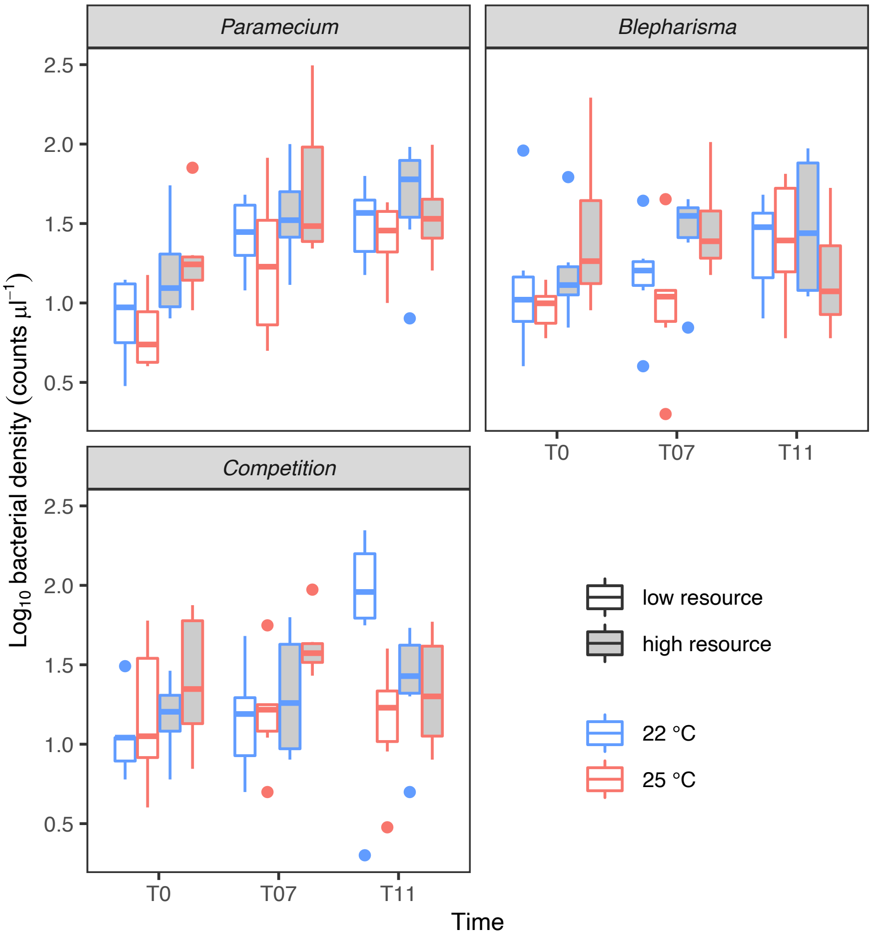

Figure S4.1. Log$_{10}$-transformed bacterial densities (ind µl^{-1}) across the experimental treatment combinations at the start (T0), on day seven (T07) and at the end of the experiment (T11). Using a linear mixed effect model with bacterial density as the response variable, temperature, resource level and competition as fix effects and time point as a random variable, we found a significant resource influence on bacterial density ($p = 0.001$). In general, bacterial densities are higher in the high resource level treatments. Interspecific competition did not negatively influence bacterial densities in comparison with monocultures, which suggests that treatment effect might relate to species-specific ability of monopolising resources from the environment.

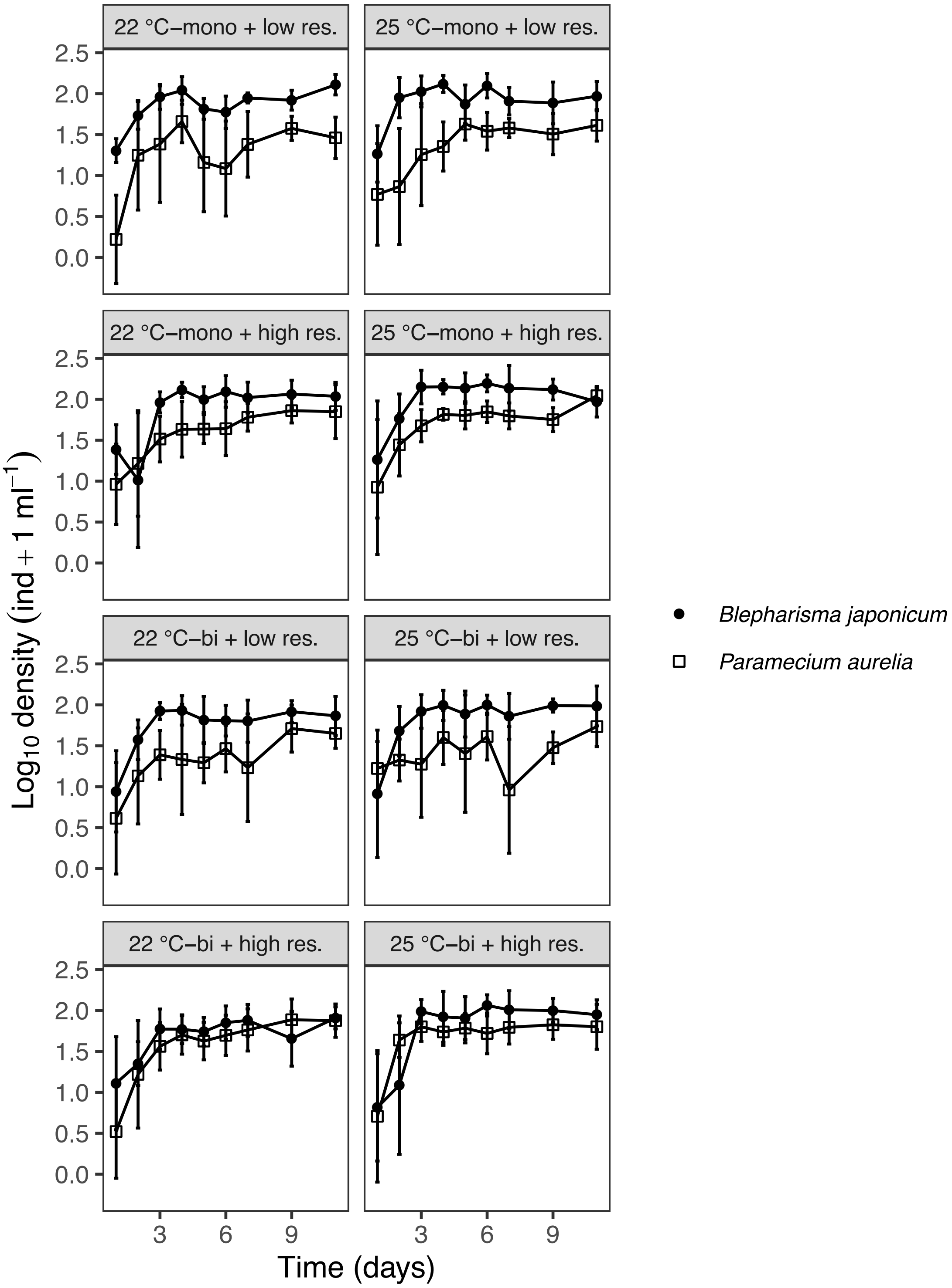

Figure S4.2. Log_{10}-transformed population density (calculated as ind ml^{-1} + 1 to account for 0 counts) across all experimental treatment combinations over the course of the experiment. Symbols represent the mean of 6 replicates and error bars represent the standard deviation.

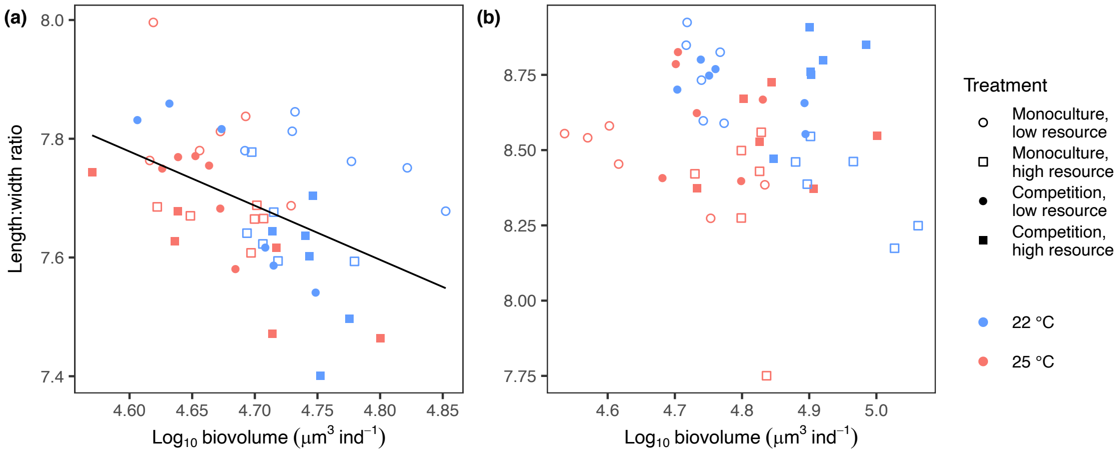

Figure S4.3. The relationship between length:width ratio and body size for the two model species. **(a)** There is a negative relationship between length:width ratio and body size of *Paramecium aurelia* ($F_{1,46} = 12.41$, $p = 0.001$, $r^2 = 0.213$, L:W ratio = 11.975 – 0.912 × $\log_{10}$ biovolume). This indicates that larger individuals are relatively less elongated. **(b)** There is no relationship between relative elongation and body size of *Blepharisma japonicum* ($F_{1,46} = 1.35$, $p = 0.252$). Data points represent the mean of 9-11 measurements per treatment combination.

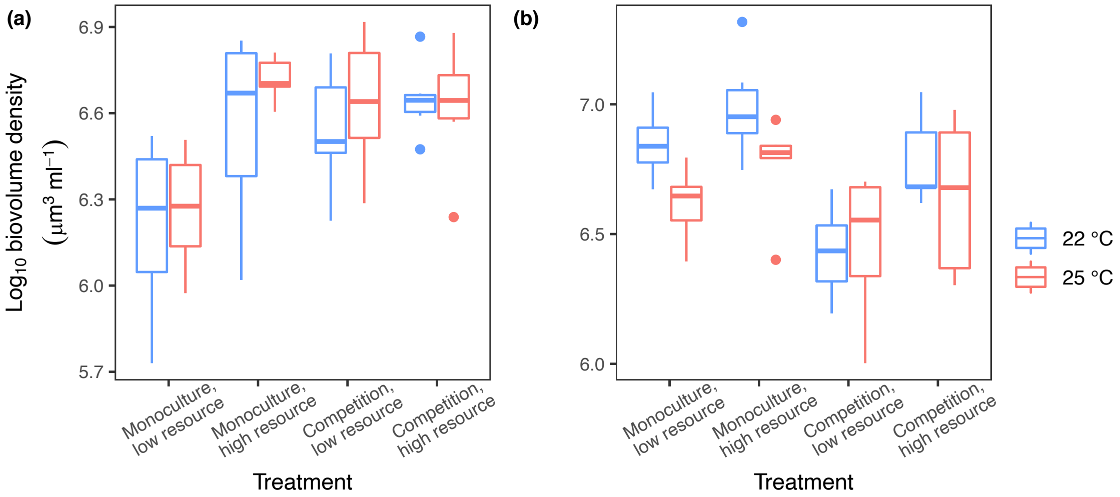

Figure S4.4. The population biovolume density of **(a)** *P. aurelia* and **(b)** *B. japonicum* across experimental treatments. Trends observed in population densities are similar as that reflected in the population biovolume densities. Competition increased *P. aurelia* population biovolume density, but only at low resource level ($F_{1,40} = 7.27$, $p = 0.010$). Interspecific competition ($F_{1,40} = 14.14$, $p < 0.001$) and low resource levels ($F_{1,40} = 11.69$, $p = 0.001$) both negatively influenced *B. japonicum* population biovolume density. Experimental warming also significantly decreased the population biovolume density of *B. japonicum* ($F_{1,40} = 5.28$, $p = 0.027$).

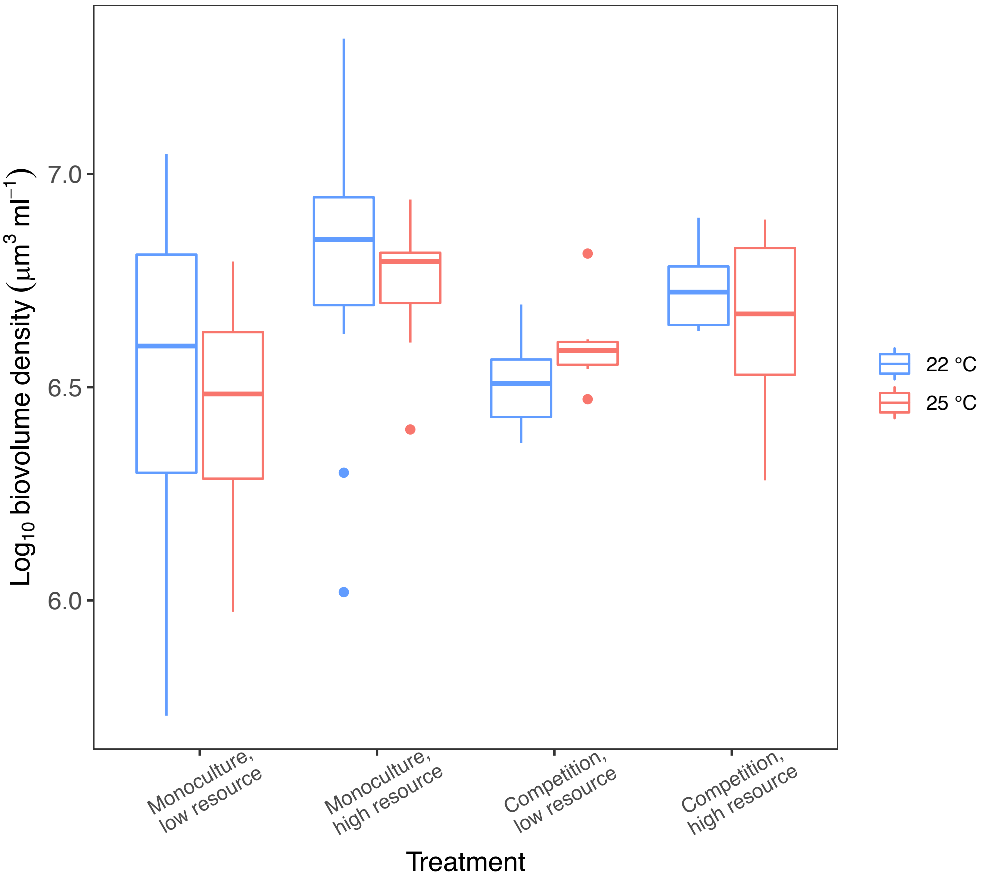

Figure S4.5. Assemblage biovolume density is similar across single-species and competition treatments. Competition did not affect the total assemblage biovolume density ($F_{1,64} > 0.01$, $p > 0.90$). There is a significant effect of resource level ($F_{1,64} = 13.37$, $p < 0.001$), indicating that resource manipulation affected resource availability.

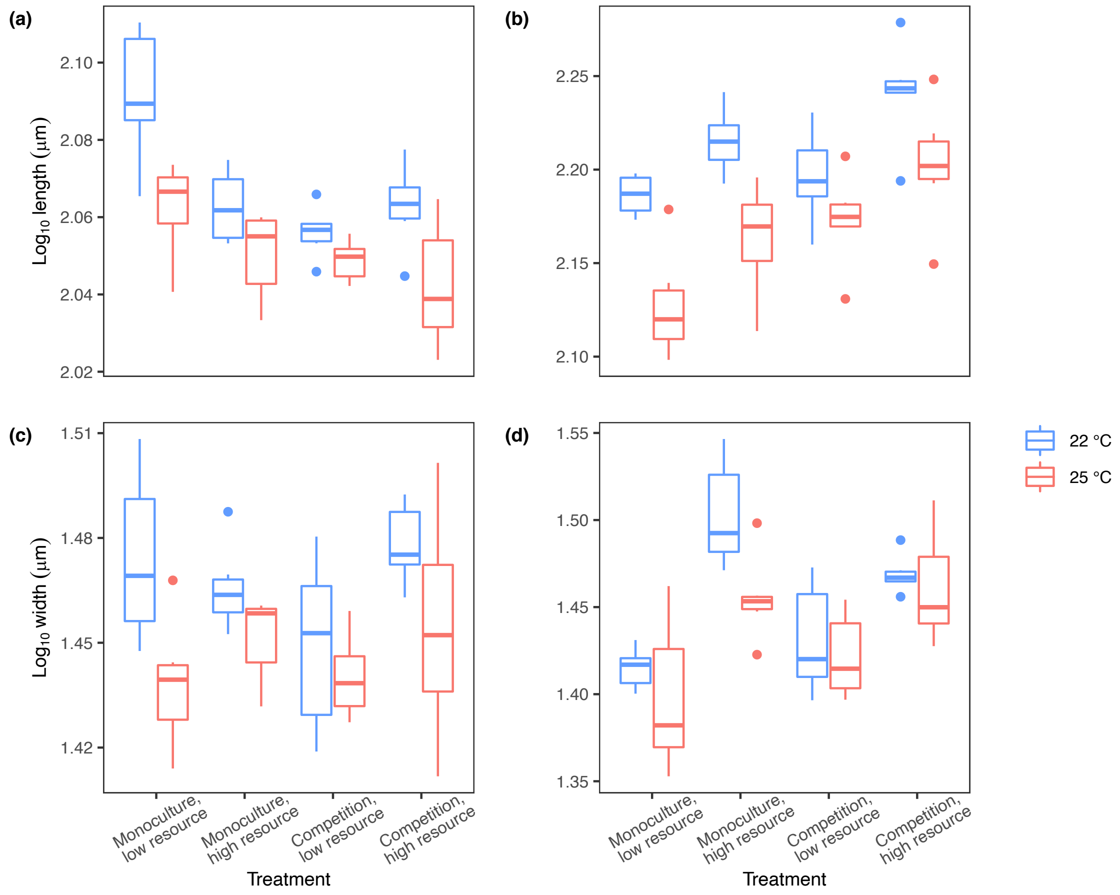

Figure S4.6. Protists body length and width across the experimental treatment combinations. **(a)** There is a significant interactive effect of temperature, competition and resource level on *P. aurelia* body length ($F_{1,40} = 4.89$, $p = 0.033$). Experimental warming reduces *P. aurelia* body length, and the effect of experimental warming was strongest in low resource monoculture. **(b)** Experimental warming also reduces *B. japonicum* body length ($F_{1,40} = 35.31$, $p < 0.001$). In contrast, high resource level ($F_{1,40} = 22.70$, $p < 0.001$) and interspecific competition increase *B. japonicum* body length ($F_{1,40} = 17.05$, $p < 0.001$). **(c)** Experimental warming ($F_{1,40} = 13.045$, $p = 0.001$) and low resource level ($F_{1,40} = 4.33$, $p = 0.045$) significantly reduce *P. aurelia* body width. **(d)** Experimental warming also significantly reduces *B. japonicum* body width ($F_{1,40} = 6.51$, $p = 0.015$). Resource level and competition interactively influence *B. japonicum* body width ($F_{1,40} = 4.47$, $p = 0.041$). Low resource level decreases *B. japonicum* body width, while competition reduces the negative effect of low resource level on body width.

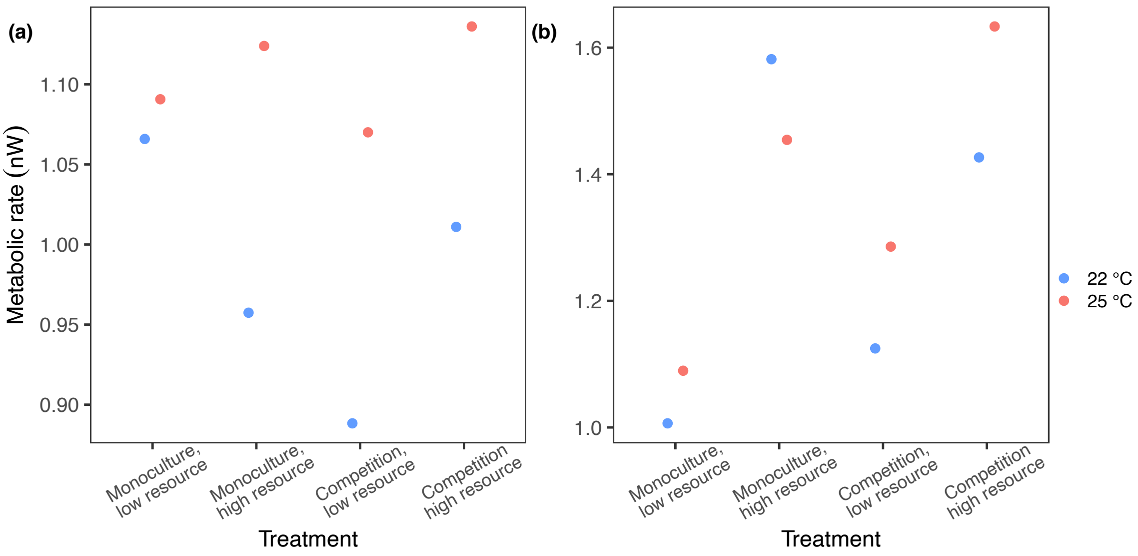

Figure S4.7. Predicted mean metabolic rates of **(a)** *P. aurelia* and **(*b*)** *B. japonicum* across treatment combinations, calculated from the allometric equation $R = 0.003M^{0.902}$ and assuming an activation energy of 0.61 eV (DeLong *et al.*, 2010). With the exception of *B. japonicum* in high resource monocultures, predicted metabolic rates are higher at 25 °C, although warmer temperature reduces body size, which suggests that size reduction under experimental warming could occur to reduce metabolic demands.

Supplementary information Chapter 5 (SI 5)

Table S5.1. The list and coordinates of regional lakes sampled for establishing the regional microbial and zooplankton inoculum to simulate dispersal.

Lake	Maximum depth (m)	Coordinates
Grabensee	14	N 47°59'28" E 13°5'46"
Obertrummersee	36	N 47°57'40" E 13°4'50"
Mattsee	42	N47°59'10" E 13°7'30"
Irrsee	32	N47°54'44" E 13°18'25"
Mondsee	68	N47°49'36" E 13°22'48"
Obinger See	45	N 48°0'11" E 12°24'56"
Brunnsee	20	N 47°59'03" E 12°26'11"
Griessee	12	N 47°59'11" E 12°26'33"
Simsee	23	N 47°52'03" E 12°13'48"
Klostersee	16	N 47°58'25" E 12°27'03"
Pelhamer See	21	N 47°56'01" E 12°20'54"
Hartsee	39	N 47°55'35" E 12°21'54"
Langbürgenersee	37	N 47°54'08" E 12°20'51"
Thalersee	47	N 47°04'11" E 15°22'02"
Chiemsee	73	N 47°53'50" E 12°27'49"

Table S5.2. Species list and species density of the zooplankton inoculum. On average, the zooplankton inoculum introduced 106 cladoceran and 412 copepod individuals to the mesocosms.

Subclass	**Species**	**Density (ind L^{-1})**
Cladocera	*Diaphanosoma brachiurum*	54.9
	Daphnia cuculata	47.1
	Ceriodaphnia reticulata	3.9
Copepoda	*Eudiaptomus gracilis Fem*	66.7
	Ediaptomus gracilis Male	19.6
	Copepodites of Calanoida	74.5
	Mesocyclops leuckarti Fem	27.5
	Mesocyclops leuckarti Male	11.8
	Thermocyclops crassus Fem	11.8
	Thermocyclops crassus Male	7.8
	Thermocyclops oithonoides Fem	23.5
	Thermocyclops oithonoides Male	31.4
	Copepodites of Thermocyclops	62.7
	Copepoda Nauplii	74.5

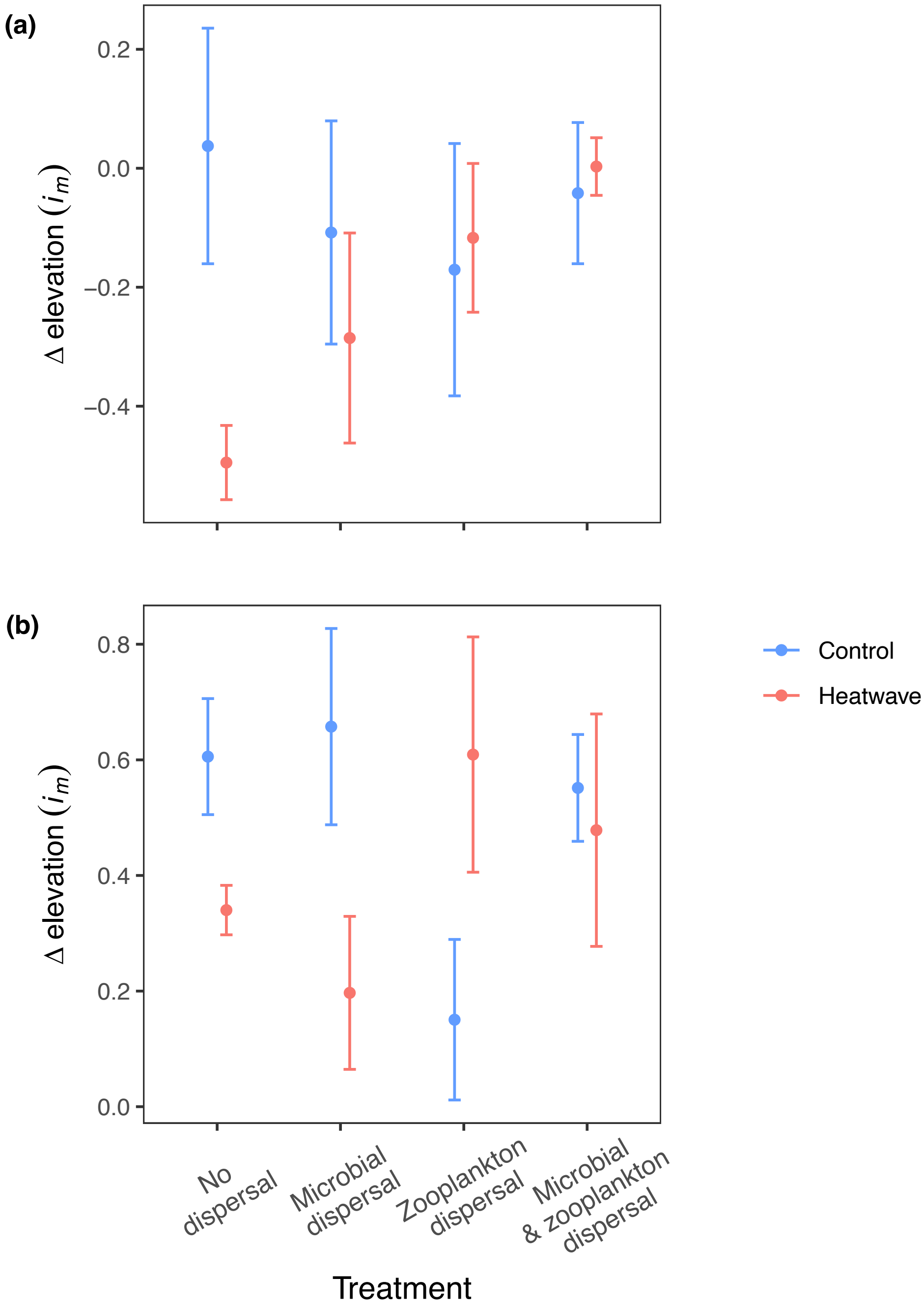

Figure S5.1. (a) Unlike comparison of elevation changes (Δi_m) based on the geometric midpoint mass of each size spectrum, there were no experimental treatment effects on the changes in the elevation of the size spectra (Δi_m) at the mean midpoint mass of all size distributions during the heatwave (H × Z, $F_{1,16} = 3.50$, $p = 0.080$). **(b)** However, the heatwave and zooplankton dispersal interactively influenced Δi_m ($F_{1,16} = 7.36$, $p = 0.015$) after the heatwave, and zooplankton dispersal increased i_m, as indicated by higher positive Δi_m values in the heated mesocosms. This is similar to the results obtained based on the geometric midpoint mass of individual size spectrum (Figure 5.3b), which indicates that differences in Δi_m after the heatwave are robust.

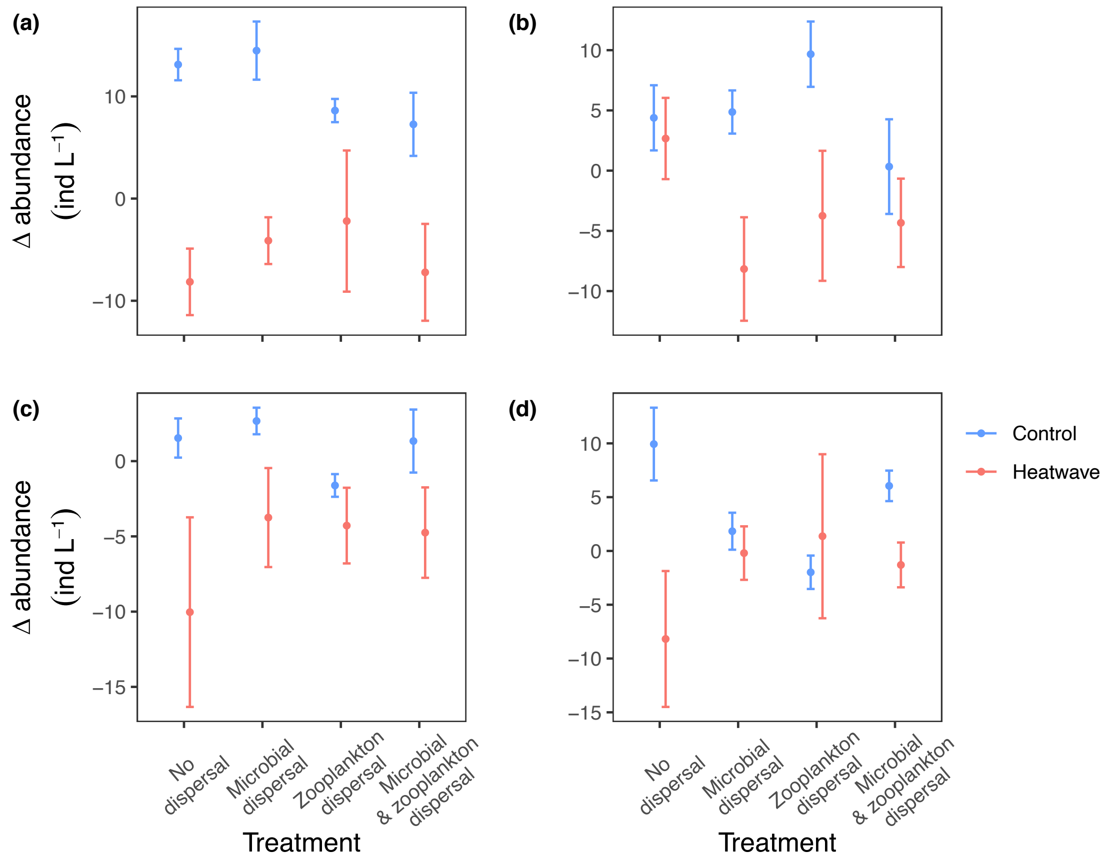

Figure S5.2. (a) Heatwave enhanced the decrease of cladoceran densities during the heatwave (t_h) relative to before the heatwave (t_0) ($F_{1,\,16} = 39.58$, $p < 0.001$), and **(b)** after the heatwave (t_{ah}) relative to t_0 ($F_{1,\,16} = 10.20$, $p = 0.006$). **(c)** The heatwave also enhanced the decrease of copepod density at t_h relative to t_0 ($F_{1,\,16} = 9.76$, $p = 0.007$). **(d)** There is a three-way interaction effect between heatwave, microbial and zooplankton dispersal on changes in copepod densities at time t_{ah} relative to t_0 ($F_{1,\,16} = 5.64$, $p = 0.031$). Zooplankton dispersal and microbial dispersal improved the recovery of copepod densities post-heatwave in heated mesocosms (as indicated by Δdensity ≥ 0), but decreased copepod densities in unheated mesocosms. Heated and unheated mesocosms that received only microbial dispersal or zooplankton dispersal are most similar after the heatwave, as indicated by copepod densities that are similar to, or higher than t_0 (Δdensity ≥ 0).

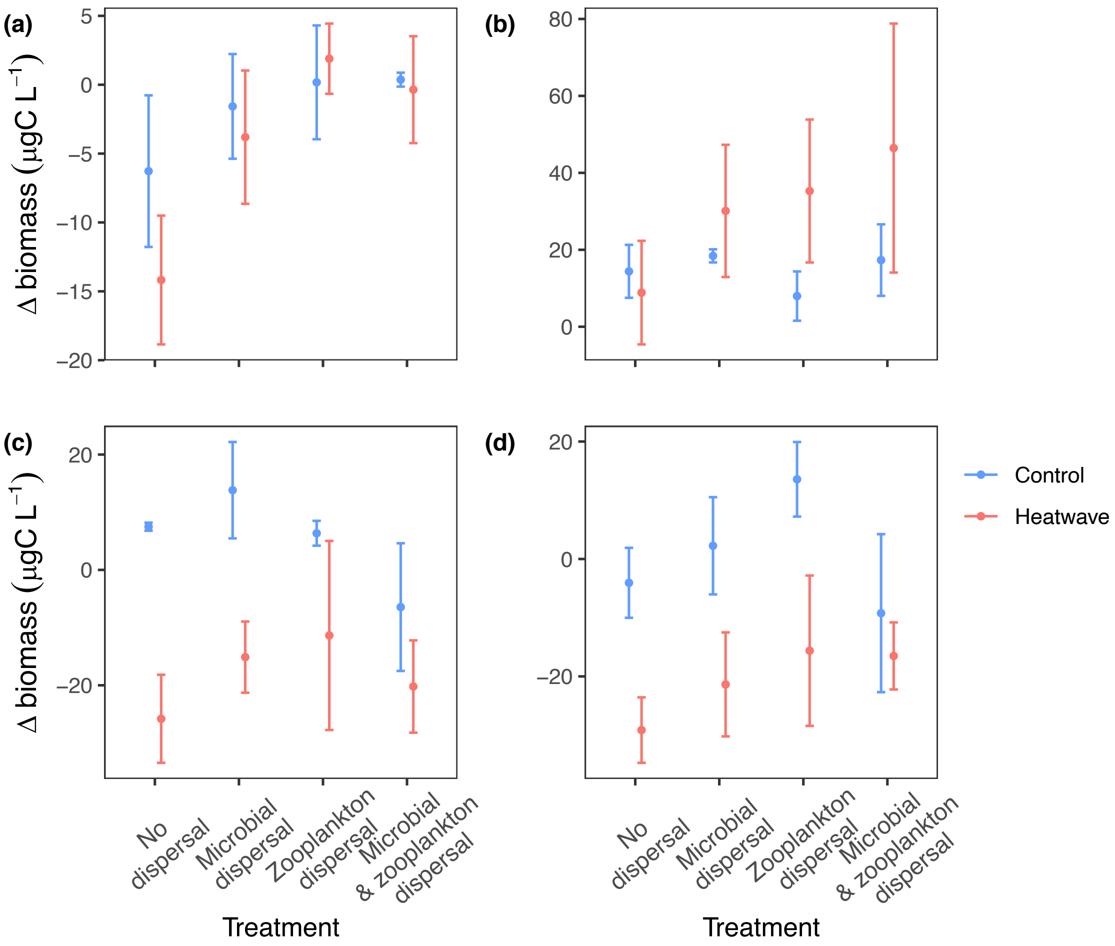

Figure S5.3. Heatwave did not influence biomass changes (Δbiomass) in **(a)** phytoplankton ($F_{1, 16} = 0.65$, $p = 0.432$). However, zooplankton dispersal dampened negative changes in phytoplankton biomass during the heatwave relative to before the heatwave (t_0) ($F_{1, 16} = 6.04$, $p = 0.026$). **(b)** There is no significant treatment effect on changes in phytoplankton biomass after the heatwave. In contrast, the heatwave had a negative influence on changes in zooplankton biomass both **(c)** during the heatwave relative to time t_0 ($F_{1, 16} = 14.03$, $p = 0.002$), and **(d)** after the heatwave relative to time t_0 ($F_{1, 16} = 11.49$, $p = 0.004$).

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