2017

THE VULNERABILITY OF DIFFERENT POPULATIONS OF THE COMMERCIALLY-IMPORTANT SHRIMP PANDALUS BOREALIS TO ENVIRONMENTAL STRESS

Hall, Emilie Florence

THE VULNERABILITY OF DIFFERENT POPULATIONS OF THE COMMERCIALLY-IMPORTANT SHRIMP *PANDALUS BOREALIS* TO ENVIRONMENTAL STRESS

by

EMILIE FLORENCE HALL

A thesis submitted to Plymouth University in partial fulfilment for the degree of

MASTER OF PHILOSOPHY

School of Marine Sciences & Engineering

August 2016

THE VULNERABILITY OF DIFFERENT POPULATIONS OF THE COMMERCIALLY-IMPORTANT SHRIMP *PANDALUS BOREALIS* TO ENVIRONMENTAL STRESS

THESIS ABSTRACT

The present study adopted an integrative approach to conduct a population comparison of vulnerability to environmental stress in a commercially important species of ectotherm. Specifically, I investigated how differing environmental conditions in native habitats may drive intra-species divergence and alter performance when conditions shift. This study used northern prawn (*Pandalus borealis* Krøyer 1838) populations with known morphological differences from two spatially proximate fjord sites differing in oxygen regime as a model system. The genetic population structure was analysed and whole organism, physiological, and metabolomic performance under hypoxia and thermal stress were assessed. Genetic analyses displayed no significant dissimilarities between *P. borealis* from the normoxic and the seasonally hypoxic site. It was hypothesised that phenotypic plasticity may act as mechanism by which *P. borealis* may persist in the seasonally hypoxic fjord. Subsequently, a common garden experiment, in which individuals from the two fjords were exposed to hypoxia and the additional stress of elevated temperature, was carried out. The populations did not show significantly different physiological performance as determined by metabolic rates and stamina. However, the experiment did confirm the negative impacts of hypoxia on this species. Finally, *P. borealis* were exposed to hypoxia in the field in a translocation experiment. As the laboratory methods used would not have been possible to replicate, performance was assessed by survivorship and metabolite regulation. *P. borealis* from the two fjords showed significantly different levels of survivorship and the metabolomic profiles demonstrated that both populations possess different levels or forms of phenotypic plasticity, as they responded differently to translocation. This thesis presents the first use of the mtDNA control region of this species being used to determine its genetic variation and emphasises the need for multidisciplinary, holistic and multi-population approaches to assessing species vulnerability.

Contents

List of Tables

List of Figures

AUTHOR'S DECLRATION

At no time during the registration for the degree of Master of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment.

This study was financed with the aid of a University Research Studentship paid by the School of Marine Science and Engineering.

A programme of advanced study was undertaken and postgraduate courses on molecular biology, laboratory skills, teaching and engagement skills, and scientific methodology courses were attended. All intellectual interpretation and work in this study is that of the author's except where specified otherwise.

Relevant scientific seminars were attended; one successful award and two successful grant applications were contributed to; external institutions were visited for consultation, training, field and laboratory work; and several papers are in preparation or have been published in conjunction with the research themes of this thesis.

Publications:

Dupont, S., **Hall, E**., Calosi, P. and Lundve, B., 2014. First evidence of altered sensory quality in a shellfish exposed to decreased pH relevant to Ocean Acidification. *Journal of Shellfish Research*, *33*(3), pp.857-861.

Harvey, B.P., Al-Janabi, B., Broszeit, S., Cioffi, R., Kumar, A., Aranguren-Gassis, M., Bailey, A., Green, L., Gsottbauer, C.M., **Hall, E.F**. and Lechler, M., 2014. Evolution of marine organisms under climate change at different levels of biological organisation. *Water*, *6*(11), pp.3545-3574.

Word count of main body of thesis: 25,938

Signed: ……  …….

Date:….. 01/08/2016……

ACKNOWLEDGEMENTS

I would like to express my gratitude to the University Research Studentship paid by the School of Marine Science and Engineering that made this project possible and for the funds and resources supplied by ASSEMBLE and the Sven Lovèn Marine Research Station - Kristineberg, the Kungliga Vetenskapsakademien (Swedish Academy of Sciences), the Natural Environment Research Council Biomolecular Analysis Facility – Birmingham, and the Primer Design Student Sponsorship Scheme.

I am also indebted to my supervisory team, Dr Manuela Truebano, Professor John Spicer, Dr Piero Calosi, Professor Paul Shaw, and Dr William Cheung, for their support and guidance throughout the project. I would especially like to thank my two Directors of Study, Manuela (2014-2016) and Piero (2013 – 2014), for their tireless patience, dedication and enthusiasm and to John for so kindly joining the team and becoming an invaluable part of it.

I would also like to extend my thanks to the many colleagues with whom I had the great fortune to work with on this project. Their contributions, advice, generosity, and help throughout the project have been hugely appreciated and it has been a real pleasure to work with them all. Thank you to my collaborators in Sweden, Dr Susanne Eriksson, Dr Hannah Wood, Dr Lucy Turner; to my collaborators at NBAF-B, Dr Ulf Sommer and Dr Jasper Engel; and to my collaborator in Aberystwyth, Dr Niall McKeowen. Thank you to everyone who helped me at MBERC, especially Marie Palmer, Roger Haslam and Mike Jarrold. Thank you to all the staff and students who helped me in Kristineberg: Bengt Lundve, Pia Engström, Linda Svanberg, Lars Ljungqvist, Marita Nyberg, the skippers of the RV Oscar von Sydow - Ursula Schwarz, Berne Petersson, and Silve, the skippers of the Gullbris - Karl-Erik and Royne Robertsson, Charlotte Alvord, Sam Dupont, Fredrik Berggren, Danny Cowing, Emma Hedlund, Vishnu Subramanian, Alexander Falkenstam, and Guo Xueli - tack så mycket!

Special thanks are due to Lotti, Niall, Sanna, Hannah, Bengt, Marie. Lotti, I am lucky to be able to call you my friend as well as my colleague; thanks for always going above and beyond. Niall, thank you for fielding all my questions with a smile and joke and taking such a genuine interest in the project. Sanna and Hannah, thank you for taking me under your wing during my time in Sweden and for all your help in developing what became the core of this thesis. Bengt, thank you for being my Go-To person in Sweden, for your enthusiasm, and for generally helping our experiments to become a reality. Marie, thank you for your patience, humour and all your help in developing my tools and the mesocosm.

Finally, my partner, my family and my friends (old and new) who may as well be family… for every pep talk, every hug, meal, G&T, laugh, distraction, motivation, adventure, for your loyalty and many kindnesses, you guys are the best! Dan, my parents, my Granddad, little Algie, Dr Griff, Leah, K, Jess, Sarah C, Sarah P, Dave, Jack, Laura, Albie, Jon, Nikki, Fraser, Amy, Rachel, Rachele, Sam M, Lucy, Joe, Molly, Dani and the many other people I am so lucky to have in my life, thank you for seeing me through to the end of this with a smile on my face.

Chapter 1: Thesis introduction

1.1 Population comparisons and environmentally-induced divergence

Recent research into evolutionary dynamics using genomics and other experimental approaches have indicated that environment-dependent selection plays a key role in divergence and speciation. However, current study is still working towards fully integrating the field of speciation genetics and environmental stress physiology (Lexer & Fay 2005). Environmental stress has long been investigated in concurrence with population extinction, but, despite its long established association with directional selection pressure, much work remains to be done investigating the evolutionary role of environmental stress (Hoffmann & Parsons 1997; Lexer & Fay 2005). A growing number of authors have cited environmental selection as the primary cause for phenotypic novelty (see Hoekstra et al. 2001; Merilä & Crnokrak, 2001; and Rieseberg et al. 2002 for reviews). Given the increasing prominence of environmental stress as a driver for divergence and evolution, it is reasonable to assume that populations from species occupying habitats with differing environmental conditions would be under selection pressure to adapt to their native conditions. Such stress induced adaptation has been observed in *Drosophila melanogaster* exposed under laboratory conditions to different microclimates (Michalak et al. 2001; Greenberg et al. 2003).

Investigating population level responses to environmental stressors is crucial to our understanding of how these stressors may impact fitness and shape species and communities (Harvey et al. 2014). Population comparison studies can offer insight into meta-population dynamics, species resilience, probability of local extinction, adaptive capacity, and fitness consequences of stress (Willi et al. 2006; Lande 2009; Johannesson et al. 2011; Reusch 2014). Population level study on physiology (Fangue et al. 2006; Smith-Keune & Oppen 2006; Kelly et al. 2012) , morphometrics (Marcil et al. 2006 a; Calosi et al. 2013), genetic structure and activity (Osovitz & Hofmann 2005; Schmidt et al. 2011), survivorship (Reusch et al. 2005; Kelly et al. 2012; Jin et al. 2013) and ontogeny (Sanford & Kelly 2011; Foo et al. 2012; Pecorino et al. 2014) of marine organisms has yielded a wealth of information regarding the vulnerability of marine ecosystems to environmental stressors.

Historically, in the marine environment, assumptions regarding connectivity and gene flow have caused population-level study of environmentally induced divergence to be dismissed (Hellberg et al. 2002). However, our knowledge of oceanographic heterogeneity continues to grow and gene flow amongst many marine invertebrate species is more restricted than previously thought (Sanford & Kelly 2011). As a result, populations inhabiting environmentally differentiated habitats

within a species range may be functionally or physiological divergent in order to maintain performance within their habitat.

Divergence between populations may manifest as differing levels of performance when exposed to stress or a shift environmental conditions, this is often termed phenotypic plasticity (see section 1.1.1). Should the traits associated with this phenotype have undergone fixation, differences in the frequencies of genotypes prevalent in each population would arise, leading to local adaption (see section 1.1.2). Such divergence may be driven by differing environmental conditions in the habitats occupied by each population or it may be driven by exposure of a population to novel environmental conditions.

1.1.1 Phenotypic plasticity

Differing levels of performance may be the result of phenotypic plasticity among individuals. Phenotypic plasticity refers to the capacity of set genotypes to react to environmental conditions by expressing changes (known as phenotypes) in form, state, behaviour or rates of activity (Pigliucci 2001; West-Eberhard 2002; DeWitt & Scheiner 2004; Whitman & Agrawal 2009). Phenotypic plasticity constitutes an important mechanism to cope with environment change. For instance, the cichlid *Pseudocrenilabrus multicolor* increases its total gill surface area by almost 20 % of its normoxic phenotype to maintain oxygen acquisition when exposed to hypoxic conditions (Chapman et al. 2000). This capacity to shift between phenotypes allows survival in a range of environmental conditions provided that the conditions an organism encounters do not exceed its plasticity.

While beneficial for tolerating a varied geographical range, phenotypic plasticity may be limited and incur costs. Prior exposure to a stressor known to induce phenotypic plasticity may enhance response time and confer a fitness advantage under the conditions which induce it or may be deleterious to the overall condition of the organism (Leroi et al. 1994; Bennett & Lenski 1997; Wilson & Franklin 2002; Woods & Harrison 2002). Plasticity is largely thought to occur due to the allelic sensitivity of structural genes to the external environment or from environmental stimuli causing shifts in expression by regulatory genes (Via et al. 1995). Many traits are associated with suites of genes and may, therefore, be interlinked with other traits. For instance, an environmentally induced change in morphology is often accompanied by changes in behaviour and physiology (Cornwallis & Birkhead 2008; Bourdeau 2009). Genetic costs may be incurred as a result of phenotypic plasticity if the gene(s) directly controlling the advantageous plastic trait are also linked to genes controlling other costly traits or if the advantageous plastic response gene

confers negative effects on other genes or alters the expression of other genes in a potentially deleterious manner (Stearns 1989; Moran 1992; Newman 1992). Additionally, the efficacy of phenotypic plasticity may be limited by a lag in stimulus exposure to phenotype expression (Levins 1968; West-Eberhard 1989; Moran 1992; Padilla & Adolph 1996).

Historically, research has focused on the genetic and ecological basis of diversification (Schluter 2000; Grant & Grant 2002; Coyne & Orr 2004; Rundle & Nosil 2005). Phenotypic plasticity has frequently been assumed to dampen selective pressure and reduce the likelihood of local adaptation. If plasticity is sufficiently large, it will nullify selective pressure and it is unlikely that there will be any shift in the frequency of genotypes within populations occupying different environmental conditions (Price et al. 2003). However, phenotypic plasticity may promote as well as inhibit divergence and local adaptation. Indeed, if selection pressure is sufficiently stable, phenotypic plasticity may enable local adaptation by fostering diverse genetic material and by exposing interlinked traits to novel selection pressures (Price et al. 2003; Cornwallis & Birkhead 2008; Bourdeau 2009; Pfennig et al. 2010).

1.1.2 Local adaptation

Acclimation through phenotypic plasticity may buffer against the immediate effects of stressors, but it can incur an energetic cost (Pörtner et al. 2004; Snell-Rood et al. 2010; Stumpp et al. 2012), and so may not be viable over extended periods. If the available phenotypes have sufficient underlying genetic variation, and gene flow and genetic drift are outweighed by stable environmental selection, local adaptation may prevent or delay local extinction (Lynch & Gabriel 1987; Mousseau & Roff 1987; Lande 1994; Travisano et al. 1995; Stanton et al. 1997; Whitlock et al. 2000; Sultan & Spencer 2002; Geber & Griffen 2003; Kawecki & Ebert 2004; Kritzer & Sale 2006; Hereford 2009).

Local adaptation is discussed in further detail in Chapter 2, but to summarise, local adaptation is a dynamic process whereby populations occupying an environmental gradient or mosaic become specialised to cope with their local abiotic and biotic constraints (Grant & Grant 1995). Natural environments across different spatial scales are heterogeneous as a result of spatial variation in abiotic and biotic factors. These differing factors may drive genetic fixation and heritable change between populations, selecting for differences in physiology, morphology, behaviour, or life history traits that contribute to overall fitness within their specific habitat (Williams 1966; Taylor 1991; Thompson 1999, Thompson & Cunningham 2002, Jormalainen et al. 2008; Sanford & Kelly 2011). The magnitude of these changes depends upon the balance between selective forces and factors

countering local adaption or specialisation (Sanford & Kelly 2011; Blanquart et al. 2013). Should selective forces acting upon a population outweigh the inhibiting factors, resident genotypes should have a higher fitness in their native habitat than genotypes from non-resident populations (Williams 1966; Grant 1995; Kawecki & Ebert 2004).

Local adaptation may be driven by any number of biotic and abiotic factors varying in the natural environment. Studies on coastal regions highlight how abiotic features, such as oceanographic structures and processes, can generate significant variation in oxygen, temperature, nutrients, pH, and other parameters over a range of spatial scales (Cooper & Brush 1993; Bustamante et al. 1995; Menge et al. 1997; Menge 2000; Feely et al. 2008). The amphipod *Orchestia gammarellus,* the frog *Rana arvalis,* the marine polychaete *Platynereis dumerilii,* the sockeye salmon *Oncorhynchus nerka, the* red abalone *Haliotis rufescens,* and the purple sea urchin *Strongylocentrotus purpuratus* are examples of species where populations have shown evidence for local adaptation manifesting in different levels of environmental tolerance (Gaston & Spicer 1998; Merilä et al. 2004; Calosi et al. 2013; Chen et al. 2013; De Witt & Palumbi 2013; Pespeni et al. 2013). These differences indicate that sensitivity to environmental change is not uniform across populations within a species, which raises many questions in the face of ongoing and anticipated climate change and habitat destruction.

Evolutionary processes have played a fundamental role in structuring current biodiversity patterns. Climate change and anthropogenic impacts are expected to impose strong selection pressures on a range of fitness-related traits (Somero 2005; Untersee & Pechenik 2007; Widdicombe & Spicer 2008; Somero 2010; Van de Putten et al. 2010; Dupont & Thorndyke 2013). Depending on adaptive capacity, some species may be able to find evolutionary rescue from environmental stressors (Sunday et al. 2014). To gain a clear view of the biogeographic and functional consequences of climate change and anthropogenic habitat degradation it is imperative that we understand levels of adaptive capacity, physiological plasticity and tolerances of populations occupying different zones within an environmental gradient (Bozinovic et al. 2011 Sunday et al. 2014).

1.3 Investigating differentiation

Two experimental approaches have generally been employed when investigating local adaptation (see Table 1.1 for examples): common garden laboratory experiments and field-based reciprocal transplants or translocations. The study outlined below will use both approaches.

Common garden experiments minimise the influence of undesirable environmental fluctuations and are used to identify the genotypic component of phenotypic variation. This is achieved by

exposing subjects to identical conditions in the laboratory (Sanford & Kelly 2011; see Conover 1998; Kawecki & Ebert 2004; and Conover et al. 2006 for reviews). In this study, specimens will be housed in a mesocosm to manipulate the environmental stressors in question and exposed according a crossed experimental design. With this approach, it is possible that field-collected organisms may retain the induced features of the environment they were collected from. Adequate acclimation to the laboratory or rearing specimens in the laboratory may negate this problem. Additionally, by their very design common garden experiments may inadvertently induce, overlook or exclude key factors that control specimen performance. Frequently, it is not feasible to replicate an organisms' entire habitat in the laboratory, which may lead to behavioural, nutritional, or other deficiencies that could confound results. Through *in situ* experimentation, reciprocal translocation experiments circumvent this limitation and allow the study organism access to many, if not all, resources and stimuli that it would habitually encounter (Sanford & Kelly 2011).

Field-based translocations allow us to assess whether local adaptation is universal across populations by testing whether each source population performs better in its native environment than do external populations (Kawecki & Ebert 2004; Leimu & Fischer 2008; Hereford 2009). Only six out of the fifteen translocation studies surveyed by Sanford and Kelly (2011) had results that indicated universal patterns of local adaptation. Nevertheless, this approach is not without its limitations. As with common garden experiments, the non-genetic variation induced by a population's native environment may continue to be expressed even after the specimens have been transplanted in their test environment. Furthermore, it may be difficult to extricate the influence of local adaptation versus phenotypic plasticity when interpreting reciprocal translocation data (Sanford & Kelly 2011).

Table 1.1 Studies that have identified either physiological differentiation or local adaptation using common garden (CG) or translocation (TE) experimental techniques. Drivers: temperature (T), salinity (S), hypoxia (H), habitat characteristics known to contribute towards natural selection (NS – including, but not limited to predation, nutrient level, light exposure, disturbance, wave action and desiccation). Outcomes: local adaptation (LA), phenotypic plasticity (PP)

Taxon	Experimental approach	Driver	Response variable	Outcome	Authors
Actinopterygii					
Danio rerio	CG	T, NS	Growth, thermal tolerance	PP	Schaefer & Ryan 2006
Pseudocrenilabrus multicolor victoriae	CG	H	Hematocrit and enzyme activity	LA	Martinez et al. 2009
Anthozoa					
Acropora millepora	CG	T	Coral bleaching	LA	Smith-Keune & van Oppen 2006
Metridium senile	CG	T	Respiration, enzyme activities	LA	Walsh & Somero 1981
Pocillopora damicornis	CG	T	Zooxanthellae density (coral bleaching), polyp behaviour	LA or acclimation PP; LA	D'Croz & Maté 2004; Ulstrup et al. 2006
Bivalvia					
Crassostrea virginica	CG	T	Growth, ciliary activity	LA	Dittman 1997; Dittman et al. 1998

Copepoda					
Acartia tonsa	CG	H	Habitat selection	LA	Decker et al. 2003
Scottolana canadensis	CG	T	Morphology –body size, growth, development rate	LA	Lonsdale & Levinton 1985
Tigriopus californicus	CG	S, T; T	Reproduction, life history; thermal tolerance	LA	Dybdahl 1989
Gastropoda					
Littorina obtusata	CG	T	Enzyme activity	LA	Sokolova & Portner 2001
Nucella canaliculata	CG	T	Heat tolerance, survival	LA	Kuo & Sanford 2009
Nucella ostrina	CG	T	Development rates	LA	Palmer 1994
Polychaeta					
Nereis diversicolor	CG	T	Survival	LA	Bryan & Hummerstone 1971
Anthozoa					
Porites lobata	TE	T	Growth, stress proteins	PP; LA	Barshis et al. 2010; Smith et al. 2007
Cirripedia					
Semibalanus balanoides	TE	T	Survival; genetic polymorphism	LA	Bertness & Gaines 1993; Schmidt et al. 2011
Gastropoda					
Littorina obtusata	TE	T, NS	Growth	LA	Trussell 2000
Magnoliopsida					
Suaeda maritima	TE	H	Metabolite levels, protein expression	PP	Weston et al. 2012

1.4 Environmental stressors

In the context of this study, the term *environmental stressor* is used to refer to environmental conditions that may increase fitness-related organismal costs or a shift in environmental conditions with the potential to increase organismal costs.

Coastal systems undergo dramatic changes in biotic and abiotic environmental factors as a result of natural and anthropogenic processes (Domenici et al. 2007). Persistent environmental gradients or regimes may constrain species performance and ranges or impose divergent selection, resulting in shifts in behaviour, morphology, physiology or life history traits providing a fitness advantage under local conditions (Brown et al. 2004; Peck et al. 2006; Sanford & Kelly 2011).

Studies on coastal regions highlight how features, such as oceanographic structures and processes, can generate significant variation in oxygen, temperature, nutrients, pH, and other parameters over a range of spatial scales (Cooper & Brush 1993; Bustamante et al. 1995; Menge et al. 1997, Menge 2000; Navarrete et al. 2005, Feely et al. 2008). In addition to this natural variability, anthropogenic impacts and, in particular, climate change, are liable to shift these environmental factors beyond their historic parameters and, potentially, existing organismal tolerance limits. Indeed, in a meta-analysis by Poloczanska et al. (2013) of 1735 observations of

biological responses of marine organisms in which environmental stressors were reported to be the driver, > 80 % of the responses were consistent with the expected impacts of climate change.

A number of key threats to marine life that are related to climate change have been identified by the scientific community (see IPCC 2014 for review). Amongst these, a reduction in marine oxygen availability (hypoxia) and increases in sea water temperatures have been particularly highlighted (Halpern et al. 2007; Diaz & Rosenburg 2008; Burrows et al. 2011; Poloczanska et al. 2013) as have their interactions (Pörtner et al. 2005; Vanquer-Sunyer & Duarte 2008; Zhang et al. 2010). A downward trend in oxygen availability has already been documented as a result of rising temperatures altering dissolution and increased extreme weather events (i.e. storms and heavy rainfall) that increase terrestrial input increasing the risk of eutrophication events (see FAO 2014 a for meta-analysis).

1.4.1 Hypoxia

1.4.1.1 Historical and current

The end of the Permian period was punctuated by mass extinction (Erwin 1993; Bowring et al. 1999). Modelling, isotope and paleontological evidence suggest that this was largely attributable to an acute climate crisis (Knoll et al. 1996; Benton & Twitchett 2003) involving climate warming and plummeting oxygen levels (Graham et al. 1995; Lasaga & Ohmoto 2002; Sheldon et al. 2002; Berner et al. 2003; Berner 2004; Kidder & Worsley 2004; Huey & Ward 2005). Observations from the field show that oxygen is once again in decline globally in the marine environment (Diaz 2001; Levin 2003; Whitney et al. 2007; Chan et al. 2008; Diaz & Rosenberg 2008) and ocean models project declines of 1-7 % global oceanic oxygen by 2100 and predict that this drop will continue for at least a millennium (Keeling et al. 2010). As a consequence of such dramatic and rapid environmental changes, mass shifts in phenology and distribution and extinctions across phyla have been documented in conjunction with observed climate change and on concurrent trajectories with predicted climate change (see Parmesan 2006 for review).

In addition to climate related hypoxia, coastal oxygen depletion is also caused by eutrophication. Dissolved organic matter in terrestrial run off causes nutrient fluxes in coastal systems. This produces excess particulate organic matter, which uses up dissolved oxygen in the water column as it degrades (Gray et al. 2002). Despite reductions in dissolved oxygen consistently being documented in all major ocean basins throughout the last 50 years (Bindoff & McDougall 2000; Matear et al. 2000; Keller et al. 2001; Ono et al. 2001; Stramma et al. 2010) and the known links with previous mass extinctions, the risks of oxygen depletion have received relatively little of the climate change research effort until recently (Scicurve 2014).

1.4.1.2 Biological and physiological responses

Many of the biological and physiological response mechanisms involved are not yet well understood (Wu 2002). Oxygen is directly linked to the rate at which organic matter is produced, redistributed, and decomposed in the ocean (Keeling et al. 2010). In addition, energetics, motor activity, behaviour, feeding, digestion, growth, and reproduction are all influenced by oxygen availability at the organismal level (Pörtner et al. 2005).

Hypoxia has been shown to affect metabolism, energy budgets (i.e. investment into growth and reproduction) and homeostatic processes (Stickle et al. 1989; Spicer & El-Gamal 1999; McMahon 2001; Spicer & Eriksson 2003; Eriksson et al. 2006; Clark et al. 2013), alter behaviour (Gerhardt & Baden 1998; Riedel et al. 2013) and lower tolerance in marine organisms to other environmental stressors (Frederich & Pörtner 2000; Pörtner & Knust 2007). At the species-level, organisms from chronically or periodically hypoxic habitats are known to be more tolerant of hypoxia than those from consistently normoxic habitats (Hagerman 1998; Diaz & Rosenberg 1995; Diaz & Breitburg 2009). Mechanisms, such as induced metabolic depression, increased ventilation rates, increased production of respiratory pigments or alteration of respiratory pigments to increase oxygen affinity (Baden et al. 1990; McMahon 2001; Leiva et al. 2015) in order to tolerate hypoxic conditions have been observed across taxa. Oxygen levels are a key determinant of organismal performance and, ultimately, fitness. It therefore seems intuitive that it should drive population divergence or local adaptation, as previously documented in the copepod *Acartia tonsa* (Decker et al. 2003), the molluscs *Amygdalum anoxicolum, Pitar sewelli, Indocrassatella indica,* and *Haliotis rufescens* (Oliver 2001; Gibson & Atkinson 2003; De Wit & Palumbi 2012), and cichlid fish *Pseudocrenilabrus multicolor* (Crispo & Chapman 2011).

1.4.2 Elevated temperature

Global temperature patterns fluctuate naturally as a result of solar irradiance and volcanism (Crowley 2000). However, the level of global warming observed over the last century cannot be explained by natural forcing (Mitchell et al. 2001) leading the IPCC to conclude that human activities - in particular green house gas and aerosol emission – are having a significant impact on the climate (Griggs & Noguer 2002). The rate at which global temperature levels are rising may outpace the ability of many organisms to adapt or shift their phenologies or biogeographic ranges (Root et al. 2003; Edwards & Richardson 2004; Schweiger et al 2008; Somero 2010).

1.4.2.1 Historical and current

Ice core analysis shows relatively stable levels of atmospheric greenhouse gases over the past 1 kyr (approximately 700 ppb CH4 and 280 ppm CO_2) until the 19th Century (MacFarling Meure 2006; Le Treut et al. 2007; IPCC 2013). Following the industrial revolution and rapid population growth, methane and carbon dioxide levels have risen to > 1830 ppb and 400 ppm respectively in the present day (IPCC 2013; Dlugokencky & Tans 2016). This peak abundance in methane far exceed the range of 400 to 700 ppb seen over the last half-million years of glacial-interglacial cycles, and the timing and rate of increase can be readily explained by anthropogenic emissions (Le Treut et al. 2007).

Greenhouse gases trap radiation, raising global temperatures; the increases in methane and carbon dioxide have been accompanied by an upward trend in temperature over the past one hundred years. Over the last 50 years, the mean rate of warming has been 0.13°C ± 0.03°C per decade, approaching twice that for the previous 100 years (Solomon 2007). The majority of this additional thermal energy has been absorbed by the oceans. The heat content of the upper 700 m of the global ocean has increased by 14×10^{22} J since 1975 (Levitus et al. 2009) and thermal stress has received much of the climate change research effort to date.

1.4.2.2 Biological and physiological responses

Temperature has already been seen to cause shifts in marine ecosystem composition and function (Hiscock et al. 2004; Perry et al. 2005; Pörtner & Knust 2007; Poloczanska et al. 2013). Shifts in and biodiversity, geographical, metabolic and life history along with phenological mismatches between functional groups and across trophic levels, have arisen as different taxa are driven by a variety of temperature related cues and governed by varying tolerance limits (Pörtner et al. 2001; Beaugrand et al. 2002; Edwards & Richardson 2004; Rand et al. 2006; Parmesan et al. 2013).

Beyond disrupting ecological interactions, increases in temperature directly affect an individual organism's physiology from inducing protein damage, to altering membrane fluidity and organ function (Hochachka & Somero 2002). As many marine organisms already live close to their thermal maxima (Somero 2002; Hughes et al. 2003; McWilliams et al. 2005), even minor changes to current temperature levels may be highly deleterious. Thermal stress appears to drive local adaptation or divergence within species with populations that persist in areas impacted by elevated temperature. For example the seagrass *Zostera marina* (Hämmerli & Reusch 2002), anthozoan *Acropora millepora* (Smith-Keune & van Oppen 2006), coral *Pocillopora damicornis*

(D'Croz & Maté 2004), blue mussel *Mytilus trossulus* (Yanick et al. 2003), red abalone *Haliotis rufescens* (De Wit & Palumbi 2013), amphipod *Orchestia gammarellus* (Gaston & Spicer 1998), acorn barnacle *Semibalanus balanoides* (Schmidt et al. 2011), copepod *Tigriopus californicus* (Kelly et al. 2012), fiddler crab *Uca pugnax* (Sanford et al. 2006), the echinoderms *Heliocidaris erythrogramma armigera* (Lymbery & Evans 2013), *Centrostephanus rodgersii* (Foo 2012; Pecorino et al. 2014) and *Strongylocentrotus purpuratus* (Osovitz & Hofmann 2005), the Atlantic cod *Gadus morhua* (Marcil et al. 2006 b), and the killifish *Fundulus heteroclitus* (Fangue et al. 2006) have all exhibited local adaptation or specialisation correlating with thermal stress. Some of these adaptations may mitigate thermal stress and achieve metabolic compensation by means of acclimatisation, the synthesis of heat shock proteins to tolerate short term temperature fluctuations, behavioural thermoregulation, altering enzyme concentration and form, altering cardiac and respiratory rates, gill structure, or respiratory pigment concentrations (Jueterbock et al. 2013; Narum et al. 2013; Newell 2013; Tepolt & Somero 2014).

1.3.1 Stressors in combination

Single stressor studies make controlling for that variable, analysis of the resulting data and causal inference more straightforward. However, as no environmental driver acts in isolation, the relevance of these studies is limited and a growing body of evidence indicates that the levels of stress induced by these environmental variables when combined may not simply be additive. Darling and Côté (2008) found that, across a meta-analysis of 112 factorial experiments studying the effects of multiple drivers of ecological change, more than three quarters of the experiments demonstrated interactions between stressors. Crain et al.'s (2008) meta-analysis of 171 multi-stressor marine and coastal studies found that 26 % of cases documented additive, 36 % synergistic, and 38 % antagonistic interactions between stress responses. For example, choice tests on the Atlantic cod, *Gadus morhua*, revealed that preferred water temperature fell from 13.9 to 13.1, 10.0 and 8.8 °C when exposed to normoxia, 30, 20 and 15 % oxygen air saturation respectively (Shurmann & Steffensen 1992) and reviews of fish across species suggest that hypoxia and thermal stress interact synergistically with regards to metabolic rate (McBryan et al. 2013).

Hypoxia is intrinsically linked with global warming. Henry's Law (1803) dictates that gas dissolution has an inverse relationship with the temperature of the liquid medium. Hence, as sea temperatures increase, less free oxygen can be accommodated and therefore acquired by respiring organisms. Furthermore, elevated sea temperatures may cause stratification (Cerrano et al. 2000; Romano et al. 2000; Sparnocchia et al. 2006) and changes to marine circulation (Joos et al. 1999; Matear et al. 2000; Keeling & Garcia 2002), altering oxygen availability. Thermal stress

and hypoxia in combination cause oxygen consumption levels to increase (Daoud et al. 2007); and beyond a critical point raise lactate and glucose concentration and deplete glycogen (Taylor & Spicer 1987) as respiratory processes shift from aerobic to less efficient anaerobic metabolism (Finke et al. 1996; Pörtner 2001; Clarke et al. 2014). This combination may also induce changes in substrate oxidation and respiratory pigments, mitochondrial complexes, proton leak, ventilation rates and heart mass in order to improve energetic efficiency (Spicer & Baden 2001; Melzner et al. 2006; Oellermann et al. 2012); cause metabolic depression or hypometabolism (St-Pierre et al. 2000) or reductions in aerobic scope (Dupont-Prinet et al. 2013).

1.4 Overall aim, hypotheses, chapter aims and objectives

The aim of this study is to investigate whether environmental stressors can drive divergence between populations and how such divergence may alter responses to other environmental stressors. More specifically, this thesis investigates the mechanisms underpinning the relative vulnerability to reduced oxygen and elevated temperature of two populations of *Pandalus borealis* from sites that experience different oxygen regimes.

The subsequent chapters adopt an integrative approach to investigate environmental stress-driven vulnerability and divergence in two spatially proximate populations (< 50 km distance) of the northern shrimp, *Pandalus borealis*, from two environmentally distinct sites. Taking what was discussed above (Sections 1.1-1.5), the following is predicted concerning *Pandalus borealis* at Gullmarfjord (seasonally hypoxic) and Brofjorden (normoxic):

- The two studied populations are genetically distinct.
- The two studied populations differ in tolerance to environmental stressors.
- That oxygen regimes in the natural environment have induced difference physiological responses between the populations studied.

These predictions will be tested using a multi-disciplinary approach involving different levels of biological organisation, in order to gain a holistic view of *P. borealis* responses. To investigate whether hypoxia has driven differentiation in two populations of *P. borealis* and the vulnerability of those populations to multiple environmental stressors, the subsequent chapters will cover the following:

- In **Chapter 2**, this thesis will discuss the genetics of local adaptation of populations from the same species and tests for differences in genetic structuring between the Brofjorden and Gullmarfjord populations.

- **Chapter 3** focuses on physiological differentiation and vulnerability to multiple environmental stressors. Following exposure to elevated temperature and hypoxia in a laboratory-based common garden experiment, measures of metabolic rate will be used to discern any difference in performance between the two populations.
- To ensure that the results of this thesis are sufficiently robust, the common garden investigation into phenotypic plasticity and physiological responses to environmental stressors, will be followed a field-based translocation experiment in **Chapter 4**. This approach allows us test our differentiation hypothesis in the natural environment. Due to the technical limitations of using the same parameters as the common garden experiment in the field (Resting Metabolic Rate and stamina), survival and metabolomics are employed as a proxy for physiological performance.
- The results of the three experimental chapters will be compared and evaluated in the context of the existing literature regarding local adaptation and environmental stress responses, climate change, and species management in **Chapter 5**.

1.4.1 Site choice

Coastal areas, are characterised by complex oceanographic features that have caused them to be neglected by researchers in the past due to their relative inaccessibility (Menge 1992; Sandford & Kelly 2011). This inherent heterogeneity is linked with persistent abiotic parameters, such as temperature, and salinity boundaries, which have more recently been proposed as barriers to population connectivity for various marine species in this region (e.g. cod, Gadus *morhua*, Jorde et al. 2007; corkwing wrasse, *Symphodus melops*, Knutsen et al. 2013; and sprat, *Sprattus sprattus*, Glover *et al.*, 2011).

Fjord systems undergo stratification as a result of freezing and reduced circulation. This can lead to periodic hypoxia or anoxia. The fjords on the west coast of Sweden are home to multiple populations of *P. borealis* (Andre pers. comms. 2014) which, according to the Swedish Meteorological and Hydrological Institute records, encounter very different oxygen regimes. Hypoxic events and cycles in the past 50 years in the fjords studied in this thesis and those adjacent have been associated with a range of significant ecological changes at depths typically occupied by *Pandalus borealis*. These include reduced biodiversity, planktonic communities and benthic foraminifera dominated by hypoxia-tolerant clades, total collapse of sensitive macrofauna – including Crustacea - in benthic communities, disrupted bioturbation and organic cycling (Josefson & Widbom 1988; Lindahl & Hernroth 1988; Austen & Widbom 1991; Gustafsson & Nordberg 1999; Gustafsson & Nordberg 2000; Nordberg et al. 2001; Filipsson & Nordberg 2004).

Gullmarfjord, on the west coast of Sweden 80 km north of Gothenburg (58°20’26.1”N 11°33’31.7”E), is subject to periodic hypoxic events typical of the inner fjords close to the coastline (Rosenberg 1985; Rosenberg et al. 1990; Gustafsson & Nordberg 2000; Andersson et al. 2009). This is primarily due to seasonal freezing and limited tidal activity increasing the stratification of the water column. This effect is compounded by naturally high productivity together with anthropogenic eutrophication leading to increased microfaunal oxygen consumption and decay, particularly in the later seasons (Gustafsson & Nordberg 1999). Hydrography in the Gullmarfjord has been monitored since the late nineteenth century by the Fishery Board of Sweden, the Kristineberg Marine Biological Station and the with data made available by the Swedish Meteorological and Hydrological Institute (SMHI) (Josefson & Widbom 1988). Gullmarfjord has a sill at 42 m water depth and a basin with a maximum depth of 120 m. The mean residence time for the water mass above the sill is approximately one month (Rydberg 1977), whereas the residence time for water in the *Pandalus borealis* habitat in the deep basin is 8 to 10 months (Lindahl 1987). Deep-water renewal of the fjords in this area occurs during winter to early spring during upwelling of more saline water occurs along the coast and when the fjord thermoclines are weakest. During upwelling, saline water, oxygenated from the sea spills over the fjord sills and flows into the fjord basins (Gustafsson & Nordberg 1999).

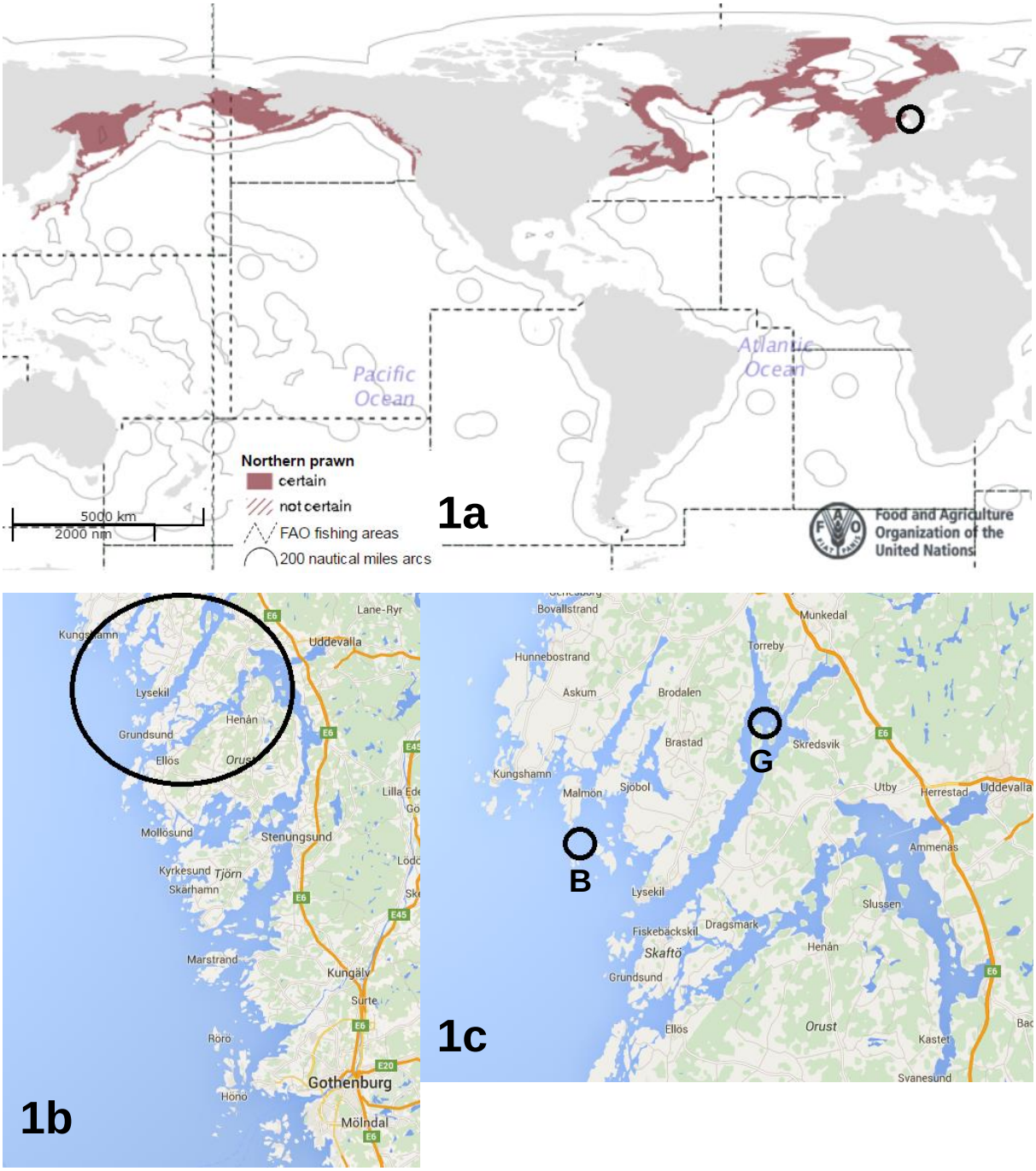


Figure 1.1 Map of *Pandalus borealis* population locations. 1a (FAO 2016) displays *P. borealis* global distribution with a black circle indicating the location selected for this study with reference the species geographical range. 1b (Google Maps V. 3.24 2016): the study location (indicated with a black circle) with reference to global landmarks). 1c (Google Maps V. 3.24 2016): the population sampling sites; Brofjorden individuals were collected from south of Malmön at the black circle labelled B, Gullmarfjord individuals were collected from west of Skredsvik at the black circle labelled G.

By contrast, the outer fjords, such as Brofjorden (58°18'12.4"N 11°17'59.7"E, 100 m deep at the mouth sample point), have greater water exchange with the open ocean, maintaining oxygen levels. The Gullmarfjord, Brofjorden and their surrounding localities are home to multiple populations of *Pandalus borealis.* Populations occupying the inner and outer fjords are reported to be morphologically distinct in size and pigmentation (Eriksson, pers. comms 2013), which may be related to local adaptation associated with their respective oxygen regimes.

The study populations are situated in relatively close proximity to one another – approximately 20 kilometres apart (over > 45 km *via* waterways). However, research has shown that at mesoscales (tens of kilometres), populations have evolved a range of tolerance levels to mirror the environmental conditions that they inhabit (Sokolova & Boulding 2004; Untersee & Pechenik 2007). Indeed, in a survey of 59 population comparison studies that cite local adaptation as an explanation for population response, Sandford and Kelly (2011) found that 23 of these studies sampled populations that were situated less than 20 km apart.

Hypoxia in fjord systems is not temporally constant, but it is temporally consistent and long lasting due to the stable features of the coastal bathymetry. These fjords are interspersed with centres of urbanisation - including a port situated in inner Brofjorden and settlements either side of Gullmarfjord - which are sources of eutrophication and other pollutants. Between the strong and persistent gradients induced by the local bathymetry and anthropogenic stressors, this environment comprises a complex mosaic with the potential to drive local adaptation within its communities.

1.4.2 Species choice

Marine ectotherms, in particular invertebrates, are taxonomically diverse and also exhibit a wide array of life histories and dispersal abilities, making then particularly appealing test subjects for investigating the interplay between divergent selection and gene flow (Sanford & Kelly 2011).

With reference to the environmental variables considered in this study, temperature elevation in conjunction with oxygen depletion poses a particular threat to ectotherms (Paaijams et al. 2013), whose metabolic rates and ability to meet oxygen demands are governed by temperature and oxygen concentrations. Crustaceans represent an ectotherm clade exhibiting high sensitivity to hypoxia, similar to that of echinoderms, with LT50 considerably shorter than molluscs and annelids (Gray et al. 2002; Vanquer-Sunyer & Duarte 2008). As such, the present study focuses on a commercially important crustacean species, *Pandalus borealis*, with a broad biogeographic range, which covers highly varied environmental conditions.

The northern shrimp *Pandalus borealis* (Krøyer, 1838) represents one of the most important harvested species in the Arctic Circle and sub-Arctic regions. *Pandalus borealis*, yielded almost 300,000 tonnes in 2013, dropping by more than 150,000 tonnes from its 2004 peak (FAO 2016). This species is also important as a primary food source for cod and other high value fish (Albers & Anderson 1985), and represents a large proportion of the biomass in the habitats it occupies in the Barents Sea and areas around Svalbard (Butler 1964; Garcia and Sims 2007). *P. borealis* profile

as a commercially important species means that its populations are subject to current management policies and some work has already been undertaken to determine population structure.

Pandalus borealis, is a prime candidate for investigating genetic specialisation and divergence with reference to environmental stress. This circumpolar demersal species has a highly varied habitat, occupying open ocean soft mud or sand demersal zones to inshore regions usually at around 50 – 500 m depth on the continental shelves of the North Atlantic (Shumway et al. 1985; Barr 2011). This species is thought to be adapted to locally occurring temperatures; 6 – 9 °C at its southern range edge (41 degrees latitude, Schlüter & Jerosch 2009) to 0 – 4 °C in the Barents Sea (80 degrees latitude, Furevik 2001; Boitsov et al. 2012; FAO 2016).

Pandalus borealis has a relatively long life span and a complex life history, requiring three to six years to undergo all life stages*. Pandalus borealis* are protandric hermaphrodites with five zoeal stages, one megalopa stage, and one post-larval stage, that once adult, change from male to female (Haynes & Wigley 1969; Rasmussen & Aschan 2011). The size at which individuals change sex is negatively correlated with temperature (Wieland 2004). They spawn throughout the autumn with the majority of shrimp only producing eggs once, usually at 3.5 years old. Eggs are brooded (800 – 3400 per clutch), attached to the pleopods, for approximately six months and then hatch in the spring (March – April). They drift as pelagic larvae for 45 – 90 days depending on sea temperature before settling on the sea floor (Shumway et al. 1985). This complex life history has significant implications for facilitating local adaptation; the larvae may drift for several hundred kilometres (Pederson et al., 2003), which implies extensive gene flow along currents. In addition, egg-brooding females migrate from offshore to shallower inshore waters during maturation and, consequently, may make up more than 90 % of the commercial yields (Bøhle 1977; Haynes & Wigley 1969; Koeller 2000).

Reflecting this statistic and given the practicalities of specimen collection, the present study used exclusively female shrimp. Females have also been documented to be less tolerant of hypoxia; they undergo a greater decrease in specific activity of enzymes involved in anaerobic biochemical pathways and greater reductions in aerobic scope compared to males (Dupont-Prinet et al. 2013). The anthropogenic sex and size skewed selection pressure in conjunction with *P. borealis* gender-correlated vulnerability and a complex temperature-mediated life history (Wienberg 1982; Rasmussen & Tande 1995) make it a particularly interesting seafood species to study in the context of climate change and local adaptation.

Pandalidae exhibit genetic structuring at a regional scale (Kartavtsev et al. 1991; Kartavtsev et al. 1993; Rasmussen et al. 1993; Jonsdottir et al. 1998; Drengstig et al. 2000; Martinez et al. 2006;

Jorde et al. 2015; Knutsen et al. 2015) down to populations only tens of kilometres apart (Jonsdottir et al. 1998; Drengstig et al. 2000; Martinez et al. 2006). A survey by Knutsen et al. (2014) of *Pandalus borealis* from 20 different oceanic and inshore sites found shrimp occupying the fjords to have significantly more population structuring than their oceanic counterparts.

Pandalus borealis are moderately well studied though information regarding their responses to hypoxia is limited. Across all life stages at temperatures of 5 and 8 °C, oxygen consumption at rest is largely explained by individual *P. borealis* weight and the treatment temperature (Daoud et al. 2007) and *P. borealis* northward distributional shifts have been documented concurrently with temperature increases (Wieland 2005). for embryo survival, hatching size and age at sex change, *Pandalus borealis* has been found to have an inverse relationship with temperature levels (Apollonio et al. 1986) and a positive relationship with larval growth, feeding and metabolic rates (Arnberg et al. 2013). Conversely, acidification delays zoeal progression in *P. borealis* (Bechmann et al. 2011). Adult *Pandalus borealis* are capable of a moderate pH regulation under acidified conditions without significantly increasing the ion-regulating activity of total ATPase and Na^+/K^+-ATPase, but this does cause a significant increase in ammonia excretion rates (Hammer & Pederson 2013).

In a survey of the metabolic performance and thermo-tolerance of caridean shrimp inhabiting British waters, Magozzi and Calosi (2015) found that *P. borealis* sister species, *P. montaguii*, had the highest oxygen consumption at the lowest temperatures, the lowest upper thermal tolerance limit, most restrictive phenotypic plasticity, and consumed significantly higher quantities of oxygen at elevated temperature than the other sub-tidal species studied. Across other caridean shrimp, hypoxia increases L-lactate and glucose in the haemolymph and tissues, reduces glycogen levels (Taylor & Spicer 1987), causes pronounced hyperventilation and acid-base disturbances (Taylor & Spicer 1991), and upregulation of gene expression coding for proteins such as muscle (Li & Brouwer 2009). Elevated temperature has a negative effect on maintaining hypo-osmoregulatory capacity (Janas & Spicer 2010). There also appears to be a notable degree of inter-species variability in vulnerability within this Caridean shrimp. However, much remains to be discovered regarding the adaptive capacity of pandalids. NGS studies into the sister species, *Pandalus latirostris*, inhabiting two separate lagoons revealed two fold difference in gene expression, suggesting local adaptation between the two populations (Kawahara-Miki et al. 2011). Other than this, very little has been tested regarding *Pandalus* local adaptation.

Chapter 2: Investigation of population genetic structure of *Pandalus borealis* at Brofjorden and Gullmarfjord

2.1 Abstract

There is a growing body of evidence documenting local adaptation between populations of marine organisms occupying sites across environmental gradients or mosaics. This study compared the genetic population structure of individuals from two morphologically-distinct populations of the northern prawn, *Pandalus borealis*, one from a normoxic and one from a periodically hypoxic site. For the first time, the mtDNA control region was used to assess genetic population structure in *P. borealis*. MtDNA and microsatellite data analyses did not detect any significant genetic dissimilarity between the two populations from which it was inferred that there are likely to be high levels of interbreeding. Consequently, I conclude that any selective forces arising from the different habitats are outweighed by gene flow and so other mechanisms must be utilised to allow *P. borealis* to persist in the periodically hypoxic fjord.

2.2 Introduction

Numerous species have been found to respond non-uniformly at the population level to environmental stressors (e.g. Dunlap & Wingfield 1995; Bacanskas et al. 2004; Knight et al. 2006; Pearson et al. 2009; see Sanford & Kelly 2011 for a review). Increasingly, evidence suggests that many marine species are spatially structured into discernibly reproductively isolated populations (Pereyra et al. 2012) and that microevolution is not restricted to marine organisms with low dispersal abilities (Sandford & Kelly 2011). Local adaptation, manifesting as environmental tolerance trait variation, has been documented on numerous occasions using physiological and -omics approaches (Gaston & Spicer 1998; Merilä et al. 2004; Stillman & Tagmount 2009; Calosi et al. 2013; Chen et al. 2013; De Witt & Palumbi 2013; Pespeni et al. 2013). Characterising the genetic structure of populations in the marine environment could lead to more effective conservation and commercial stock management policy by enabling the investigation of relative vulnerabilities of different populations and individual action plans for selected populations (Begg & Waldman 1999; Booke 1981).

In the marine environment, genetic homogeneity within species is maintained through the movement of gametes, individuals, or populations (Slatkin 1987). Most marine species have at least one highly dispersive stage (Cowen et al. 2000), for example gametes or pelagic larvae and

eggs during broadcast spawning. These dispersive stages will be subject to transport *via* ocean currents leading to extensive mixing of individuals (Scheltema 1971). Many species also undergo active migrations associated with environmental variations or internal conditions, such as reproductive maturity (Pittman & McAlpine 2003). These species are highly varied and include, but are not limited to, decapod crustaceans, such as the rock lobster *Palinurus spp.* (Kanciruk & Herrnkind 1978; Groeneveld & Branch 2002), blue crabs *Callinectes sapidus*, spider crabs *Maia squinado*, and Christmas Island crab *Gecarcoidea natalis* (Hines et al. 1995; Adamczewska & Morris 2001), numerous squid species (see for examples Ballantyne et al. 1981; Ehrhardt 1991; O'Dor 1992; Arkhipkin 1993; Basson et al. 1996), and at least 401 species of fish, 319 species of marine birds, 102 species of marine mammal, and seven species of marine reptile (Lascelles et al. 2014).

By contrast, marine populations are subject to five key factors that may serve to limit gene flow in the marine environment in spite of this potential for high connectivity. Firstly, larval behaviour may constrain dispersal by counteracting passive transport *via* ocean currents (Levin 2006; Butler et al. 2011). Secondly, vertical stratification, eddies and gyres operating within the aforementioned currents may act as retention mechanisms for pelagic larvae (Leis 1991; Cowen & Castro 1994; Banks et al. 2007; Knutsen et al. 2007; Myksvoll et al. 2014). Thirdly, passive transport may cause larvae to settle in a habitat in which their phenotype might be selected against, leading to isolation of the parent population or adaptation amongst the newly recruited population (Nosil et al. 2009). Fourthly, once settled, subsequent life stages may undergo a return migration from nursery or feeding areas to spawning grounds, as seen in northeast Arctic cod (Hjort 1914; Westgaard & Fevolden 2007). Finally, spatial distance alone may be sufficient to isolate populations as it may not be feasible for individuals or gametes to mix within the time constraints of their dispersive life stages (Slatkin 1993). Local adaptation may take place when environmental gradients within a species range impose selection on phenotypes and gene flow is inhibited by the above factors.

The northern prawn, *Pandalus borealis,* is a prime candidate for investigating genetic specialisation and divergence. *Pandalus borealis* is a circumpolar, ectothermic crustacean of high economic value, meaning that its populations are subject to current management policies and some work has already been undertaken to determine population structure. It has a highly varied habitat, occupying open ocean demersal zones to inshore regions, typically at depths between 50 and 500 km, and occurring from 41 to 80 degrees latitude (Shumway et al. 1985; Barr 2011; FAO 2016). *Pandalus borealis* is subject to all the above factors concerning gene flow and has a relatively long life span and a complex life history, requiring three to six years to undergo all life stages, and is protandric. Eggs are brooded for four to ten months and *P. borealis* has five pelagic

larval stages subject to oceanic current dispersal for up to three months (Shumway et al. 1985; Ouellet & Chabot 2005; Ouellet & Allard 2006). This complex life history has significant implications for facilitating local adaptation; the larvae may drift for several hundred kilometres (Pederson et al. 2003), which implies extensive gene flow along currents.

A number of studies investigating population genetic structure at local (tens of kilometres apart) and regional scales (hundreds to thousands of kilometres) have been carried out on *Pandalus borealis* over the past quarter century. Through allozyme, RAPD and microsatellite analyses these studies found broad scale dissimilarities between populations occupying two different regions in the Pacific and ten to twelve regions covering the North Atlantic and Arctic (Kartavtsev et al. 1991; Kartavtsev et al. 1993; Rasmussen et al. 1993; Jonsdottir et al. 1998; Drengstig et al. 2000; Martinez et al. 2006; Jorde et al. 2015; Knutsen et al. 2015). Frequency of differentiation at the local scale in each of these studies appears to be relatively variable. Drengstig et al.'s (2000) allozyme analysis found genetic divergence between eight Norwegian fjords, but widely geographically separated samples from the Barent Sea appeared genetically homogeneous. Martinez et al. (2006) studied two of the Norwegian fjord groups Drengstig et al. (2000) identified as distinct, but did not discern any differentiation using RAPD analysis between the two. However, their analysis was sufficiently sensitive to indicate divergence between two populations on the coast of Jan Mayen. Geographic distance and temperature gradients were the key drivers of differentiation posited by these studies. Indeed, Jorde et al.'s (2015) Regional Ocean Modelling System, based on microsatellite data from across the species' range in the North Atlantic, showed that temperature explained almost 26 % of the genetic differentiation in *P. borealis* compared with larval drift only accounting for 2.5 – 4.7 %. Both Jonsdottir et al. (1998) and Drengstig et al. (2000) uncovered genetic differences between inshore and offshore localities. These and Knutsen et al.'s (2015) study suggest that oceanographic features, such as temperature and salinity gradients, or bathymetry, may act as barriers to population connectivity (Knutsen et al. 2015). Evidence of such barriers leading to heterogeneity between populations has already been found in several other marine species: mussels *Perna perna* (Nicastro et al. 2008; Teske et al 2012), and in the north east Atlantic – the region where this study was conducted - cod *Gadus morhua* (Jorde et al. 2007) corkwing wrasse *Symphodus melops* (Knutsen et al. 2013), and sprat *Sprattus sprattus* (Glover et al. 2011).

Pandalus borealis occupy the full western coastline of Scandinavia (FAO 2016), a highly bathymetrically complex region, with variations in oxygen regimes over a small geographical area. Oceanographic features along the Scandinavian coastline that generate oxygen minimum zones and periodic oxygen depletion have had significant impacts on local fauna. Fjord hypoxic events

and cycles in the past 50 years have been associated with a range of significant changes, including reduced biodiversity, community shifts and disrupted ecological processes, at depths typically occupied by *Pandalus borealis* (Josefson & Widbom 1988; Lindahl & Hernroth 1988; Austen & Widbom 1991; Gustafsson & Nordberg 1999; Gustafsson & Nordberg 2000; Nordberg et al. 2001; Filipsson & Nordberg 2004).

Gullmarfjord, an enclosed fjord on the west coast of Sweden 80 km north of Gothenburg, is subject to periodic hypoxic events typical of the inner fjords close to the coastline (Rosenberg 1985; Rosenberg et al. 1990; Gustafsson & Nordberg 2000; Andersson et al. 2009). The fjord deep water has a residence time of eight to ten months and a full description of relevant Gullmarfjord bathymetry is provided in Chapter 1. By contrast, open fjords, such as Brofjorden (located 15 km north of Gullmarfjord), have greater and continual water exchange with the open ocean, maintaining oxygen levels in these fjords.

Populations from Gullmar and Brofjorden are reported to be morphologically distinct in size and pigmentation (Eriksson, pers. comms. 2013) with larger, more intensely pigmented specimens typically found in Gullmarfjord (see Appendices for comparison). Morphometric differences in segment, tail and limb lengths between four genetically similar groups of *P. borealis* in two adjacent fjords in Iceland have been previously described (Jornsdottir et al, 2016). By contrast morphological differences based on similar measures were found to correlate well with genetic population structure for *Pandalus borealis* in the Pacific (Kartavtsev et al., 1993) and, more recently, for tail shape in *Crangon crangon* in the UK (Beaumont & Croucher 2006).

Several studies have posited that environmental factors influence morphometric divergence of crustacean species (Chow and Sandifer 1991; Debuse et al. 2001; Dimmock et al. 2004). The differences documented in the locations used in this study may be the result of localised differences in turbidity or other factors. However, hypoxia is one such factor that has been attributed to morphological shifts across clades (Chapman et al. 2000; Lamont & Gage 2000; Schaack & Chapman 2003) and is known to impact foraging behaviour with direct consequences for growth (Pihl 1994; Cisterna et al. 2008). In addition to impacting energy budgets (i.e. investment into growth and reproduction), hypoxia has been shown to affect metabolism, homeostatic processes (Stickle et al. 1989; Spicer & El-Gamal 1999; McMahon 2001; Spicer & Eriksson 2003; Eriksson et al. 2006; Clark et al. 2013), alter behaviour (Gerhardt & Baden 1998; Riedel et al. 2013) and lower tolerance in marine organisms to other environmental stressors (Frederich & Pörtner 2000; Pörtner & Knust 2007).

Here, I investigate genetic structure within and between populations of *Pandalus borealis* inhabiting environments with different oxygen regimes. Individuals were collected from two spatially proximate sites; a periodically hypoxic fjord (Gullmarfjord) and a well circulated open fjord with no known hypoxic episodes (Brofjorden). Genetic analysis was carried out by sequencing of microsatellite loci and mtDNA haplotypes. Based on existing morphological differences, and natural exposure to different oxygen regimes, I predict that genetic differentiation will accompany this morphological divergence and spatial separation.

2.3 Methods

2.3.1 Sampling

Adult female of the northern shrimp *Pandalus borealis* (n = 300; body length range 61 – 89 mm) were collected by benthic trawl from inner Gullmar fjord (58°20'26.1"N 11°33'31.7"E, maximum depth 120 m) and close to the mouth of Brofjorden (58°18'12.4"N 11°17'59.7"E, maximum depth 100 m) on the west coast of Sweden between March and May 2014. Trawls were run for a total of 30-60 min (allowing maximal yield with minimal damage to individuals) at > 85 m depth. Harvested shrimp were immediately transferred to insulated, aerated, chilled tanks filled with sea water from the fjord (93.2 ± 0.3 % a.s. O_2; T = 8.4 ± 0.03 °C; pH = 7.9 ± 0.0 S = 34.0 ± 0.2) in order to minimise thermal shocks and fluctuations of environmental parameters during transit to the laboratory.

Within five hours of the *P. borealis* being returned to the laboratory, 70 individuals from each population were haphazardly selected as representatives for genetic population structure analysis. The cephalothorax, carapace, appendages and gut were removed in order to minimise genetic contaminants that could confound the results of the population structure analysis. Approximately one third of the abdominal muscle tissue was dissected out and preserved in molecular grade ethanol (99 %) awaiting analysis.

2.3.2 Molecular analyses

Molecular analysis was carried out at the Institute of Biological, Environmental and Rural Sciences (IBERS) , University of Aberystwyth, Wales.

2.3.2.1 DNA extraction and processing

Total DNA was extracted using a standard CTAB-chlorofrom/isoamylalcohol method (Winnepenninck et al., 1993). Mitochondrial DNA (mtDNA) variation was assessed by sequencing

of the entire control region. Briefly, the primers Pbor-CR-F [5'- TCACAAACGGATCCACCTTAC-3'] and Pbor-CR-R [5'- CAAGGCGATCTTTTTGTCAAG-3'] were designed from the mtDNA genome sequence deposited on GenBank (accession number FJ403244) and used in polymerase chain reaction (PCR) to amplify the control region. PCR reactions were performed in a total volume of 30 ul, containing ~100ng of template DNA, 1 μM of each primer, 1X PCR Buffer, 2.0 μM $MgCl_2$ and 0.5 U Taq DNA polymerase (Bioline UK). The PCR thermoprofile was 3 min at 95 ^{o}C, followed by 35 cycles of 30 s at 95 ^{o}C, 30 s at 58 ^{o}C and 45 s at 72 ^{o}C; followed by a final 5 min extension at 72 ^{o}C. PCR products were purified using ExoSAP IT and sequenced using the internal primers Pbor-CR-830R [5'-AAGGATAGTAAATAATCCCAAAAGAGG-3'] and Pbor-CR-1016F [5'-GTCAGTCGGCATTTCAGCTC-3'] on an ABI 3130 DNA sequencer. CONTIG assembly and sequence alignment was performed in BIOEDIT (Hall, 1999).

Nuclear genetic variation was assessed at eight microsatellite loci (PbA1, SD1-41, PbC8, PbD9, SD-2-14, PbA110, SD3-62, PbC-105) developed by Pereyra et al. (2012) and using the recommended PCR conditions. Amplicons were separated using a ABI 3130 DNA sequencer and alleles inferred using the GENEMAPPER software

Table 2.1 Microsatellite loci characteristics. B refers to Brofjorden samples and G to Gullmarfjord.

Locus	Group	Size range (bp)	Expected heterozygosity	Observed heterozygosity
PbA1	B	182 - 216	0.72	0.66
	G	188 - 252	0.74	0.64
SD1-41	B	332 - 365	0.86	0.86
	G	259 - 392	0.85	0.84
PbC8	B	292 - 356	0.66	0.55
	G	256 - 368	0.79	0.63
PbD9	B	251 - 311	0.83	0.80
	G	275 - 399	0.82	0.81
SD-2-14	B	143 – 169	0.65	0.53
	G	143 - 175	0.69	0.60
PbA110	B	250 – 266	0.72	0.69
	G	256 - 270	0.66	0.68
SD3-62	B	151 – 153	0.21	0.19
	G	151 – 163	0.18	0.16
PbC-105	B	227 – 263	0.86	0.81
	G	235 - 351	0.85	0.78

2.3.3 Statistical analyses

2.3.3.1 Analysis of mtDNA data

All analyses were performed using ARLEQUIN 3.1 (Excoffier et al. 2005) unless stated otherwise. Genetic variation was described using indices of haplotype and nucleotide diversity (*h* and π respectively; Nei & Tajima 1981; Nei 1987) and their variances. A Minimum Spanning Tree (MST) was constructed in NETWORK (www.fluxus-engineering.com/sharenet.htm). Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) tests were used to test for deviations from mutation-drift equilibrium that could be attributed to selection and/or population size changes. Mismatch distributions (Harpending 1994), the frequency distribution of numbers of pairwise differences between haplotypes within a sample, and simulated distributions under a model of demographic expansion were compared with the sum of squared deviations (SSD) between observed and expected distributions (significance assessed after 10 000 bootstrap replicates) used as a test statistic, and the expansion parameter τ estimated. The partitioning of variation was analysed using AMOVA (Excoffier et al. 1992) derived estimates of various Φ-statistics (and their variance components), the significance of which were assessed by 10,000 permutations. Differentiation between pairs of samples was further tested by exact tests of haplotype frequency homogeneity and pairwise Φ_{ST} (significance assessed by permutation).

2.3.3.2 Analysis of microsatellite data

Numbers of alleles (N_A), allelic richness (A_R; El Mousadik and Petit 1996), observed heterozygosity (H_O), and expected heterozygosity (H_E), were calculated using FSTAT 2.9.3.2 (Goudet 1995). Genotype frequency conformance at individual loci to Hardy-Weinberg equilibrium (HWE) expectations and genotypic linkage equilibrium between pairs of loci were tested using exact with default parameters in GENEPOP 3.3 (Raymond & Rousset 1995). Multilocus values of significance for HWE tests were obtained using Fisher's method (Sokal & Rohlf 1995) to combine probabilities of exact tests. Genetic structuring was assessed using a number of approaches. Single- and multi-locus values of the unbiased F_{ST} estimator, θ (Weir & Cockerham 1984), were calculated using FSTAT, with the significance of estimates tested by 10 000 permutations of genotypes among samples (Goudet et al. 1996). Genotypic differentiation was tested using the log likelihood (G) based exact test, and genic differentiation by Fishers exact test, both implemented in GENEPOP (with default settings). Genetic structure was also investigated without *a priori* sample information included using the Bayesian clustering method implemented in STRUCTURE (Pritchard et al. 2000) to identify the most probable number of genetic groups (*K*) represented by

the data through a comparative assessment of models (and their posterior probability) of *K* ranging from 1-4. Both the 'no admixture model' (as recommended for low F_{ST}; Pritchard et al., 2000) and 'admixture model with correlated allele frequencies' were employed. Each MCMC run consisted of a burn in of 10^6 steps followed by 5 X 10^6 steps. Three replicates were conducted for each *K* to assess consistency. The *K* value best fitting the data set was estimated by the log probability of data [Pr(X/K].

2.4 Results

Analyses across all haplotypes and loci indicate that there is no significant dissimilarity between the Brofjorden and Gullmarfjord populations. Population comparisons show no significant differences in genetic diversity or mutation rates.

2.4.1 MtDNA data

The mtDNA 980 base pair control region yielded eight haplotypes from the 140 individuals sampled across the two sites.

Genetic variation in each population, as described by haplotype and nucleotide diversity, was almost identical (see Table 2.2) and relatively low (≈ 0.5, Grant & Bowen 1998; Goodall-Copestake et al. 2012).

Table 2.2 Standard and molecular diversity indices for the mtDNA at a single locus

Sample group	Sample size	Number of haplotypes	Haplotype diversity	Nucleotide diversity
Brofjorden	61	8	0.55 ± 0.06	0.0007 ± 0.0006
Gullmarfjord	55	5	0.53 ± 0.45	0.0006 ± 0.0006

These low diversity values are also seen in Figure 2.1 and Figure 2.2. Figure 2.1 displays the percentage frequency of haplotypes identified and Figure 2.2 is the Minimum Spanning Tree (MST) of the identified haplotypes with branches representing the mutations structurally separating them. In both figures, Haplotype 1 and Haplotype 2 dominate with the small number of remaining five haplotypes appearing in very low frequencies and only differing from the dominant haplotypes by one to two nucleotide mutations.

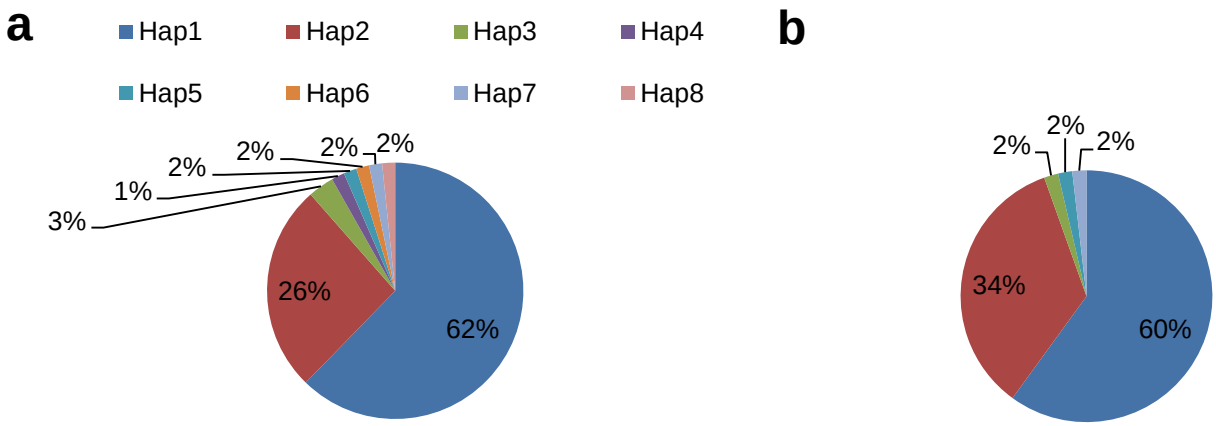


Figure 2.1 Percentage haplotype frequencies. a represents haplotypes present in the Brofjorden population. b represents the Gullmarfjord population, lacking haplotypes 5, 6 and 8.

In Figure 2.2, the most dominant haplotype (Haplotype 1) is located at the centre of a star shaped network with five (in the Brofjorden population where all haplotypes are represented) of the remaining haplotypes only differing from Haplotype 1 by a single nucleotide mutation. Of the two other remaining haplotypes, Haplotype 3 only differs from Haplotype 1 by two nucleotides and Haplotype 8 only differs from the second most dominant haplotype (Haplotype 2) by a single nucleotide.

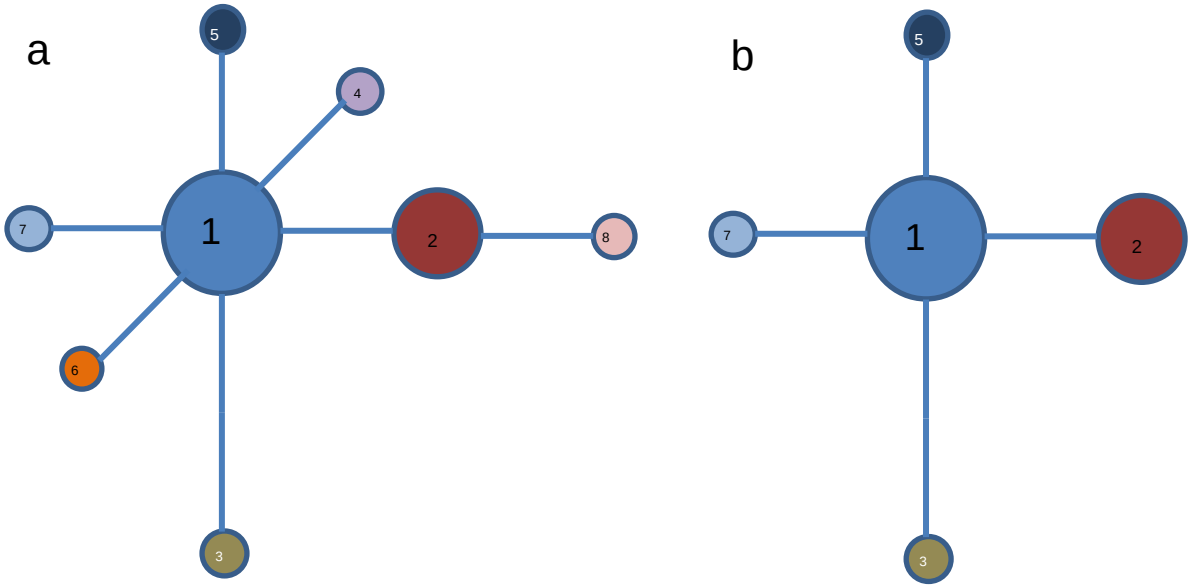


Figure 2.2 Minimum Spanning Trees for the haplotypes of the Brofjorden population (a) and the Gullmarfjord population (b). Length of branches indicates the number of nucleotide deviations from a connecting node.

2.4.2 Microsatellite data

Eight of the ten loci selected by Jorde et al. (2015 – loci designations after Pereyra et al. 2012) were successfully screened from the microsatellite samples for this study.

Analysis of allele richness (A_R) and number (N_A) showed that the shrimp collected from both sites were extremely similar in terms of diversity, standing population size and capacity to respond to long term selection (see Table 2.3; Nei et al 1975; Petit et al. 1998). The only locus that showed substantial deviation between the two populations is PbC8.

Table 2.3 Allelic richness across microsatellite loci for each population

Locus	Brofjorden		Gullmarfjord	
	Richness	Number	Richness	Number
PbA1	8.93	11	9.37	13
SD1-41	16.72	21	15.56	24
PbC8	13.32	15	22.00	27
PbD9	9.33	11	8.41	11
SD-2-14	9.84	13	7.99	15
PbA110	9.17	10	8.75	10
SD3-62	3.50	4	2.99	4
PbC-105	10.43	12	10.39	14

Hardy-Weinberg equilibrium (HWE) assumes that for large populations with no migration, natural selection, no net mutations and random mating, allele frequency will remain constant across generations. Deviation from the HWE infers drift or the impact of selective processes (Wiggington et al. 2005). None of the loci showed significant deviation from the HWE expectations other than one locus from the Brofjorden samples (SD-2-14) and two loci from the Gullmarfjord samples (PbC8 and PbD9 – see Table 2.4). The most significant deviation from the HWE was observed for PbC8 ($P < 0.001$) as with the A_R and N_A results. The p-value across all loci and all population for Hardy-Weinberg is heavily skewed by locus SD-2-14 and therefore its significance is not representative.

As with the A_R and N_A results and similar to the HWE analysis, the Exact tests for genotypic linkage equilibrium between pairs of loci only showed significant divergence at one locus, PbC8 ($P < 0.05$ – see Table 2.4). Across all other loci, there was no significant difference. Fisher multilocus values of significance for HWE, combining probabilities of exact tests, displayed no significant differences between the Brofjorden and Gullmarfjord samples (see Table 2.4). Indeed the high values *Fis* (inbreeding coefficient: up to 0.186) indicate high levels of interbreeding between Brofjorden and Gullmarfjord individuals.

Table 2.4 Observed genetic variability statistics for screened microsatellites

Metric	Sample group	Microsatellite loci								
		PbA1	SD1-41	PbC8	PbD9	SD-2-14	Pb A110	SD3-62	PbC-105	All loci
HWE	B	> 0.05	> 0.05	> 0.05	> 0.05	< 0.005	> 0.05	> 0.05	> 0.05	< 0.05
P value	G	> 0.05	> 0.05	< 0.001	< 0.005	> 0.05	> 0.05	> 0.05	> 0.05	
Exact Test	Paired	> 0.05	> 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

P value										
Fis	B	0.097	-0.001	0.161	0.040	0.186	0.051	0.116	0.049	0.078
	G	0.133	0.013	0.202	0.021	0.132	-0.028	0.096	0.082	0.080
F_{ST}	Paired	-0.0061	-0.0010	0.0096	-0.0020	0.0003	0.0008	-0.005	-0.0048	-0.0013

No significant genetic structuring between the two populations was found using F_{ST}. Additionally, the low F_{ST} values (-0.0061 to 0.0096*)* indicate high levels of interbreeding and heterozygosity between Brofjorden and Gullmarfjord individuals. As with the previous tests, F_{ST} highlighted some low level structuring at the PbC8 locus. This similarity between the output of these tests indicates that sample sizes were adequate and results are therefore robust.

2.4 Discussion

On the basis of morphological differences and differing oxygen regimes in their respective habitats, it was predicted that *Pandalus borealis* from Brofjorden and Gullmarfjord may have genetically diverged. Contrary to that prediction, no significant differences in genetic population structure between *P. borealis* at Brofjorden and Gullmarfjord were found. Diversity indices and the haplotype frequencies for both groups are near identical and both groups appear to have undergone the same haplotype radiations, as evidenced by the patterns of haplotype mutation. Genetic variance statistics indicate high levels of interbreeding between individuals at Brofjorden and Gullmarfjord and no detectable divergence or selective forcing between individuals sampled at each site.

Allelic richness is a measure of genetic diversity indicative of a population's adaptive potential and persistence (Petit et al. 1998) and can be a useful as an indicator of a previous selective sweeps or population crashes (Nei et al. 1975). Though there are insufficient data here to conclude the adaptive capacity of the populations sampled, it can be inferred from the diversity analyses in this chapter that neither group has undergone independent specialisation, experienced independent selective sweeps or diverged from one another (see Table 2.3 and 2.4; Caicedo & Schaal 2004). Levels of genetic diversity within both populations are low (< 0.5 – Grant & Bowen 1998). In fact, haplotype diversity and nucleotide diversity calculated in this chapter were lower than mean levels for all nine species surveyed by Goodall-Copestake et al. (2012) calculated from a homologous 456-base region of *cox1.* Nevertheless, low genetic diversity is characteristic of marine invertebrate species in the North Atlantic. It results from species with individuals with a low likelihood of survival to maturity undergoing range expansion since the most recent glacial period (Maggs et al. 2008; Hedgecock & Pudovkin 2011).

The haplotype frequencies and MSTs (see Figure 2.1 and Figure 2.2) also indicate a complete shared evolutionary history between the two populations. Both populations display small number of dominant haplotypes and a star-shaped MST showing minimal mutational differences between the most frequently observed haplotypes and the lower frequency haplotypes. This MST structure of an interior node surrounded by haplotypes with minimal nucleotide differences is also consistent with Sweepstakes Reproductive Success (SRS) theory (Hedgecock 1994), which acts on many marine invertebrate species. SRS describes a mode of reproduction and recruitment impacting genetic diversity. SRS refers to species with individuals that have extremely large variance in reproductive success; adults produce high numbers of eggs, very few of which survive to maturity due to sweepstake-like probability of matching survivorship factors, such as oceanographic conditions. The key genetic consequence of this is the reduction of the ratio of effective to actual population size to a value much smaller than 0.01 (Hedgecock & Pudovkin 2011) as can be seen in the nucleotide diversity values in Table 2.2. As so few eggs survive to maturity, very few mutations become fixed to high proportion within the population, resulting in a star-shaped network or geneology (Eldon & Wakely 2008; Hedgecock & Pudovkin 2011).

The star-shaped MST is also characteristic of marine species that have undergone post-glacial range expansion (Maggs et al. 2008; Strasser & Barber 2009). The dominance of a small number of haplotypes is thought to have been achieved through expansion following a massive selective sweep, such as would have occurred during the last glacial maximum. In the relatively short time since that period (approximately 10 ka; Blunier et al. 2001) there has been limited scope for high levels of mutational change to become fixed in post-glacial populations, leading to shallow genealogies (Maggs et al. 2008; Strasser & Barber 2009). This genetic structure associated with selective sweeps and rapid expansions has been inferred multiple on occasions in Atlantic cod, *Gadus morhua*, (Pogson et al. 1995; Arnason 2004; Carr & Marshall 2008; Pampoulie et al. 2008; Fevolden et al. 2012), salmon, *Oncorhyncus tshawytscha,*(Wilson et al 1987) sticklebacks, *Gasterosteus aculeatus,* (Orti et al. 1994) and in various species of invertebrate (Remerie et al. 2005; Riginos & Henzler 2008; Espinosa et al. 2010).

That the Gullmarfjord dataset lacks three of the haplotypes present in the Brofjorden cohort is unlikely to represent a selective sweep in the Gullmarfjord habitat. It is far more likely to be an artefact of the low genetic diversity of these two populations and the extremely low frequencies in which the non-dominant haplotypes are found (Excoffier et al. 1992; Tishkoff et al. 2000). Furthermore, as the allelic richness, diversity indices, measures of population structure, and haplotype frequency and linkages show no significant differences from one another, it can be assumed that both the Brofjorden and Gullmarfjord ancestral lines experienced the same selective

sweep and have not experienced any genetic bottleneck events independently from one another since.

Previous genetic studies conducted on *Pandalus borealis* at similar geographical scales have found local adaptation to be somewhat non-uniform and stochastic. Polymorphic microsatellite research conducted on *P. borealis* across the entire north Atlantic found populations located between Nova Scotia at 45 ° N and northern West Greenland at 68 ° N to be more closely related to one another than single populations at the Flemish Cap (47 ° N) and the Gulf of Maine (43 ° N) (Jorde et al. 2015). Similarly, microsatellite screening of *P. borealis* at 20 locations across the North Sea only yielded nine distinct groups, though the Skaggerak fjord samples did appear to have greater genetic population structure than other regions (Knutsen et al. 2015). Drengstig et al. (2000) only found local differentiation between Norwegian fjords, having found no such patterns within the Barents sea or Svalbard regions. North Sea RAPD analysis found genetic divergence between spatially proximate samples from Jan Mayen, but not from those within the Barents Sea, Svalbard or the Norwegian coastline (Martinez et al. 2006). Martinez et al. (2006) posited a difference in water currents and the thermal forcing associated with those currents could be at the root of the Jan Mayen divergence. The other localities experienced similar within-region temperature levels throughout sampling.

In this study, we assumed divergence would be driven by oxygen regime differences between Brofjorden and Gullmarfjord. Hypoxia in Gullmarfjord fluctuates (Josefson & Widbom 1988; SMHI 2016 – see Appendices Section i for oxygen regime in Gullmarfjord locale) and may not be a sufficiently stable force to select for hypoxia-tolerant genotypes amongst *Pandalus borealis* populations that occupy such temporally heterogeneous habitats (Sanford & Kelly 2011; Volis et al. 2015). Extreme hypoxic events in the Gullmar and adjacent fjords have been known to cause local population collapse especial of mobile, sensitive macrofauna, including Crustacea (Josefson & Widbom 1988; Austen & Widbom 1991; Filipsson & Nordberg 2004). After a period of recovery, these clades were seen to return to the fjords, albeit in greatly reduced abundance (Josefson & Widbom 1988). This suggests that there is either substantial immigration into these areas and/or that these particularly sensitive macrofaunal clades are able to physically avoid hypoxic zones when oxygen is most depleted (Josefson & Widbom 1988). *Pandalus borealis* is highly vagile and known to undertake diel migrations (Barr 1970) and hypoxic minima in these fjords are known to be at their most severe in the deep basin (Gustafsson & Nordberg 1999; Gustafsson & Nordberg 2000), so this species may well have the capacity to behaviourally avoid substantial exposure to hypoxia.

Even if hypoxia levels were sufficiently stable as to drive genotypic selection, immigration levels and general gene flow may outweigh selective forcing and maintain homogeneity between Brofjorden and Gullmarfjord. This genetic homogeneity may be facilitated by several aspects of *P. borealis* life history and the oceanographic processes of the fjord system. During the three to six month larval period, *Pandalus borealis* may drift 200 – 400 km along currents (Pederson et al. 2002). In a comprehensive local adaptation literature review, Sandford & Kelly (2011) found that the 66 % of marine invertebrates that present highly adaptive differentiation at the population level predominantly have brief (less than one week) planktonic life stages, limiting their dispersal range. Indeed, theory suggests that local adaptation should be particularly common in species with direct development (Struhsaker 1968; Yamada 1989). In accordance with Hollanders' (2008) meta-analysis, we would expect plasticity to dominate over adaptation for a species such as *P*. *borealis* with a high dispersal capacity.

In addition, though the fjord deep water residence time is approximately eight to ten months; water exchange in the Gullmarfjord occurs predominantly from December to May with the highest rates taking place between January and March (Lindahl & Hernroth 1988), which coincides with the *Pandalus borealis* breeding season (Skuladottir et al. 2007). With reference to *P. borealis* planktonic larval stages, Lindahl and Hernroth (1988) found these advective processes - as opposed to temperature, salinity, oxygen and food supply - to be the main regulator of the zooplankton community composition within Gullmarfjord by preventing adaptation to single environmental regime and mixing gene pools internal and external to the fjord. In 1991, Bergström found that shifts in patterns of catch per unit effort (as a proxy for adult abundance) and length frequency distributions of adult *Pandalus borealis* in Gullmarfjord were consistent with the timing of strong advective flows over the fjord sill. This water renewal also coincides with when offshore adult females approach coastal regions to brood (Bergström 1991; Bergström 2000), which would serve to increase gene flow between inner fjords, such as Gullmarfjord and areas with full oceanic circulation, such as Brofjorden.

In addition to the differing oxygen regimes of the fjords studied in this chapter, the divergence hypothesis was based on the differing morphologies of *Pandalus borealis* from the two sites. Morphological differences are not always tied to genetic population structure. For example, no distinct difference in morphometric traits was observed between kuruma shrimp, *Penaeus japonicus,* despite being genetically distinct (Tsoi et al. 2005) and morphological differences may be caused by numerous non-genetically fixed factors, such as food availability, environmental conditions and physiological stress, and carry-over effects (Pechenik et al. 1990; Verberk et al. 2011; Zhang et al. 2012; Dupont et al. 2013). Colouration is a known health and diet indicator in other crustacean species (Chien et al. 1991; Main & Laramore 1999; Bondad-

Reantaso et al. 2001; Floreto et al. 2001). Diet and fatty acid content have been found to impact hypoxia tolerance in a number of fish species by affecting cardiac, kidney, gill and plasma function (Ishibashi et al. 1992; McKenzie 2001; McKenzie et al. 2008; Zambonino-Infante et al. 2013). Furthermore, Swedish populations of Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*), plaice (*Pleuronectes platessa*), dab (*Limanda limanda*), and American plaice (*Hippoglossoides platessoides*) have been shown to alter their diets in response to hypoxia (Pihl 1994).

The morphological differences observed between *P. borealis* in Gullmarfjord and Brofjorden may reflect functional phenotypic differentiation. This study demonstrates that any local differentiation exhibited by *Pandalus borealis* occupying the two sites has not undergone broad scale genetic fixation. Despite being highly mobile, it is still highly likely that *P. borealis* in Gullmarfjord are exposed to low level hypoxia for extended periods throughout the year. It is possible that *P. borealis* may be reliant on phenotypic plasticity to survive this environmental variability. It is also possible that different populations may exhibit differing levels of performance under stress due to previous exposure; this will be explored in Chapters 3 and 4.

Chapter 3: Do populations of *Pandalus borealis* from different oxygen regimes exhibit physiological differentiation under environmental stress? – Common garden approach

3.1 Abstract

A common garden experiment was used to test for differences in performance under hypoxia and thermal stress between *Pandalus borealis* collected from a normoxic and a periodically hypoxic fjord. No significant interactions between the stressors were observed and *P. borealis* individuals from the two populations did not show significant differences in metabolic rates or stamina. Hypoxia and elevated temperature did, however, have a significant impact on metabolic rates in *P. borealis* and hypoxia caused stamina to be reduced to up to a quarter of its normoxic capacity. Common garden testing of metabolic rates and stamina did not show any significant population differences in performance under stress, so I conclude that *P. borealis* in the periodically hypoxic Gullmarfjord may utilise other physiological or behavioural mechanisms to persist.

3.2 Introduction

Broad-scale microsatellite and mtDNA analysis did not detect divergent genetic structuring (see Chapter 2) between individuals of *Pandalus borealis* collected from two different locations: Brofjorden and Gullmarfjord. Brofjorden is a well oxygenated site with continual oceanic circulation. The Gullmarfjord has limited circulation and seasonal stratification resulting in hypoxic zones (Eriksson & Baden 1997; Hjerpe et al. 2004; Enebjörk & Fränne 2006). While this genetic homogeneity between individuals at the two sites could be interpreted as an absence of local adaptation, it is still possible that selective forces, specifically historic and current exposure to different environmental hypoxia, may be acting on phenotypic plasticity and driving non-genetically fixed selection or divergence at non-neutral loci that were not targeted in the analyses covered in Chapter 2. In spite of the results of the genetic analyses, in order to maintain consistency, sample groups from Gullmarfjord and Brofjorden will continue to be referred to as ‘populations’ throughout this thesis.

Pandalus borealis is a wide-ranging species (Bergstrom 2000). Environmental conditions between the extremes of its latitudinal range (approximately 42 – 81 ° latitude; FAO 2016) vary greatly and *P. borealis* undertakes diel vertical migrations causing it to be exposed to wide ranging conditions within its own habitat (Barr 1970). As, such *P. borealis* likely exhibits at least a moderate level of phenotypic plasticity to cope with this heterogeneous environment.

Phenotypic plasticity is the capacity of set genotypes to react to environmental conditions by expressing changes in form, state, behaviour or rates of activity (Pigliucci 2001; West-Eberhard 2002; DeWitt & Scheiner 2004). As explained in Chapter 1, phenotypic plasticity allows survival in a range of environments. If plasticity is sufficiently large, it will nullify selective pressure and it is unlikely that there will be any shift in the frequency of genotypes within populations occupying different environmental conditions (Price et al. 2003). Evidence of phenotypic plasticity in Pandalid shrimp has been found with reference to the timing of sex change to match breeding opportunities across the Pandalid family (Charnov & Anderson 1989), in differing levels of gene expression across different populations of *P. latirostris* (Kawahara-Miki et al. 2011), and, following exposure to hypoxia, in enzyme activity and gene expression in *P. borealis* (Pillet et al. 2016). However, sister species *P. montagui* was shown to have very limited phenotypic plasticity when exposed to elevated temperature (Magozzi & Calosi 2014).

Both the Gullmarfjord and the Brofjorden populations may have sufficient – and equal - plasticity to acclimatise to hypoxic events and mitigate the negative impacts (Mandic et al. 2009). Should this be the case, one might predict that performance would improve with increasing exposure time for both populations. However, the morphological differences observed between *P. borealis* in Brofjorden and Gullmarfjord (Eriksson pers. comms. 2013), which when views alongside their habitat differences, may suggest that the Gullmarfjord population may has been impacted by its oxygen regime. Prior exposure or acclimatisation and acclimation to stressful conditions leads to improved tolerance in many organisms (Drew 1997; Fangue et al. 2009). If *P. borealis* in Gullmarfjord have had prior exposure to oxygen minimum zones, we might predict that their stress tolerance levels under hypoxic treatments, and therefore their performance, would be better after short term exposure than their Brofjorden counterparts.

The levels of hypoxia that occur in Gullmarfjord are potentially challenging to *P. borealis*. Phenotypic plasticity may be one mechanism by which *P. borealis* in the Gullmarfjord habitat achieve persistence. Acutely, the hypoxia responses are characterised by systemic changes in respiratory and cardiovascular physiology, aimed at the maintenance of oxygen delivery to tissues (Holeton & Randall 1967; Fritsche & Nilsson 1990; Bushnell & Brill 1992; McMahon 2001; Capossela et al. 2012). For example, hypoxia has been documented as resulting in significant decreases in aerobic scope (the difference between maximum oxygen consumption during maximal exertion and minimum oxygen consumption a rested state - Clark et al. 2013) and increases in the specific activities of enzymes involved in anaerobic biochemical pathways (Meyerhof & Lohman 1928; Taylor et al. 1977; Bridges & Brand 1980; Dupont-Prinet et al. 2013).This kind of metabolic disruption tends to be energetically costly (Bushnell & Brill 1992; Larade & Storey 2002). Hypoxia-induced hypometabolism and a switch to less efficient anaerobic

ATP generation may allow survival in the short term (Burke 1979; Vandenthillart et al. 1994; Drew 1997). However, after more prolonged exposure, oxygen carriage is often optimised by modifications in the concentration and intrinsic oxygen affinity of extracellular respiratory pigments (Taylor 1976; Magnum & Van Winkle 1973; Lallier & Truchot 1989; McMahon 2001; Spicer & Baden 2001).

Thermal stress is intrinsically linked with hypoxia. Henry's Law (1803) dictates that gas dissolution has an inverse relationship with the temperature of the liquid medium. Hence, as sea temperatures increase, less free oxygen can be accommodated and therefore acquired by respiring organisms. Furthermore, elevated sea temperatures may cause stratification (Cerrano et al. 2000; Romano et al. 2000; Sparnocchia et al. 2006) and changes to marine circulation (Joos et al. 1999; Matear et al. 2000; Cubasch et al. 2001; Keeling & Garcia 2002), altering oxygen availability.

Ectotherms, such as *Pandalus borealis,* are particularly at risk from hypoxia and thermal stress in combination as their metabolic rates and ability to meet oxygen demands are governed by both temperature and oxygen availability (Pörtner 2001). Organismal performance is generally set by the aerobic scope and energy budget (Pörtner & Knust 2007; Pörtner & Farrell 2008). Reduced aerobic capacity may result in a shift to less efficient, anaerobic means of ATP production, thus limiting the energy budget. This reduction in available energy coupled with additional energetic requirements from stress responses alters the investment of energy into essential processes, such as growth and/or reproduction (Chabot & Dutil 1999; McNatt & Rice 2004). Consequently, organismal fitness may be negatively affected.

Thermal stress and hypoxia in combination may cause significant physiological perturbations (see Section 1.3.1). In caridean shrimp, the stressors have been shown to synergistically increase the dependence on anaerobic respiration (González-Ortegón et al. 2013) and to induce or depress expression of heat shock proteins in peneid shrimp depending on the duration of exposure (Li et al. 2009).

Pillet et al. (2016) suggest that *P. borealis* is resilient to hypoxia up to a threshold dissolved oxygen level of 40 % air saturation (a.s.) after which the shrimp undergo significant shifts in enzyme activity for aerobic, anaerobic and lactate dehydrogenase pathways. Experiments with adult *P. borealis* under hypoxia demonstrated lower tolerance in females and, across both sexes, that severe hypoxia (22 % a. s.) did not affect standard metabolic rate (oxygen consumption at rest), but significantly reduced maximum metabolic rates compared to normoxia (Dupont-Prinet et al. 2013). In a survey of the metabolic performance of caridean shrimp inhabiting British waters, Magozzi and Calosi (2014) found that *Pandalus borealis* sister species, *P. montagui*, was

particularly vulnerable to elevated temperature relative to the other shrimp. *Pandalus montagui* had the highest oxygen consumption at the lowest temperatures, the lowest upper thermal tolerance limit, most restrictive phenotypic plasticity, and consumed significantly higher quantities of oxygen at elevated temperature than the other sub-tidal species studied.

As a result of the interrelatedness of hypoxia and thermal stress, elevated temperature has been selected as an additional stressor in this study to amplify the physiological impacts of hypoxia and help define any possible differences between the populations.

This chapter investigates whether the Brofjorden and Gullmarfjord populations exhibit phenotypic plasticity under hypoxic and thermal stress and if their ability to express plasticity in response to multiple stressors differs from one another. Elevated temperature has been selected as an additional stressor to amplify the effects of hypoxia and thereby highlight any possible performance differences between the populations. If elevated hypoxia and temperature elicit a physiological shift, it would be consistent with the view that these stressors are potential selective forces for this species and provide some insight as to the capacity of *Pandalus borealis* to tolerate predicted climate change. Furthermore, if the two populations show different levels of plasticity, this may indicate that these two populations have begun to functionally diverge in response to their respective oxygen regimes.

Pandalus borealis from both Brofjorden and Gullmarfjord were exposed to elevated hypoxia, elevated temperature and both stressors in combination as part of a mesocosm-based quadratic experimental design for either one or four weeks. After exposure, oxygen consumption (as a proxy for metabolic rates) and stamina were measured. This chapter posits the following hypotheses: 1) the Gullmarfjord population is more capable of tolerating limited oxygen availability i.e. its metabolic rate should increase to a lesser extent than that of the Brofjorden population and stamina should be maintained under stress; 2) *Pandalus borealis* is able to acclimate to hypoxia and elevated temperatures as evidenced by improved performance with increased exposure; and 3) elevated temperature and hypoxia have a synergistic detrimental effect on the metabolic rates of *P. borealis* from both populations.

3.3 Methods

3.3.1 Animal collection and husbandry

Adult female northern shrimp *Pandalus borealis* were collected as described in Section 2.3.1.

The shrimp were kept in deep fjord water flow-through stock tanks (1800 L capacity; T =7.6 ± 0.02 °C; S= 33.2 ± 0.9) at the Sven Lovén Centre for Marine Sciences – Kristineberg for 48 h to adjust to the laboratory conditions and to ensure that only healthy, undamaged shrimp were selected for the experiments. Subsequently, shrimp were transferred to a mesocosm (as described below: see Section 3.3.2) and kept under control conditions (93.2 ± 0.3 % a.s. O_2; T = 8.4 ± 0.03 °C; pH = 7.9 ± 0.0 S = 34.0 ± 0.2) for five days before initiating the experimental exposure. Animals were fed *ad libitum* throughout the adjustment period and during the experimental period on a combination of live *Artemia* nauplii, *Mytilus edulis*, *Clupea harengus* and fish food (Marine Flake, New Era, Thorne, UK). Forty eight hours prior to measuring metabolic rate and stamina, feeding was ceased in order to negate dietary induced thermogenesis, as energy expenditure exceeding RMR due to the cost of processing food for direct use and storage (e.g. Rosas et al. 1999; Lannig et al. 2008; Dupont-Prinet et al. 2013). Residual food was regularly removed to prevent water fouling.

3.3.2 Mesocosm experimental design

A factorial experimental design incorporating two levels of seawater oxygen and two levels of seawater temperature was used. Oxygen levels were maintained at either full aeration (hereon referred to as “normoxic”: 93.15 ± 0.26 % air saturation (a.s.) O_2) or at hypoxic levels (46.03 ± 0.52 % a.s. O_2). Conventionally the definition for hypoxic waters is < 2 mg O_2 / L (approximately 20 % a.s.). However, Vaquer-Sunyer and Duarte (2008) found that across 872 published experiments, this level was below the empirical sublethal and lethal oxygen threshold concentrations for half the species tested. In light of this, we wished to test whether reductions in oxygen that do not constitute the general definition of “hypoxia” are harmful to shrimp. Preliminary experiments suggested that oxygen levels below 30 % a.s. are lethal to *P. borealis*. Accordingly, 45 % a.s. was selected as the hypoxia treatment. Temperature levels were selected to represent local conditions for that spring (“ambient”: 8 °C) and near-future temperature projections for the North Atlantic and the Baltic Sea (“elevated” temperature: 10 °C). The IPCC Fifth Assessment Report (Stocker et al. 2013) predict temperature increases of 1 -3 °C in the winter months and increases of 0.5 – 2 °C in summer between 2016 – 2036 with respect to 1986-2005 average values for the Swedish Atlantic coastline.

Individuals from the Gullmarsfjord and Brofjorden population were exposed to one of these four treatments (normoxic (N) ambient (A) temperature; N elevated (E) temperature; hypoxic (H) A; and HE), for either one or four weeks before testing the effect of exposure on shrimps’ resting metabolic rates and stamina as outlined below

The shrimp were haphazardly allocated, two *per* tank, one from each population, across 60 closed-system 5-L tanks filled with 4.5 L of aerated deep fjord sea water. This water was changed three times a week and oxygen conditions regulated to the desired levels *via* bubbling of pure N_2 gas during the entire duration of the experimental period. The shrimp were separated from each other by a perforated plastic barrier to aid identification and prevent aggression or competition for food, whilst guaranteeing maximum circulation of water within the aquarium.

Barometric pressure was obtained from the University of Gothenburg Sven Lovén Centre for Marine Sciences, Kristineberg weather station data bank in order to express measurements of oxygen consumption at standard temperature and pressure. In order to maintain the desired temperature, the 60 tanks were distributed across nine flow-through water-filled trays which, with the addition of heaters and circulation pumps, acted as water baths (see Appendices Section ii).

3.3.3 Measurement of rates of oxygen uptake and stamina

Oxygen consumption (MO_2) has long been used as a proxy for metabolic rate (Keys 1930; Clausen 1936; Fry & Hart 1948; Fry 1971; Spicer & Eriksson 2003). In order to establish the aerobic scope of *P. borealis* under different combinations of hypoxia and elevated temperature, measures of both Resting Metabolic Rate (RMR i.e. the rate of oxygen consumption of a fasted, rested animal participating only in voluntary movement; Walker et al. 2009) were taken.

MO_2 was measured using closed bottle respirometry. Shrimp were placed inside plastic, blacked-out closed-cell experimental chambers (80 x 200 x 130 mm; Klip Lock, Sainsbury, London, UK). Each chamber was supplied with sea water from the oxygen * temperature combination of exposure and sealed underwater to prevent air bubbles being trapped in the chamber. To ensure moderate and continuous water mixing, preventing the formation of a hypoxic layer around the shrimp, each experimental chamber was equipped with a magnetic flea and placed over a multi-channel magnetic stirrer (MS-53M, Jeio Tech, Chalgrove, UK). Each chamber was supplied with plastic blocks reducing volume from 1.5 L to 990 mL sea water in order to reduce the trial time and the shrimp placed inside a perforated plastic cage. This cage also served to prevent the shrimp from being injured or damaged by the spinning flea, and provide each shrimp with substrate to attach itself to and limit its activity, forcing the shrimp to rest in order to measure its RMR. Oxygen consumption was measured every 15 min for 1 h using an optical oxygen sensor (Fibox4, PreSens, Regensburg, Germany).

After the RMR was recorded, they were exercised in their respective treatment water until they were fully fatigued: here defined as maximum sustainable rate of locomotion achieved

spontaneously or through coercion (Weibel & Hoppeler 2005; Dupont-Prinet et al. 2013). If the shrimp began to settle, they would be disturbed into swimming once more using a rod with a small panel of mesh attached, which was designed as a disturbance tool that would not cause damage to the shrimp. If the shrimp would still swim with only intermittent coercion after 3.5 h, it was deemed that the shrimp had reached an elevated rate of metabolic activity comparable to those individuals that had become fatigued after a much shorter period. Exhaustion time was recorded *per* individual and as a proxy for stamina.

Atmospheric pressure and experimental water salinity were measured each day. Their values were used in conjunction with temperature, the volume of each individual shrimp, Green and Carritt's (1967) oxygen solubility coefficients and Weiss' (1970) vapour pressure values to determine the absolute quantities of oxygen consumed during each experiment (see Appendices Section iii for formulae).

Biometric data gathering was considered essential for metabolic rate interpretation. Daoud et al. (2007) models on adult *Pandalus borealis* exposed to elevated temperature found temperature and individual wet weight explained 97 % of resting metabolic rate (RMR) variability. Before introducing the shrimp to the experimental chamber for RMR determination, wet weight (g) and body volume (mL) of each shrimp was recorded using an electronic high precision scale (Mettler-Toldeo B502, Leicester, UK), and specimen volume was determined by immersing each prawn in a Pyrex graduated cylinder (100 mL, accuracy 1 mL, Fischer Scientific UK Ltd) filled with sea water, and measuring the water displaced by the shrimp.

3.3.4 Statistical analyses

All analyses were conducted using the statistical program R version 3.1.2.

The effect of oxygen concentration, temperature, length of exposure, population origin and their interactions on RMR and stamina were analysed using GLM tests. The RMR data proved highly non-normal after investigation with Shapiro-Wilk tests and histograms and Levene's test indicated unequal variances (RMR: $F = 1.61$, $p = 0.09$). Consequently, GLMs were run on log transformed data. Individual weight was also included as a covariate in the maximal model to account for possible variation in metabolic needs between larger and smaller individuals. Post hoc analyses were conducted to identify significant differences between mean RMR.

A similar GLM was run for stamina, but the data were normal, so no transformation was performed.

In the laboratory experiment, the two populations studied did not exhibit significant differences in their responses to elevated temperature and hypoxia during analysis, so their results were pooled for ease of interpretation.

3.4 Results

There were no significant differences between the populations in MO_2 responses or stamina across all treatments, so the data for each treatment were pooled for subsequent analysis to strengthen modelling power. Data collected for both MO_2 responses and stamina were highly variable. For MO_2, individuals in the hypoxic treatments exhibited a greater range of oxygen consumption compared to individuals in the normoxic treatments (see Figure 3.1 and Figure 3.2). General Linear Model analysis did not show any significant interaction between oxygen level and elevated temperature (see Table 3.1). Exposure to elevated temperature was initially associated with a decrease in RMR MO_2 and then an increase in RMR MO_2 after four weeks of exposure, but there were no significant changes in stamina upon exposure to elevated temperatures (see table 3.1 and Figure 3.1). Stamina reduced significantly under low oxygen treatments (see Table 3.1 and Figure 3.3). Length of exposure had a significant influence on metabolic rates (see Table 3.1), though the patterns were more complex than for temperature or oxygen as discussed below.

Table 3.1 Results for F-tests conducted on minimal adequate GLMs for RMR and stamina data for shrimp exposed to experimental mesocosm conditions. Degrees of freedom (df), mean of square (MS), F-ratio (F), and probability level (p) are reported. Bold test indicates significant factors.

Trait	Source	df	MS	F	P
RMR MO_2	**Temperature**	1	17.4	39.974	≈ 0.05
	Length of exposure	1	2702.2	-	**< 0.01**
	Oxygen level	-	-	-	> 0.05
	Population origin	-	-	-	> 0.05
	Individual weight	-	-	7.971	> 0.05
	Temperature : Length of exposure	1	2718.3	-	**< 0.01**
	Length of exposure : Individual weight	-	-		> 0.05
Stamina	Temperature	-	-	-	> 0.05
	Length of exposure	-	-	-	> 0.05
	Oxygen level	1	56788	18.22	**< 0.01**
	Population origin	-	-	-	> 0.05
	Individual weight	-	-	-	> 0.05
	Temperature : Length of exposure	-	-	-	> 0.05

Length of exposure : Individual weight	-	-	-	> 0.05
Oxygen : Length of exposure	-	-	-	> 0.05

Table 3.2 Mean values for RMR and stamina with standard errors and group size per treatment.

Treatment	Mean RMR ± standard error (mmol O_2 L^{-1} h^{-1})	Group size
RMR		
NAB1	43.13 ± 4.15	4
NAG1	32.18 ± 6.25	3
HAB1	63.98 ± 13.11	4
HAG1	38.25 ± 13.64	5
NEB1	25.41 ± 5.60	7
NEG1	29.32 ± 6.22	6
HEB1	43.91 ± 11.87	2
HEG1	41.24 ± 8.21	3
NAB4	13.40 ± 6.71	3
NAG4	15.43 ± 8.56	3
HAB4	NA	NA
HAG4	12.22 ± 7.57	4
NEB4	30.08 ± 7.76	5
NEG4	30.83 ± 7.74	5
HEB4	15.57 ± 15.57	3
HEG4	29.30 ± 12.74	
Stamina	Mean time to exhaustion (min)	
NAB1	101.46 ± 17.62	5
NAG1	73.59 ± 34.70	5
HAB1	20.21 ± 7.60	3
HAG1	74.37 ± 45.11	5
NEB1	90.98 ± 23.36	7
NEG1	62.50 ± 30.39	6
HEB1	22.23 ± 6.98	3
HEG1	15.37 ± 0.19	2
NAB4	94.40 ± 13.12	4
NAG4	102.14 ± 29.08	5
HAB4	NA	NA
HAG4	15.73 ± 9.98	4
NEB4	86.76 ± 12.23	7
NEG4	107.13 ± 41.37	5
HEB4	14.88 ± 7.17	3
HEG4	2.13 ± 2.67	4

3.4.1 Metabolic rates

Temperature treatment had a significant influence on RMR MO_2 and either increased or decreased MO_2 depending on the length of exposure to mesocosm conditions. Generally, when comparing elevated temperature treatments to ambient, elevated temperature was associated with

a higher mean MO_2 after one week of exposure and then a subsequent downturn in MO_2 after four weeks of exposure (F_1 = 7.971, P < 0.01; see Table 3.1, Table 3.2 and Figure 3.1). When investigating responses within treatment groups, mean MO_2 dropped from the first sampling time point (one week exposure) to the second (four weeks exposure; F_1 = 7.923, P < 0.01, see Table 3.1, Table 3.2 and Figure 3.1) for all treatments except NE (normoxic and elevated temperature).

Oxygen level did not have a significant influence on RMR. The minimum mean MO_2 at rest was observed in *P. borealis* from the Gullmarsfjord population incubated at ambient temperature under hypoxic conditions for four weeks (HAG4: 12.22 ± 7.57 µmol L^{-1} h^{-1}, n = 4), while the maximum was observed in the Brofjorden treatment group incubated at ambient temperature levels under hypoxic conditions for one week (HAB1: 63.98 ± 13.11 µmol L^{-1} h^{-1}, n = 4; see Table 3.2). All mean hypoxic RMR MO_2 were higher than their corresponding normoxic controls except for the NE treatments after one week of exposure and HA (hypoxic, ambient temperature) for the Gullmarfjord individuals after four weeks exposure where mean MO_2 was lower than NA.

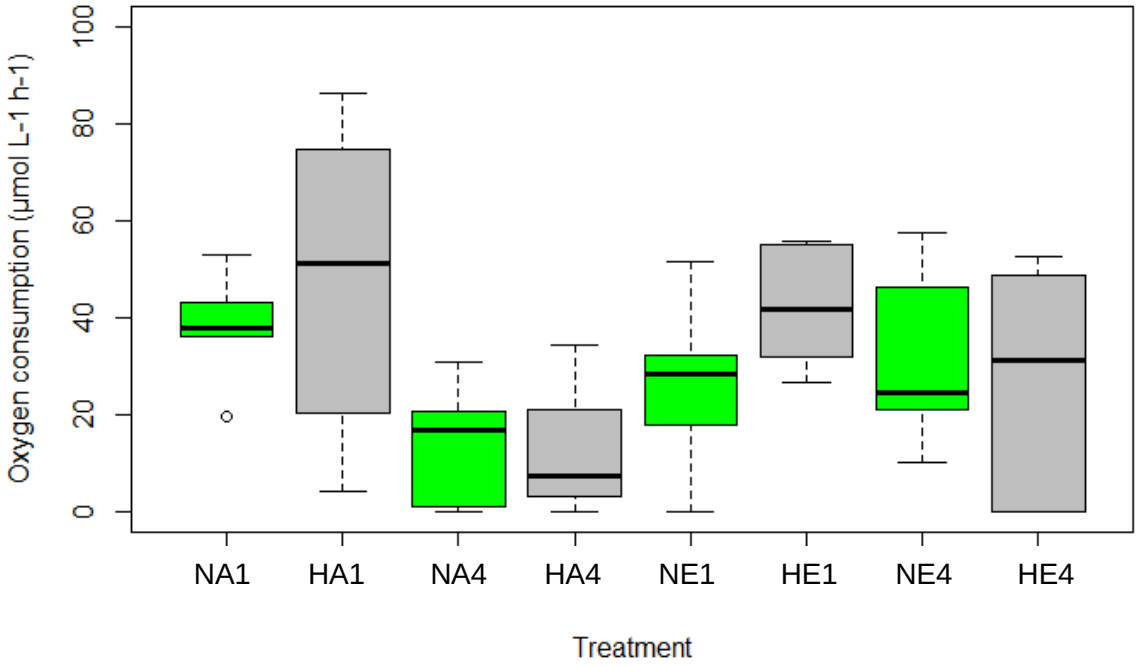


Figure 3.1 RMR box and whisker plot with pooled population data. N: normoxia; H: hypoxia; A: ambient temperature; E: elevated temperature; 1: one week of exposure to mesocosm conditions; 4: four weeks of exposure to mesocosm conditions.

3.4.2 Stamina

General linear modelling indicated that oxygen level was the sole significant explanatory variable for stamina (F_1 = 18.22, P < 0.01; see Table 3.1). Across all treatments, oxygen concentration had a positive relationship with the time taken to reach fatigue (see Figure 3.3). Under hypoxic conditions, stamina was reduced to as little as a quarter of its normoxic capacity.

Means ± SE for stamina (time taken to reach exhaustion (min)) are given in Table 3.2. In general, hypoxic treatments exhibited a narrower range of exhaustion times *per* treatment compared to normoxic treatments. The minimum mean exhaustion time was observed in the Gullmarsfjord treatment group incubated at elevated temperature under hypoxic conditions for four weeks (HAG4: 2.13 ± 2.67 min, n = 4), while the maximum was observed in the Gullmarsfjord treatment group incubated at elevated temperature under normoxic conditions for four weeks (NEG4: 107.13 ± 41.37 min, n = 5; see Figure 3.3).

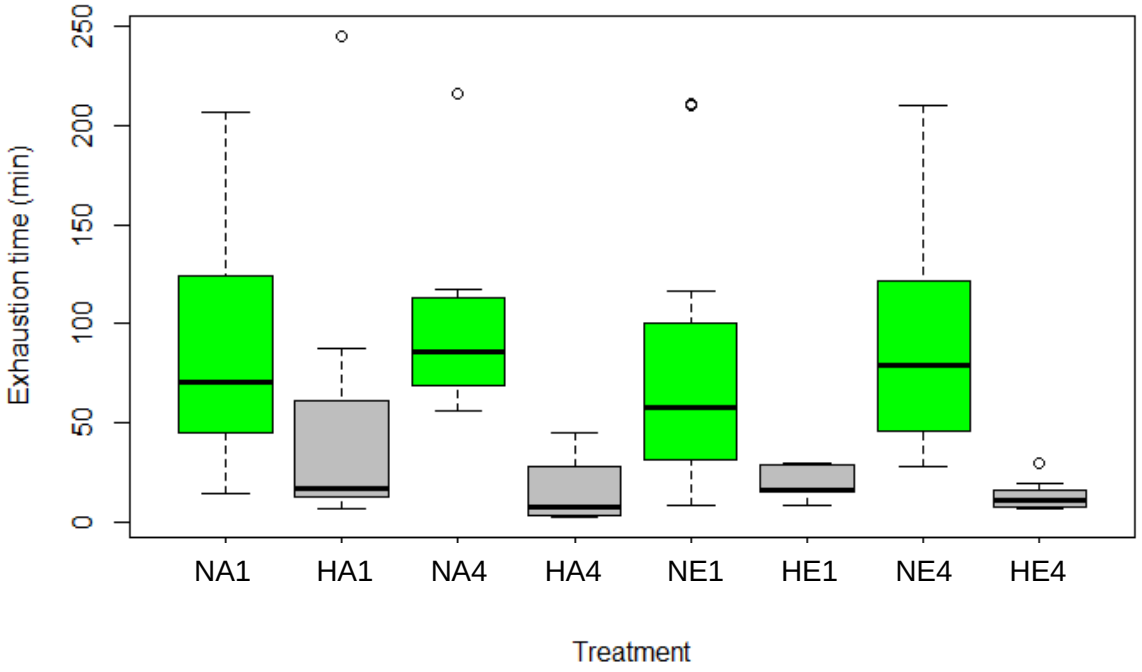


Figure 3.3 Stamina box and whisker plot with pooled population data. N: normoxia; H: hypoxia; A: ambient temperature; E: elevated temperature; 1: one week of exposure to mesocosm conditions; 4: four weeks of exposure to mesocosm conditions.

3.5 Discussion

No significant differences in the physiological performance of *Pandalus borealis* from Brofjorden and Gullmarfjord were observed. As such, the data for both populations were pooled for the GLM analysis. The capacity for *P. borealis* to acclimate to elevated temperature and hypoxia was unclear. Metabolic rates altered after four weeks exposure compared to one week. However, this effect was also seen in individuals from the control treatment. Elevated temperature had a significant impact on metabolic rates and hypoxia significantly reduced stamina. However, neither response demonstrated a significant interaction between the stressors. The three predictions outlined in Section 2.2 are addressed independently in further detail in the following sections.

3.5.1 Population differentiation

The first prediction put forward in this chapter posited that the Gullmarfjord and Brofjorden populations have undergone phenotypic divergence as a result of the different oxygen regimes in the habitats they occupy. No significant difference between the two populations in performance for metabolic rates or stamina was observed. This suggests that the periodic hypoxia in Gullmarfjord has not driven a shift in performance under stress. In general, mean stamina fell in hypoxia and elevated treatments when compared to control (NA) treatments (see Figure 3.3 and Table 3.2). There was, however, one population-specific anomaly in this pattern. There was a distinct difference in the mean stamina response of the Gullmarfjord population under normoxia and elevated temperature (NE) compared to the control treatment after both one and four weeks of exposure.

Our experiments exposed *P. borealis* to chronic stress. There is evidence to suggest that the mechanisms for dealing with periodic or acute hypoxia are different to those that allow organisms to cope with chronic hypoxia (deFur & Pease 1988; Taylor & Miller 2001; see Burnett & Stickle 2001 for review). For example, under acute hypoxia, organisms may increase ventilation rates or increase blood flow to respiratory surfaces. Whereas under chronic hypoxia, physiological responses may include elevated respiratory pigment production, altering respiratory pigment structure to increase oxygen affinity, or adopting anaerobic metabolism (Burnett & Stickle 2001). It is also possible that those mechanisms for coping with periodic hypoxia carry an inherent physiological cost that may reduce overall fitness or leave organisms competitively inferior under alternate conditions (St-Pierre et al. 2000).

3.5.2 Phenotypic plasticity and acclimation

In its second prediction, this study proposes that *P. borealis* may be able to acclimate to hypoxia and elevated temperature and that this could be demonstrated by improved performance with increased exposure. Length of exposure had a significant negative relationship with RMR However, we also see this pattern across control treatments, indicating that it has not been induced by the stressors selected for this study or that the control treatments induced similar levels of stress. Laboratory confounding effects were not found to be widely reported in the literature. Obernier and Baldwin (2006) cite changes in carbohydrate, protein and lipid metabolism, elevated heart rates and weight loss as a result of insufficient acclimation time after transportation. However, as their review suggests that most of these measures return to baseline within one to

seven days, I believe our five day acclimation period was sufficient and that the temperature levels adopted may have confounded the results as discussed in section 3.5.3.

A further possibility is that *Pandalus borealis* are sufficiently vagile to avoid hypoxic conditions. It is known that they undertake vertical migrations (Barr 1970) and this may negate the need to develop hypoxia tolerance mechanisms or prevent sufficient exposure as to allow an acclimatory response. Equally, the more stable conditions in Brofjorden may have resulted in shrimp that were in better condition and therefore more resilient compared to their Gullmarsfjord counterparts (Zhang et al. 2006).

3.5.3 Environmental stressors

The physiological damage of elevated temperature and increase in metabolic rates combined with the performance limitation induced by hypoxia observed in other organisms (Sharpe and DeMichele 1977; Schoolfield et al. 1981;Taylor & Spicer 1987; Finke et al. 1996; Spicer et al 1999; Daoud et al. 2007; Hochachka & Somero 2002; Daniel & Danson 2010; Schulte et al. 2011), led me to propose, in my final prediction of this chapter, that elevated temperature and hypoxia would have synergistically deleterious impacts on *P. borealis*. General Linear Model analysis did not show any statistically significant interactions between oxygen and temperature level for metabolic rates or stamina performance.

Synergy between environmental stressors may not be as widespread as often assumed in ecological studies. Through a meta-analysis of 122 factorial experiments measuring faunal mortality in marine, freshwater and terrestrial systems, Darling and Cote (2008) found that mortalities from two stressors were generally not synergistic. Only a third of the experiments displayed any truly synergistic interactions. However, multi-stressor experiments are still important as more than 75 % of the experiments showed that multiple stressors interacted non-additively.

Oxygen and temperature had distinct impacts as individual stressors on metabolic rates and stamina. Oxygen level did not have a statistically significant influence on RMR. However, the hypoxic treatments were generally more variable, producing both the highest and lowest mean MO_2 (HAG4: 12.22 ± 7.57 µmol L^{-1} h^{-1}, n = 4; HAB1: 63.98 ± 13.11 µmol L^{-1} h^{-1}, n = 4). This increased phenotypic variability may be as a result of environmental stress (Leary & Allendorf 1989; Badyaev et al. 2000) as is typical of animals close to their physiological limits, but further investigations would need to be conducted to confirm this.

Contrary to expectation, oxygen consumption tended to be higher in shrimp from hypoxic treatments. Many organisms increase their ventilation rates and cardiac output to compensate for low oxygen availability (Herreid 1980). However, this is unlikely to raise oxygen consumption above normoxic levels. The percentage utilization of dissolved oxygen by the rainbow trout, *Oncorhynchus mykiss*, under progressive hypoxia decreased from 55 to 20 % despite a 13-fold increase in ventilation volume (Holeton & Randall 1967) and oxygen extraction efficiency in *Nephrops norvegicus* only rose as high as 40 % under hypoxia (25 % a.s.) though gill beat rate doubled (Hagerman & Uglow 1985). During the data quality control phase of this study, it was discovered that a sensor issue resulted in some of the post-exercise shrimp being introduced to higher oxygen concentrations than originally intended. To compensate for this, only the most reliable data points were included and the first 15 minute readings for all the data were excluded. It is possible that these measures were not sufficiently stringent and the increase in oxygen consumption for hypoxic treatments is as a continued "hyperventilation" effect, or excess post-hypoxic oxygen consumption (EPHOC), due to being introduced to a higher level of oxygen concentration than they have been conditioned to where the shrimp attempt to recover their oxygen debt (Marvin & Burton 1973; Nonnotte et al. 1993; Virani & Rees 2000). This EPHOC is generally caused by the pathways that synthesise ATP and glycogen being reactivated (Mandic et al. 2008). In fact, this increase in MO_2 upon return to higher oxygen concentrations may be a means by which the Gullmarfjord *P. borealis* recover if they encounter hypoxic zones (Svendsen et al. 2012).

By contrast, hypoxia reduced stamina to as little as a quarter of its normoxic capacity in this experiment. Neither temperature, length of exposure, nor population origin had any significant influence on stamina, suggesting that neither adaptation nor acclimatisation to thermal or hypoxia stress had any role in this experiment. It should be noted, however, that shrimp across all treatments were highly variable in their responses, so it is possible that patterns would have emerged with larger treatment groups. Nevertheless, there were some key consistencies that demonstrate the ways in which hypoxia may inhibit or constrain this species. For example, the treatment group with the most depressed mean metabolic rate (HAG4) was the same treatment with the shortest mean stamina (< 3 min; see Table 3.2 and Figure 3.3). Hypoxia induced reductions in stamina may have significant costs to fitness, hindering their ability to escape predators, forage, or even to evade unsuitable habitat such as oxygen minimum zones (Pörtner et al. 2005).

The lack of influence of temperature and population origin on stamina throughout the study could be attributed to the shrimp being close to their thermal tolerance limit already at the temperature used in the ambient treatment (Paul & Nunes 1983). Though it was not directly tested, during the

experiments, shrimp from elevated temperature treatments appeared more lethargic and required more coercion to swim.

The physiological capacities of aquatic ectotherms have evolved so that aerobic scope is maximised within a specific temperature range thus optimising fitness-related performance (Pörtner 2002). Beyond this optimal temperature range, aerobic scope diminishes with deleterious consequences regarding the energy invested in key fitness traits, such as growth, reproduction and locomotion (Pörtner 2001). The ramifications of this may have severe negative impacts on population stability and recruitment by lowering survivorship throughout *Pandalus borealis* life history. For example, elevated temperature affects yolk absorption and the rate at which yolk is turned into tissue in embryos (Heming 1982). By increasing metabolic rates, it increases larval development rates, resulting in smaller juvenile and frequently deformed shrimp (Arnberg et al. 2013). These undersized shrimp have reduced swimming and hunting ability and their unusually small size may prevent them from accessing seasonal food sources, causing trophic mismatch (Ouellet et al. 2011).

Treatment temperatures were set according to the conditions in the field at the time of sampling and near future prediction of temperature increases for the area (IPCC 5th Assessment Report 2013). Marine organisms often exist on the limit of their thermal maximum tolerance (Helberg 1998; Tomanek & Somero 1999; Stillman & Somero 2000; Stillman 2002) and it is possible that, due to the temperature selected for this experiment, *P. borealis* were exposed to their thermal maxima across all treatments.

The Swedish Meteorological and Hydrological Institute have the most complete marine environmental datasets for the area, but they generally only survey marine temperatures down to approximately 40 m. Their records from the last 56 years between 40 and 64 m depth show a minimum temperature of 2.39 °C, maximum of 10.1 °C, and a mean of 5.96 °C. The water temperature accessible below the thermocline at the beginning of the experiment was taken as the ambient temperature. The majority of thermo-tolerance studies adopt worst case scenario temperature predicted to occur by 2100. Our elevated temperature was designed to mirror the IPCC near future predictions for the Baltic and Swedish coastline within the same time frame.

The IPCC 5th Assessment Report (2013) projects temperature increases of 1-3 °C between December and February and 0.5-2 °C between June and August from 2016-36 with respect to the 1986-2005 average temperatures. *Pandalus borealis* may live more than half a decade (Skuladottir et al. 2005). The present study investigated likely physiology-driven consequences for species persistence over a time frame within which evolutionary rescue would be improbable and plasticity would be key. The fact that the “ambient” temperature selected appears already at the or

past the species environmental maximum, was an artefact of local conditions; Spring 2014 in Kristineberg was unusually mild.

Despite this methodological artefact, temperature did have a significant impact on RMR and mean values of MO_2 and stamina for NE behaved atypically compared to the other treatment groups. Initially, RMR appeared reduced under elevated temperature. It then increased after four weeks of exposure and across both sampling time points, RMR was generally higher among stress treatments than the control groups. Relative to the control samples, the mean RMR MO_2 for NE1 dropped in contrast to the other treatment means. Similarly, when we investigate the mean stamina values for each population within a treatment group, stamina only increased relative to the control group in Gullmarfjord individuals exposed to NE at both time points.

Multi-stressor experiments can be highly illuminating, but are inherently complex (Folt et al. 1999; Darling & Cote 2008). Further work should include the use of multiple temperatures at a lower level specific to the habitat of populations being studied, spanning the organisms' known tolerance range and encompassing predicted values, for future experiments wishing to investigate changes in metabolic rates and the interaction between oxygen limitation and thermal stress.

Other studies have found sub-tidal crustaceans to be particularly intolerant of environmental stress. For example, Magozzi & Calosi (2014) found that *Pandalus borealis* had the highest MO_2 at the lowest temperatures, the lowest upper thermal tolerance limit, and the lowest levels of physiological plasticity under thermal stress when compared to five other temperate species of shrimp for *Pandalus borealis*. Dupont-Prinet et al. (2013) noted that females were less tolerant of hypoxia than their earlier life stage male counterparts, that hypoxia significantly decreased the activity of enzymes involved in anaerobic biochemical pathways, and that aerobic scope was severely reduced under hypoxia. All of these factors greatly constrict *P. borealis* capacity to respond to metabolic demands, such as vertical migration, foraging or egg production. *Pandalus borealis* aerobic scope has already been seen to be compromised in present-day warm years and, though Pillet et al. (2016) observe resilience to hypoxia in terms of enzyme activity at similar oxygen levels, this study amongst others clearly demonstrates vulnerability under hypoxia.

Due to the lack of population differences, it is difficult to make specific inferences regarding phenotypic specialisation or shifts and trade-offs in energetic balance under elevated temperature and hypoxia from this particular experiment. It is important to note that not all phenotypic plasticity is adaptive in the sense of improving fitness and some traits may simply be plastic as a result of biochemical or developmental constraints (Sultan 1995). Further work investigating the balance between aerobic and anaerobic respiration *via* ATP analysis would be advised and the relative production of cellular metabolites is investigated in Chapter 4 of this thesis.

This study documents significant impacts of oxygen limitation and a clear drop in stamina under hypoxia. In conjunction with the fact that we could not run the experiment at conventional “hypoxic” levels (20 % a.s. O_2), it is vital to echo Vanquer-Sunyer and Duarte (2008) in that the vulnerability to oxygen stress is being largely underestimated.

Rising levels of eutrophication (Gray et al. 2002) through anthropogenic input, as well as global warming leading to reduced oxygen dissolution and increased marine stratification, make coastal hypoxia a mounting concern for the health of marine organisms (Levin & Dayton 2009). Vagile organisms such as *P. borealis* may be able to avoid these depleted oxygen zones (Brierley & Kingsford 2009), but only as long as their ranges are not compromised by other anthropogenic and climatic factors. Though the common garden experiment used in this chapter did not show any physiological differences between *P. borealis* collected in Brofjorden and Gullmarfjord, it is possible that physiological plasticity may manifest along metabolic pathways not directly associated with the metrics selected for this chapter. It is also possible that population differences may be more evident away from potential confounding laboratory effects. Chapter 4 will use a field translocation experiment testing whole organism and metabolomic responses to address these issues and further explore physiological plasticity among these two *P. borealis* populations.

Chapter 4: Do populations of *Pandalus borealis* from different oxygen regimes exhibit physiological differentiation under environmental stress? - Translocation approach

4.1 Abstract

A translocation experiment was used to test for differences in physiology and vulnerability under hypoxia between *Pandalus borealis* collected from a normoxic and a periodically hypoxic fjord. It was predicted that individuals from the population from the periodically hypoxic site, Gullmarfjord, would be less vulnerable to hypoxia. Contrary to the common garden investigation of phenotypic plasticity, this study did find significant differences in the responses of the two populations to hypoxia. The metabolomic profiles of each treatment showed that both populations exhibited a physiological response to translocation and, moreover, that they responded differently to the same conditions. However, *P. borealis* collected in Gullmarfjord appeared to be more vulnerable than their Brofjorden counterparts to hypoxia with significantly higher mortality levels. Fully understanding the mechanisms behind this unexpected vulnerability requires further testing, but the costs of environmental stress and differences in *P. borealis* diet at the two sites may have contributed.

4.2 Introduction

Difference in morphology and oxygen regimes of *Pandalus borealis* inhabiting Brofjorden and Gullmarfjord led me to hypothesise that periodic hypoxia in Gullarmfjord may have driven differentiation between the two populations. No significant differences between population physiological performance – as quantified by oxygen consumption and stamina – were observed in the common garden experiment detailed in Chapter 3. Exposure to hypoxia or living close to their critical oxygen threshold has routinely been found to be deleterious or energetically costly to Crustacea, including *P. borealis* (Baden et al. 1990; McMahon 2001; Dupont-Prinet et al. 2013; Leiva et al. 2015; Pillet et al. 2016; Sun et al. 2016). So, despite the lack of population differentiation found during the targeted common garden investigations in Chapter 3, phenotypic plasticity to alleviate hypoxia stress may still be a favourable mechanism for *P. borealis* persistence in the Gullmarfjord. If true, this would be evident across whole organism responses and physiological pathways not detectable from measuring oxygen consumption and stamina alone.

Chapter 3 examined oxygen consumption and stamina as proxies for metabolic rates to determine environmental stress impacts on overall fitness. This is highly challenging to achieve in the field, but by adopting an omics approach on specimens exposed to stressors in the field, the results of this experiment remain comparable with the common garden experiment and may yield physiological differences that could be overlooked with a targeted approach. This has previously been the case for the common carp, *Cyprinus carpio*, exposed to hypoxia; myoglobin production was induced in organ tissues including the gill, liver and brain despite having previously thought to be expressed exclusively in skeletal and cardiac tissue (Fraser et al. 2006; Gracey 2007). In addition, many biological processes are interlinked and disruptions in one suite of processes may have unanticipated consequences for another. Adopting a metabolomic approach with our tissue samples not only allows us to understand the impacts on metabolic rates, but also to survey all biochemical footprints left by cellular processes and characterise the interactions of *P. borealis* with its environment (Regoli et al. 2004; Bundy et al. 2009). Once this information has been obtained, we then have the opportunity to investigate in much greater detail the metabolite expression involved in the affected pathways. In addition, molecular and cellular level shifts can represent the earliest warning signals of environmental disturbance (Depledge, 1994), so a metabolomic approach may prove far more sensitive at detecting stress responses than standard physiology techniques.

Furthermore, common garden experiments are limited in that they only measure a single component of local adaptation: the spatial covariance between the phenotypic frequencies within the populations in question and the key environmental driving factor identified in their native habitat (in this case, hypoxia) (Nuismer & Gandon 2008). By contrast, reciprocal translocation experiments take into account the variability of the ecological environment not exclusive to the identified environmental driver (Nuismer & Gandon 2008). Spicer (2014) advocates the use of multiple experimental techniques to address the discrepancies that may arise in organismal performance and behaviour between the laboratory and field.

Translocation experiments tend to impose minimal handling stress compared to laboratory based common garden experiments. A field based experiment also minimises handling stress, disturbance and other laboratory confounding factors that may add noise to data or alter the result (Balcombe et al. 2004; Kawecki & Ebert 2004). Though common garden experiments appear to be more widely adopted for population comparisons (see balance of studies in review table in Appendices Section ix), reciprocal transplant experiments have provided important insights concerning the extent of population divergence and specialisation and of the factors that contribute towards adaptive differentiation (Kawecki & Ebert 2004; Leimu & Fischer 2008; Hereford 2009).

Mitchell-Olds and Schmitt (2006) state that reciprocal translocation experiments between natural populations are required in order to directly test local adaptation hypotheses.

To address some of the limitations of the common garden approach used in Chapter 3, this study investigated whether populations of *Pandalus borealis* from different oxygen regimes exhibit physiological differentiation under environmental stress using a field translocation experimental design. Due to potential prior exposure to hypoxia, it was predicted that *P. borealis* originating from a seasonally hypoxic inner fjord site (Gullmarfjord) would be less vulnerable to the negative impacts of hypoxia than individuals originating from Brofjorden (well-circulated site). Shrimp from Gullmarfjord and Brofjorden were translocated to a normoxic and a hypoxic site with a control group in each site for a period of 14 days that was sampled weekly. Survivorship and metabolomic profiles from individuals from both populations were assessed as a proxy for performance and overall fitness.

4.3 Methods

4.3.1 Animal collection and husbandry

Adult females of the northern shrimp *Pandalus borealis* were collected as *per* the protocol described in Section 2.3.1.

4.3.2 Field translocation experiment

Two candidate sites with differing oxygen regimes were selected based on historical data for the area on the Swedish Meterological and Hydrological Institute (SMHI) database (see Figure 4.1). Havstensfjord (58°19'39.2"N 11°45'34.8"E) undergoes cyclic hypoxia and the mouth of Gullmarsfjord (58°15'08.5"N 11°26'11.8"E) – more than 1 h boat journey (> 15 km) away from the inner fjord site where our shrimp population are found - experiences high currents and is well circulated all year round. The sites where the shrimp were originally harvested were unsuitable study sites. Both were unsafe for the animals and equipment as they receive heavy shipping traffic and Brofjorden was not sufficiently sheltered to avoid damage to the equipment and the caged animals. During the experiment, the shrimp were caged in creel cages with reinforced hatches and mesh over the entrance to prevent escape and a feeding pouch attached to the inside.

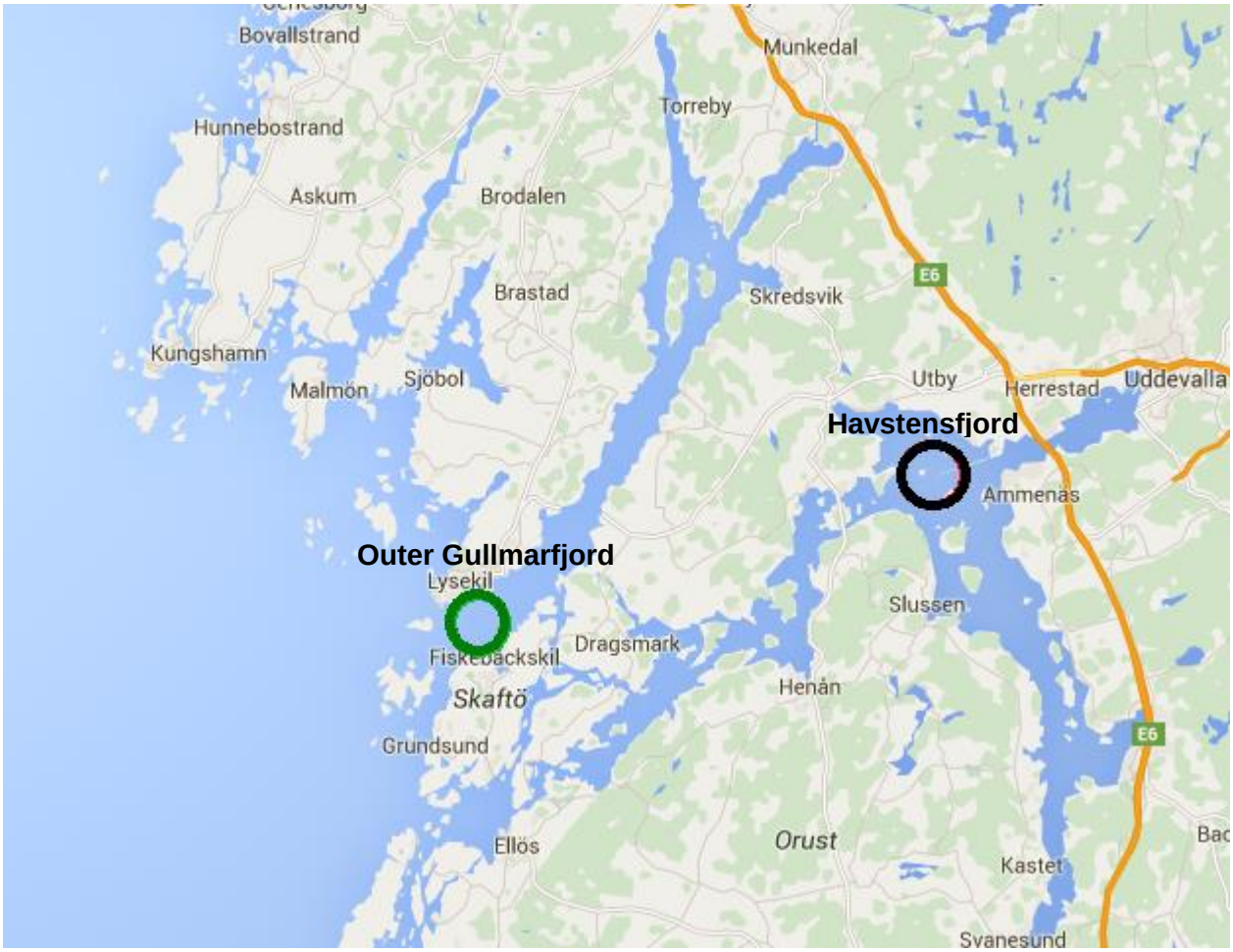


Figure 4.1 Translocation sites. Normoxic treatements were situated at the mouth of Gullmarfjord (58°15'08.5"N 11°26'11.8"E) and hypoxic treatments were situated at Havstensfjord (58°19'39.2"N 11°45'34.8"E).

Six 60 L modified creel cages (three containing Gullmarfjord individuals and three containing Brofjorden individuals) were suspended at 20 m depth in each experimental site. Each cage contained one perforated food pouch, stocked with chopped herring (*Clupea harengus*), and 20 individual shrimp. Pairs of cages were placed 50 m apart at 200 m intervals and water samples were taken from 20 m depth to record at depth temperature, pH, O_2 using a probe (Sven Go SG2, Mettler Toledo, Leicester, UK) and salinity using a refractometer (H2Ocean Precision Instrument, DD The Aquarium Solution, Ilford, UK), nutrient (API Aquarium Pharmaceuticals Saltwater Master Tester Kit, Chalfont, USA) and alkalinity levels (Titroline Alpha Plus, SI Analytics, Mainz, Germany) (see Table 4.1. for main environmental conditions).

After one and two weeks of exposure, a subsample of approximately ten individuals from each cage was sampled for subsequent tissue analysis, mortality was recorded and physico-chemical variables of seawater samples measured.

Table 4.1 Environmental variables (± s.e.) from the two field sites (Control – mouth of Gullmarsfjord; Hypoxic – Havstensfjord). Samples were taken upon deployment of the cages (week 0) and when tissue samples were taken after one and two weeks of exposure. Values are means of 18 samples per site.

Sample	% O_2	pH	temperature °C	salinity
Control week 0	95.43 ± 0.30	8.18 ± 0.03	8.58 ± 0.03	35.60 ± 0.10
Control week 1	97.48 ± 0.41	8.18 ± 0.05	9.89 ± 0.18	36.22 ± 0.26
Control week 2	100.39 ± 0.44	8.17 ± 0.03	10.64 ± 0.09	34.28 ± 0.55
Hypoxic week 0	63.13 ± 0.65	7.79 ± 0.01	6.94 ± 0.13	27.75 ± 0.17
Hypoxic week 1	60.12 ± 0.43	7.73 ± 0.03	6.85 ± 0.21	28.25 ± 0.30
Hypoxic week 2	63.00 ± 0.79	7.59 ± 0.02	7.96 ± 0.17	31.11 0.10

4.3.3 Metabolomic analysis

4.3.3.1 Sample extraction – metabolomics analyses

Samples were homogenised in Precellys tubes with methanol (4 µl/mg tissue) ad water (8.5 µl/mg tissue). A 240 µl aliquot was taken and 80 µl of chloroform and 500 µl chloroform, methanol and water solution (1 : 2 : 0.8) were added. The aliquot was vortexed, cooled for five minutes and then centrifuged for 10 min. Once cool, 500 µl of the resulting supernatant was aliquotted and dried under liquid nitrogen.

4.3.3.2 Quality control and UHPLC-MS analysis

Laboratory and computational analyses were carried out at NERC Biomolecular Analysis Facility at Birmingham.

Samples were taken up in 100 µl water / methanol 7:3 and vortexed. Each sample was transferred to a 1.5 ml plastic tube, and 10 µl each were taken to pool a QC (quality control) sample (approximately 1.5 ml). After centrifugation, (15000 rpm for 10 min at 4 °C, Biofuge), 20 µl per sample were pipetted into a 96-well plate, starting with QC samples, a blank, and further QC samples, and ending with a blank after two QC samples. The samples were run in controlled

randomised order, with QC samples equidistantly between them. They were analysed by UHPLC-MS (Ultra High Performance Liquid Chromatography - Mass Spectrometry) on a Thermo Scientific Q Exactive mass spectrometer attached to a Thermo Dionex Ultimate 3000 RS system, equipped with a Thermo Hypersil Gold column (100 x 2.1 mm, 1.9 µm particles). Samples were run for 14 min for LC from solvent 1 (0.1 % formic acid in water) to solvent 2 (0.1 % formic acid in methanol), 400 µl/min, with 18 µl injections per sample and with MS starting at 0.45 min, with the prior flow directed towards waste. Data was collected in positive ion and profile mode, *m/z* 100-1000. Two plates were required for the sequence and, as the first runs did not show sufficient quality, they were repeated using a third plate.

4.3.4 Statistical analyses

4.3.4.1 Field translocation experiment analyses

All analyses were conducted using the statistical program R version 3.1.2.

A full GLM maximal model of the field mortality data including all the environmental variables was over parameterised. Correlations of the raw data showed the strongest relationships between mortality and 'experimental site', 'population origin' and 'weeks of exposure', so I reduced the maximal model to investigate only the impacts of these three factors and their interactions. The minimal adequate model was achieved by excluding all non-significant variables.

4.3.4.2 Metabolomic analyses

Initial data exploration indicated high level of detrimental cage effects on the shrimp metabolome, so subsequent uni and multivariate analyses were only carried out on the shrimp exposed for one week.

ANOVA in combination with FDR correction ($\alpha = 0.05$) was used for univariate analysis to detect if the factors population and site and their interaction population x site were significant. Next, the significant peaks were filtered on the basis of their fold change, where peaks with absolute log2 FC-values larger than 1 were retained.

PCA + MANOVA and regularized MANOVA were used for multivariate analysis of the data. Two analyses were considered; namely a model with factors population, site and interaction population x site, and a model with factor group (e.g. normoxic population - normoxic site, normoxic

population – hypoxic site, etc.). The factor group model was mainly used for visualization of the data.

When a factor or interaction was marked as significant, multi-group sparse discriminant analysis was used to visualize the differences between the samples and to determine which peaks in the data were most related to this effect. Additionally, the FDR corrected standardized coefficients of the rMANOVA model were used for this purpose.

4.4 Results

Both the survivorship and metabolomic data show that the two populations respond significantly and significantly differently from one another under translocation to or away from a hypoxic site. The survivorship results show the inner Gullmarfjord population to be the more vulnerable population and the contrast in metabolomic peak profiles show that both populations exhibited a different response to translocation and hypoxia stress.

4.4.1 Survivorship

Overall survivorship and vulnerability to hypoxia between the two populations was significantly different. Additionally, length of exposure had a significant impact on mortality at both experimental sites.

Survivorship was highest among Brofjorden individuals across all treatments. During the first week, mortality was considerably greater in shrimp exposed to the hypoxic site (62.43± 0.41 % a. s.), especially for the Gullmarsfjord population (see Figure 4.2; see Table 4.2 population origin $p < 0.01$). Only one dead individual was recorded in the first week in the normoxic site. In the second week of exposure, almost identical levels of mortality were observed at the hypoxic site and the normoxic site (see Figure 4.2; see Length of exposure $p < 0.05$) with mortality in the Gullmarsford population being almost six times higher than that of the Brofjorden population at both sites.

Finally, pH, alkalinity, salinity and nutrient levels documented during the field experiment had negligible explanatory power in GLMs over the levels of mortality of *Pandalus borealis* recorded during the experimental period and so were excluded from the minimal adequate models.

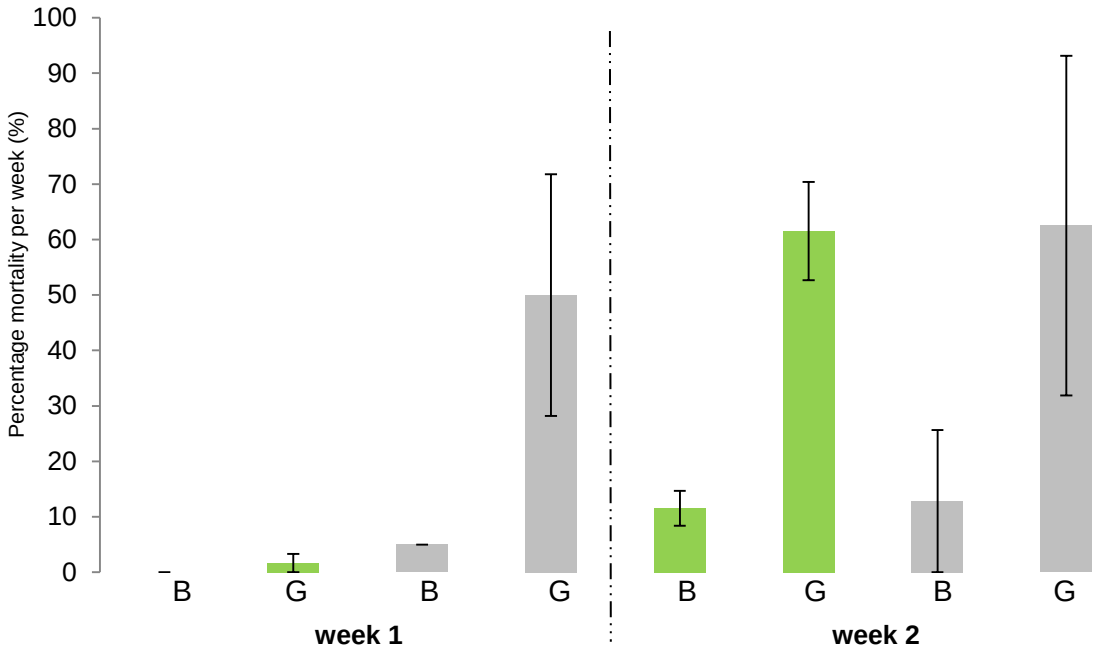


Figure 4.2 Mean percentage non-cumulative mortality per cage per week with standard errors of Pandalus borealis at one and two weeks of field exposure. The population origin of each treatment group in indicated under each histogram; "B" for Brofjorden individuals, "G" for Gullmarsfjord. Cages were suspended in a normoxic site (98.241 ± 0.836 % a. s. O_2: green bars) and a hypoxic site (62.433 ± 0.410 % a. s. O_2: grey bars).

Table 4.2 Results of GLMs to identify the main explanatory variables of Pandalus borealis mortality during translocation. df: degrees of freedom, F: F-ratio, p: probability level

Trait	Source	df	F	p
Field mortality	Population origin	19	11.160	< 0.01
	Length of exposure	20	4.683	< 0.05

4.4.2 Metabolomics

Differences in the metabolomic profiles of each treatment group after one week of exposure indicated that both the Brofjorden and the Gullmarfjord populations responded differently to translocation (see Figure 4.3). In a MANOVA model used to determine if the treatment groups were significantly different, there was clear separation between the groups. Furthermore, the directions of metabolomic shift coinciding with translocation (as represented by the arrows) are not parallel. This observed interaction effect was confirmed by a second MANOVA model (see Appendices Section v), where a significant interaction between site and population was detected ($p < 0.005$).

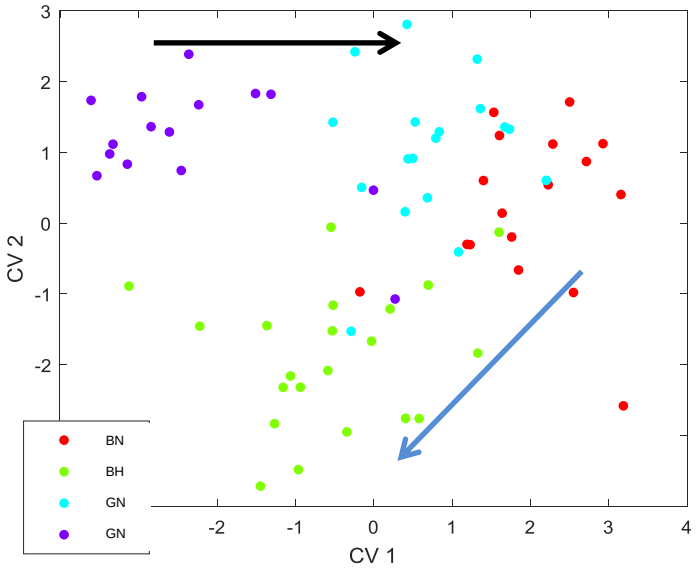


Figure 4.3 MANOVA score plot of model 1. The labels BN, BH, GN, and GH refer to samples from Brofjorden (B) and Gullmarfjord (G) translocated to either a normoxic site (N) or a hypoxic site (H). The arrows indicate the shifts of the normoxic and hypoxic populations to a change in site. The black arrow indicates the shift of the hypoxic population. The blue arrow indicates the shift of the normoxic population. CV refers to the canonical variate. The p-values of CV1 and CV2 were < 0.0001 and < 0.0001, respectively.

Univariate analysis detected significant differences in metabolite production attributable to both population origin and the translocation site. No significant interaction between population origin and site was observed using this approach (see Table 4.3). However, multivariate statistics observed significant differences in metabolome attributable to population, site, and the interaction between the two. For a large number of peaks no putative annotation was obtained. However, a complete list for both population and site effects of the top ten peaks in the data set and their significance/annotation can be found in the Appendices (see Section v).

4.4.2.1 Univariate analysis

ANOVA in combination with FDR correction ($\alpha = 0.05$) observed significant differences for population origin and site, but not for the interaction effect (see Table). This lack of interaction confers that both populations respond to translocation, but respond in the same way. As a result, subsequent univariate analysis excluded interaction effects.

Table 4.3 summary of the univariate analysis of the data describing the average difference between the normoxic and hypoxic populations (factor population) and the normoxic and hypoxic site (factor site).

Factor	Significant peaks	Significant peaks with significant FC	Up regulated	Down regulated
Population origin	295	18	6	12
Site	351	62	11	51
Population : Site	0	0	0	0

Most of the peaks putatively annotated from the univariate results refer to metabolites involved in protein or aromatic compound synthesis, neurotransmitter inhibitors, or compounds with known toxic effects for arthropods (see Appendices Table V).

4.4.2.2 Multivariate analysis

Significant differences were observed via both rMANOVA and combined PCA and MANOVA analysis for factors population and site, and interaction the interaction between the two factors (see Table 4.4). Significant differences were also observed when the four groups of samples were directly compared (see Figure 4.3 and Appendices Section v).

Table 4.4 Summary of the multivariate analysis of the data

Method	Group	Population origin	Site	Population x site
PCA + MANOVA	2.2e-14	2.4e-07	1.7e-09	0.014
rMANOVA	9.9e-04	9.9e-04	9.9e-04	9.9e-04

In contrast with the univariate statistics, the significant interaction between site and population origin effects indicates that the Gullmarfjord and Brofjorden shrimp do respond differently to translocation. Due to this significant interaction between the main effects and the nature of the models used, direct interpretation of the main effects was not possible. Therefore identification of the important peaks in the data focused on the peaks that were associated to the interaction effect (see Appendices Section v).

A large number of peaks based on the CV weights from the rMANOVA model had a similar weight. Interpretation of the canonical vectors from this analysis risks overlooking potentially important peaks or producing false positives. To circumvent this, a variable selection approach using MGSDA (Multi-Group Sparse Discriminant Analysis) was also used to select the most important peaks for a factor or interaction, which yielded a much more interpretable model (see Appendices Section v). The putative annotations for the most significant peaks identified *via* MGSDA mostly referred to compounds involved in energetics, protein synthesis or had insecticidal properties.

4.5 Discussion

As predicted, the survivorship data and the multivariate metabolomics analyses show that populations from Brofjorden and Gullmarfjord do exhibit significant levels of physiological differentiation. However, contrary to our predictions, the Gullmarfjord population appear to be more vulnerable under hypoxic conditions than the Brofjorden population. It is possible that caged conditions were too unfavourable for the shrimp and hypoxic conditions simply accelerated their decline. Alternatively, it may be that the Brofjorden population exhibited significantly higher levels of tolerance; in week 1 there was no mortality in the normoxic site and a tenth of the level of mortality experienced by the Gullmarsfjord population in the hypoxic site. Under this assumption, it would appear that the Gullmarsfjord population demonstrated no adaptive or acclimatory tolerance to chronic hypoxic conditions. During the second week of the study, mortality did not differ significantly between the two populations at the normoxic site, which indicates that hypoxia may have been the key driver of mortality during the first week.

There are several reasons why the Gullmarfjord population may be more vulnerable that *Pandalus borealis* from Brofjorden. Firstly, as our results suggest that the periodic hypoxia of the inner Gullmarfjord has not selected for hypoxia tolerance in this population of *Pandalus borealis*, we could assume that a population lacking continuous oceanic circulation has relatively low genotypic diversity. High genetic diversity increases the likelihood of tolerant genotypes being present within a population and reduces extinction risk (Reid 1994; Oostermeijer et al. 2003; Frankham 2005; Jump & Peñuelas 2005; Jump et al. 2009). However, the results from Chapter 2 show that both the Brofjorden and Gullmarfjord populations have almost identical standing genetic diversity, which may be an artefact of the methods used or evidence that no genetically fixed traits have been selected for.

Secondly, morphological differences in size and colouration have been documented in these populations of *Pandalus borealis* (Eriksson pers. comms. 2013). Colouration is a known health and diet indicator in other crustacean species (Chien et al. 1991; Main & Laramore 1999; Bondad-Reantaso et al. 2001; Floreto et al. 2001) as briefly discussed in Section 2.4. Assessment of fatty acid profiles and protein-lipid rations could be used in further work to test the assumption that *P. borealis* populations in Brofjorden and Gullmarfjord differ in diet or overall body condition to an extent where it may impact their hypoxia tolerance. Equally, the more stable conditions in Brofjorden may have resulted in shrimp that were in better condition and therefore more resilient at the beginning of the experiment compared to their Gullmarsfjord counterparts (Zhang et al. 2006).

The *Pandalus borealis* used in this study did not differ significantly in terms of body length, weight per unit length, or mortality in the laboratory (see Appendices Section vi for table of values and statistical analyses). During field translocation trials, total loss of specimens was experienced at the initial hypoxic site. This was thought to be due to a low salinity event. Salinity levels between the hypoxic and normoxic sites used in this chapter do vary, so a laboratory based trial was used to determine the salinity tolerance thresholds of *P. borealis* under low salinity and hypoxia stress. Above 27 psu, *P. borealis* appear to experience minimal negative impacts and so our chosen sites were deemed suitable. As with the translocation experiment described in this chapter, the Gullmarfjord individuals appeared more vulnerable and specimens under both normoxic and hypoxic conditions died sooner than Brofjorde specimens.

Thirdly, I posit that this is because our experiment, while providing a representation of potential stressors encountered by this species and possible future conditions, did not accurately represent the selective forces that currently act on our populations. The conditions experienced in the field transplant experiment were chronic hypoxia and, if the Gullmarsfjord population do encounter hypoxia, it may well be for much shorter periods of time as shrimps are sufficiently vagile (Barr 1970) to escape hypoxic zones. It is known that they undertake vertical migrations (Barr 1970) and this may negate the need to evolve hypoxia tolerance mechanisms or prevent sufficient exposure as to allow an acclimatory response. However, as hypoxic events become spatially and temporally more prevalent, this behavioural avoidance strategy may cease to be sustainable.

It is possible that any tolerance the Gullmarfjord *P. borealis* may have developed for these acute shocks may involve very different mechanisms to those required to tolerate chronic stress (deFur & Pease 1988; Taylor & Miller 2001; see Burnett & Stickle 2001 for review). Concurrently, if the Gullmarsfjord population have such mechanisms for dealing with short-term hypoxia, the potential energetic trade-off of this trait may leave them at a competitive disadvantage compared with other populations when facing chronic stress (St-Pierre et al. 2000).

The dramatic increase in mortality in both populations from week one to week two of the field experiment suggests that *P. borealis* can only tolerate relatively short periods of chronic hypoxia. A negative effect of long-term exposure suggests a loss of physiological performance, and that these individuals were unable to acclimatise and express a sustainable phenotype to cope with these conditions. This is further supported by the toxic nature of many of the most significant peaks putatively annotated in the metabolomic analysis. However, cage conditions themselves may have in part increased mortality levels in week two, as we also observed some level of mortality at normoxic conditions. Temperature fluctuated at both sites, with mean levels rising by 1.1 °C at the hypoxic site and by 2.0 °C at the normoxic. Given the levels of temperature related

stress observed in Chapter 3, this rise in temperature, particularly at the normoxic site, may have contributed to mortality in the second week.

Few reciprocal translocation experiments testing the effects of marine hypoxia appear to have been carried out thus far. *Mytilus edulis* exposed translocated to chronic hypoxic conditions exhibited moderate phenotypic plasticity, but – unlike our findings - physiological tolerance was still higher in mussels from intertidal zones that had had previous exposure to hypoxia (Altieri 2006). Impacts of marine hypoxia on the metabolome are also relatively understudied. Hypoxia exposure in *Haliotis diversicolor* caused disturbances in energy metabolism and osmotic balance (Lu et al. 2016). Approximately, 15 % of all the putatively annotated metabolites found in this study were directly related to glucose metabolism, demonstrating the scale of the negative impacts hypoxia has on energetics in marine invertebrates.

It is vital that studies incorporate molecular investigations in order to truly understand the threat of climate change and that we understand the relative vulnerabilities of different population directly affected by anthropogenic stressors. Molecular or omics approaches provide the opportunity to characterise an organism's capacity to acclimatise to abiotic stressors, comprehend the mechanisms behind sub-lethal stress that may have profound consequences for fitness (e.g. energy allocation). Metabolomics (metabolic profiling) allows us to characterise the metabolic, functional responses of *P.borealis* to environmental stressors and potentially discover novel biomarkers and molecular mechanisms of hypoxia and/or salinity tolerance. By using such techniques to compare the vulnerabilities of different populations, understanding of species spanning environmental gradients or mosaics could be greatly enhanced.

Both the survivorship and metabolomic data indicate negative impacts of hypoxia and that the Gullmarfjord and Brofjorden *Pandalus borealis* populations exhibit physiological differentiation. The periodic hypoxia of the inner Gullmarfjord habitat does not appear to have induced increased tolerance to hypoxia in *P. borealis*. In fact, the Brofjorden population exhibits higher tolerance under hypoxia.

This chapter, in the context of this thesis as whole, emphasises the need for a multidisciplinary approach to investigating local adaptation. Currently our understanding of the mechanisms underlying organismal responses to environmental stressors is incomplete, especially with respect to integrating processes across biological hierarchies and spatial and temporal scales (Schulte et al. 2011; Harvey et al. 2014). This creates challenges when attempting to design practicable and strongly grounded experiments to test the impacts of environmental stressors and adaptive potential.

Chapter 5: Synthesis

The overall aim of this thesis was to see whether there were population differences in response to environmental stress between *Pandalus borealis* inhabiting two coastal Scandinavian sites (Brofjorden, 58°18'12.4"N 11°17'59.7"E and Gullmarfjord, 58°20'26.1"N 11°33'31.7"E) which differ in their oxygen regimes. Such differentiation was postulated based on observed morphological differences between individuals from these populations (Eriksson, pers. comms. 2013). A secondary aim was to determine whether any differences have been driven by the periodic hypoxia occurring in one of the sites, the Gullmarfjord (Rosenberg et al. 1990; Gustafsson & Nordberg 2000; Andersson et al. 2009). We hypothesised that exposure of *P. borealis* to periodic hypoxia in Gullmarfjord would result in relatively higher levels of performance under stress than found in the Brofjorden population.

An integrative approach was adopted to address these aims, using whole organism, physiological, genetic and molecular techniques and both common garden and translocation experimental design. Firstly mitochondrial DNA and microsatellites were used to assess population genetic dissimilarity (Chapter 2). No significant differences in population structure were found in *Pandalus borealis* from Brofjorden and Gullmarfjord from which a fully shared evolutionary history can be inferred. This could be explained by the influx of *P. borealis* immigrants into Gullmarfjord from areas with high connectivity to Brofjorden driven by seasonal water exchange when the Gullmarfjord thermocline weakens in early spring (Bergström 1991). Previous genetic studies of *P. borealis* population structure have found both genetic dissimilarity and homogeneity in fjord populations at similar geographic scales (Drengstig et al. 2000; Knutsen et al. 2015; Martinez et al. 2015), inferring that the balance between gene flow and selective forces is highly dynamic in these bathymetrically complex regions.

As the populations showed no genetic differentiation, it was then hypothesised that phenotypic plasticity could allow *P. borealis* to tolerate fluctuating oxygen levels in Gullmarfjord. It was also predicted that previous exposure to hypoxia may allow Gullmarfjord individuals to elicit a more rapid or effective response to tolerate hypoxia than their Brofjorden counterparts. This pattern has been observed across a number of taxa and stressors. For example, with hypoxia and aerial exposure tolerance in the mussel *Mytilus edulis* (Altieri 2006), thermotolerance in the water flea *Daphnia magna* (Yampolsky et al. 2014), and toxicant tolerance in rainbow trout *Oncorhynchus mykiss (*as *Salmo gairdneri)* (Pascoe & Beattie 1979; Dixon & Sprague 1981a, b). Two experiments were designed to test this prediction.

Discrepancies between physiological responses to hypoxia in the laboratory and field are not uncommon and so a combination of experimental designs was deemed essential to gain a more reliable understanding of *P. borealis* responses to environmental stress (Spicer 2013). Firstly, a common garden experiment was used to investigate the impacts of hypoxia and elevated temperature on rates of oxygen consumption (MO_2) and stamina (see Chapter 3). Elevated temperature was selected as an additional stressor as metabolic rates in ectotherms are affected by both oxygen and temperature, and marine oxygen depletion is expected to worsen as a result of global warming (Pörtner 2001; Matear et al. 2000; Keeling & Garcia 2002; Paaijams et al. 2013; Clarke et al. 2014). As with the genetic analyses, no significant differences were found between populations for either oxygen consumption or stamina under any of the treatments. However, hypoxia was found to have a particular detrimental effect on stamina of individuals from both populations.

A reciprocal translocation experimental design was subsequently used to test *Pandalus borealis* responses to hypoxia in the field (Chapter 4). As measuring MO_2 and stamina would have been impracticable in the field, survivorship and metabolomic analysis were used as proxies for physiological performance. As seen in Chapter 3, shifts in metabolomic profile indicated that both populations exhibit a physiological response to hypoxia. However, in this experiment, Gullmarfjord and Brofjorden individuals responded significantly differently in terms of both survivorship and in altered metabolic processes; in other words both populations responded to hypoxia but in different ways. Interestingly the Brofjorden population exhibited better survivorship under hypoxia and generally appeared hardier throughout the experiment. This finding did not support our hypothesis. Two key factors may have contributed to this outcome.

Firstly, *Pandalus borealis* inhabiting Gullmarfjord may rely primarily on physical avoidance of hypoxia. As a result, they may have only developed mechanisms to tolerate acute exposure to hypoxia, which may not be effective under chronic exposure (deFur & Pease 1988; Taylor & Miller 2001).

Secondly, it is possible that the population differences observed were due to a disparity in body condition between *Pandalus borealis* at the two sites caused by high temporal variability in Gullmarfjord. The two populations are reported to differ in body size and pigmentation (Eriksson pers. comms. 2013; see Appendices Section vii for photographs). This, in conjunction with oceanographic differences between native habitat sites, was the basis for selecting these two populations for comparison and hypothesising divergence in the first place. Pigmentation has frequently been used as an indicator of health in other crustacean species (Chien et al. 1991; Main & Laramore 1999; Bondad-Reantaso et al. 2001; Floreto et al. 2001). Gullmarfjord shrimp may be

in poorer body condition due to environmentally-induced physiological stress or due to diet. Hypoxia has also been known to alter diet in several species (Pihl 1994). Furthermore, food sources may differ in general between the two sites; seasonal cycling in Gullmarfjord and adjacent sites has had a significant impact on community assemblage (Josefson & Widbom 1988; Lindahl & Hernroth 1988; Austen & Widbom 1991; Gustafsson & Nordberg 1999; Gustafsson & Nordberg 2000; Filipsson & Nordberg 2004). Dietary shifts and changes in food availability have been known to cause differences in physiological tolerance (Ishibashi et al. 1992; McKenzie 2001; McKenzie et al. 2008; Zambonino-Infante et al. 2013). Broad scale assessment of body condition based on a body mass: body length index found no significant differences between the individuals used in this thesis, but should further work on fatty acid content or protein-lipid ratios demonstrate population differences between Gullmar and Brofjorden, this would contribute to our understanding of the observed morphological differences.

So, to summarise this study shows that there are population differences in *Pandalus borealis* exposed to environmental stress, but not differences that we would have predicted. There is substantial evidence, from this and many other investigations, to show that both hypoxia and elevated temperature have detrimental impacts on crustacean performance and fitness (e.g. Bridges & Brand 1980; Grieshaber et al. 1993; McMahon 2001; Stillman & Hurt 2015). However, this study does not support the hypothesis that exposure to periodic hypoxia improves tolerance levels such as found by Altieri (2006). Indeed, the shrimp from Brofjorden – an open ocean circulated site – performed better than the Gullmarfjord shrimp under both normoxia and hypoxia. I have struggled to find examples of studies using similar species where prior exposure to hypoxia has reduced performance under subsequent hypoxia exposure when compared to organism for whom the stressor is novel. It is possible that this is a new finding for *Pandalus borealis* though it should be noted that prior exposure to hypoxia in this case is assumed on the basis of *P. borealis* depth ranges and the oxygen regime of the site. However, exposure of juvenile penaeid shrimp *Metapenaeus ensis* to low oxygen increased their hypoxia detection and avoidance behaviour in later life (Wu et al. 2002), and early-life stage exposure to hypoxia negatively impacted metabolic processes and impaired food assimilation in sea bass, *Dicentrarchus labrax,* (Vanderplancke et al. 2015a; 2015b).

This is not the first instance of counterintuitive outcomes regarding shrimp hypoxia responses. In a series of studies on intertidal congenitors, *Palaemon serratus* and *P. elegans*, Taylor and Spicer (1987; 1989; 1991) established that *P. elegans* possessed superior physiological hypoxia tolerance characteristics than *P. serratus*, such as higher pigment oxygen affinity and superior aerobic capacity. In spite of this physiological capacity, field observations and subsequent laboratory experiments concluded that behaviour was the primary mode of hypoxia stress

alleviation in *P. elegans.* By contrast, northern krill*, Meganyctiphanes norvegica*, was found to have poor anaerobic capacity, poor pigment oxygen affinity and a relatively high critical oxygen threshold and yet still diurnally migrated into severely hypoxic zones, undergoing exposures that were near lethal levels and incurring high concentrations of anaerobic end products (Spicer et al. 1998; Strömberg & Spicer 2000). As such, without conducting further targeted studies, it is challenging to draw firm conclusions as to how *Pandalus borealis* persists in the periodically hypoxic Gullmarfjord or why individuals from this site appear to have a lower capacity to maintain performance under hypoxia when compared to individuals from Brofjorden.

This study is unique in that it is the first example of the mtDNA control region being used to assess genetic variation in the commercially important species, *P. borealis.* This study also emphasises the need for multidisciplinary approaches to studying local adaptation and the impacts of environmental stressors as previously highlighted by Calow (1991), Block (2005), Altshuler et al. (2011), and Spicer (2013) amongst others. Multidisciplinary approaches allow researchers to integrate theoretical models with field observations, identify common responses between different experimental designs with their individual limitations and assumptions, and advance the field by investigating causes, mechanisms and responses within the same experimental model. By conducting direct experimental comparisons, researchers may uncover untested assumptions perpetuated throughout the literature or in their own work.

Historically, gathering genetic data to support inferences of local adaptation has been challenging or costly. Indeed targeted genetic methods may miss genes under selection (Tiffin & Ross-Ibarra 2014). However, as genetic methods improve and become more cost effective, it is crucial to integrate research and explore genetic variation as well as population dynamics when assessing likelihood of evolutionary rescue and the impacts of climate change and localised environmental stressors (Gonzalez et al. 2013). Furthermore, environmental stressors impact organisms across levels of biological organisation (Harvey et al. 2014). By investigating responses at a molecular, physiological, whole organism and population level, this study was able to compile a holistic assessment of hypoxia threats to local *Pandalus borealis*. Additionally, the patterns of tolerances discovered in Chapter 4 could easily have been missed if we had exclusively adopted a common garden approach. In a cross-taxa review of experimental population tolerance comparison studies from the last 50 years (see Appendices Section ix), I found only 14 out of 58 studies employed translocation techniques. Laboratory experiments are highly valuable in terms of identifying driving forces in ecology (Kawecki & Ebert 2004). However, limited ability to recreate or manipulate natural habitats can lead to behavioural, nutritional, or other deficiencies that may confound results. Field experiments circumvent this limitation and allow the study organism access to many, if not all, resources and stimuli that it would habitually encounter (Sanford & Kelly 2011). This study

adopted a multidisciplinary approach in order to gain both a population level and a more mechanistic understanding of the impacts environmental stressors on different populations of *P. borealis.* It is hoped that this method has helped to circumvent the limitations of laboratory and field studies alone. I am confident that investigating *P. borealis* responses at different biological levels and incorporating genetic data has improved the strength of this study and that my interpretation would have differed greatly had these steps not been taken.

Many species present non-uniform responses to environmental stress across different populations within their range (Whittaker & Levin 1977; Ebert & Russell 1988; Helmuth et al. 2006). This study shows that, although the *Pandalus borealis* occupying Gullmarfjord and Brofjorden do not appear to genetically distinct, they do exhibit functional physiological differences. Conservation and sustainability management frequently focuses on maintaining the most genetically distinct populations and using genetic structuring as the basis of delineating different management plans. However, this study indicates that two genetically indistinct populations exhibit differing levels of susceptibility to stress.

The shrimp studied here were collected from the lower central region of their latitudinal range. Historically, organisms found at range edges have been considered to be under the greatest stress and the most vulnerable (Kirkpatrick & Barton 1997; Stillman & Somero 2000). By contrast, this study suggests that the habitat mosaics generated by highly heterogeneous coastal bathymetry complicate this pattern and create high stress zones far from range edges (situated at 41 to 80 degrees latitude: FAO 2016). This pattern is seen across a range of coastal systems and taxa, such as upwellings associated with population size frequencies in purple sea urchin *Strongylocentrotus purpuratus* (Ebert & Russell 1988), terrestrial nutrient run-off impacting ecosystem function (Correll et al. 1992), and tide timings correlated by body temperature in the mussel Mytilus californianus (Helmuth et al. 2006). These small scale stress zones are unlikely to impact the survival of the species as a whole. However, this non-uniform vulnerability in coastal areas may prove problematic for the sustainability of commercially fished species, such as *Pandalus borealis*. The combination of stress from anticipated climate change (Rosenzweig et al. 2008; IPCC 2014) and fishing pressure (Hilborn et al. 2003; Pauly et al. 2005), which is managed at regional scales may lead to the collapse of local populations and undermine the small scale artisanal fishing practices that many advocates of seafood sustainability wish to encourage. Improving our understanding of population dynamics and vulnerability may aid effective management and mitigation.

The discovery of phenotypic differentiation and a non-uniform response to hypoxia stress between two non-genetically distinct, spatially proximate populations of *Pandalus borealis* in this study is

evidence to support the need for the following: multidisciplinary studies; a need to further our collective understanding of mechanistic stress responses; and a need to investigate species vulnerability to environmental stressors at the population level and to investigate this well within species ranges as well as at range edges.

Glossary

List of terms

Aerobic metabolism: the synthesis of ATP through the decomposition of carbohydrates, amino acids and lipids in the presence of oxygen

Anaerobic metabolism: the synthesis of ATP independent of oxygen, through the conversion of glucose to lactate or pyruvate

Genotype: the genetically fixed and heritable characteristics of an organisms

Haplotype: a suite of genes inherited concurrently from a single parent

Hypoxia: oxygen is depleted conditions

Life history: the sequence of events and development related to growth, survival and reproduction undertaken during an organism's lifespan

Local adaptation: the modification of a lineage following selection against phenotypes maladapted to the surrounding conditions

Locus: the specific location of a gene's DNA sequence on a chromosome

Metabolic rate: rate at which organisms uptake, transform and expend energy

Omics: the study of transcriptomics (gene expression), proteomics (protein expression) and metabolomics (metabolite expression)

Oxygen regime: the profile of oxygen concentrations in a given habitat over a given time

Phenotype: a set of characteristics or functions resulting from an organism's genotypic interaction with its environmental conditions

Phenotypic plasticity: Phenotypic adjustment to the surrounding conditions, without fixed genetic change

Physiology: the study of the function and activity of a given organism's biological organs, systems and pathways

Population: an interbreeding assemblage of organisms from a single species generally assumed to be isolated to some extent from other populations

Selection: non-random survival or reproduction of individuals expressing a particular phenotype

List of abbreviations

a.s.: air saturation

ANOVA: analysis of variance

Df: degrees of freedom

EPHOC: excess post-hypoxic oxygen consumption

FC: fold change

FDR : false discovery rate

Fis: the inbreeding coefficient

$F_{ST:}$ fixation index

GLM: general linear model

HA1: mesocosm treatment – exposed to hypoxia and ambient temperature for one week

HA4: mesocosm treatment – exposed to hypoxia and ambient temperature for four weeks

HE1: mesocosm treatment – exposed to hypoxia and elevated temperature for one week

HE4: mesocosm treatment – exposed to hypoxia and elevated temperature for four weeks

HWE: Hardy Weinberg equilibrium

J: joules

MANOVA: multivariate analysis of variance

MGSDA: multi-group sparse discriminant analysis

MO_2: oxygen consumption

MST: minimum spanning tree

mtDNA: mitochondrial DNA inherited maternally

n: number of specimens per treatment or analysis

NA1: mesocosm treatment – exposed to normoxia and ambient temperature for one week

NA4: mesocosm treatment – exposed to normoxia and ambient temperature for four weeks

NE1: mesocosm treatment – exposed to normoxia and elevated temperature for one week

NE4: mesocosm treatment – exposed to normoxia and elevated temperature for four weeks

P: probability value

PCA: principal components analysis

Ppb: parts per billion

Ppm: parts per million

rMANOVA: repeated measures MANOVA

RMR: resting metabolic rate

References

Adamczewska, A.M. and Morris, S., 2001. Ecology and behavior of *Gecarcoidea natalis*, the Christmas Island red crab, during the annual breeding migration. *The Biological Bulletin*, *200*(3), pp.305-320.

Albers, W. D., Anderson, P. J. 1985. Diet of Pacific cod, *Gadus macrocephalus*, and predation on the northern pink shrimp, *Pandalus borealis*, in Pavlof Bay, Alaska. Fishery Bulletin 83(4): 601-610

Altieri, A.H., 2006. Inducible variation in hypoxia tolerance across the intertidal–subtidal distribution of the blue mussel *Mytilus edulis*. *Marine Ecology Progress Series*, *325*, pp.295-300.

Altshuler, I., Demiri, B., Xu, S., Constantin, A., Yan, N.D. and Cristescu, M.E., 2011. An integrated multi-disciplinary approach for studying multiple stressors in freshwater ecosystems: Daphnia as a model organism.*Integrative and comparative biology*, p.icr103.

Andersson, S., Persson, M., Moksnes, P., Baden, S. 2009. The role of the amphipod *Gammarus locusta* as a grazer on macroalgae in Swedish seagrass meadows. Marine Biology, 156 (5): 969-981

Apollonio, S., Stevenson, D. K., & Dunton, E. E. (1986). Effects of temperature on the biology of the northern shrimp*, Pandalus borealis,* in the Gulf of Maine. [*Seattle, Wash.], U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service*. http://purl.fdlp.gov/GPO/gpo27806.

Arkhipkin, A., 1993. Age, growth, stock structure and migratory rate of pre-spawning short-finned squid *Illex argentinus* based on statolith ageing investigations. *Fisheries Research*, *16*(4), pp.313-338.

Árnason, E., 2004. Mitochondrial cytochrome b DNA variation in the high-fecundity Atlantic cod: trans-Atlantic clines and shallow gene genealogy. *Genetics*, *166*(4), pp.1871-1885.

Arnberg, M., Calosi, P., Spicer, J.I., Tandberg, A.H.S., Nilsen, M., Westerlund, S. and Bechmann, R.K., 2013. Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (Pandalus borealis) larvae. *Marine biology*,*160*(8), pp.2037-2048.

Austen, M.C. and Wibdom, B., 1991. Changes in and slow recovery of a meiobenthic nematode assemblage following a hypoxic period in the Gullmar Fjord basin, Sweden. *Marine Biology*, *111*(1), pp.139-145.

Bacanskas, L.R., Whitaker, J. and Di Giulio, R.T., 2004. Oxidative stress in two populations of killifish (Fundulus heteroclitus) with differing contaminant exposure histories. *Marine environmental research*, *58*(2), pp.597-601.

Baden, S.P., Pihl, L. And Rosenburg, R., 1990. Effects of oxygen depletion on the ecology, blood physiology and fishery of the Norway lobster Nephrops norvegicus (L.).*Marine Ecology Progress Series*, *67*, pp.141-155.

Badyaev, A.V. and Foresman, K.R., 2000. Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. *Proceedings of the Royal Society of London B: Biological Sciences*, *267*(1441), pp.371-377.

Balcombe, J.P., Barnard, N.D. and Sandusky, C., 2004. Laboratory routines cause animal stress. *Journal of the American Association for Laboratory Animal Science*, *43*(6), pp.42-51.

Ballantyne, J.S., Hochachka, P.W. and Mommsen, T.P., 1981. Studies on the metabolism of the migratory squid, *Loligo opalescens*: enzymes of tissues and heart mitochondria. *Marine biology letters*.

Banks, S.C., Piggott, M.P., Williamson, J.E., Bové, U., Holbrook, N.J. and Beheregaray, L.B., 2007. Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. *Ecology*, *88*(12), pp.3055-3064.

Barr, L., 1970. Diel vertical migration of Pandalus borealis in Kachemak Bay, Alaska. *Journal of the Fisheries Board of Canada*, *27*(4), pp.669-676.

Barshis, D.J., Stillman, J.H., Gates, R.D., Toonen, R.J., Smith, L.W. and Birkeland, C., 2010. Protein expression and genetic structure of the coral Porites lobata in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity?. *Molecular ecology*, *19*(8), pp.1705-1720.

Basson, M., Beddington, J.R., Crombie, J.A., Holden, S.J., Purchase, L.V. and Tingley, G.A., 1996. Assessment and management techniques for migratory annual squid stocks: the *Illex arge*ntinus fishery in the Southwest Atlantic as an example. *Fisheries Research*, *28*(1), pp.3-27.

Beaugrand, G., Reid, P.C., Ibanez, F., Lindley, J.A. and Edwards, M., 2002. Reorganization of North Atlantic marine copepod biodiversity and climate.*Science*, *296*(5573), pp.1692-1694.

Beaumont, A.R. and Croucher, T., 2006. Limited stock structure in UK populations of the brown shrimp, Crangon crangon, identified by morphology and genetics. *Journal of the Marine Biological Association of the United Kingdom*, *86*(05), pp.1107-1112.

Bechmann, R.K., Taban, I.C., Westerlund, S., Godal, B.F., Arnberg, M., Vingen, S., Ingvarsdottir, A. and Baussant, T., 2011. Effects of ocean acidification on early life stages of shrimp (Pandalus borealis) and mussel (Mytilus edulis). *Journal of Toxicology and Environmental Health, Part A*,*74*(7-9), pp.424-438.

Begg, G.A. and Waldman, J.R., 1999. An holistic approach to fish stock identification. *Fisheries research*, *43*(1), pp.35-44.

Bennett, A.F. and Lenski, R.E., 1997. Evolutionary adaptation to temperature. VI. Phenotypic acclimation and its evolution in Escherichia coli.*Evolution*, pp.36-44.

Benton, M.J. and Twitchett, R.J., 2003. How to kill (almost) all life: the end-Permian extinction event. *Trends in Ecology & Evolution*, *18*(7), pp.358-365.

Bergström, B.I., 1991. Yearly immigration of *Pandalus borealis* (Krøyer) into periodically isolated fjord populations. *Sarsia*, *76*(1-2), pp.133-140.

Bergström, B.I., 2000. The biology of Pandalus. *Advances in Marine Biology*, *38*, pp.55-245.

Berner, R.A., 2004. *The Phanerozoic Carbon Cycle: CO [2 and O [2*. Oxford University Press.

Berner, R.A., Beerling, D.J., Dudley, R., Robinson, J.M. and Wildman Jr, R.A., 2003. Phanerozoic atmospheric oxygen. *Annual Review of Earth and Planetary Sciences*, *31*(1), pp.105-134.

Bertness, M.D. and Gaines, S.D., 1993. Larval dispersal and local adaptation in acorn barnacles. *Evolution*, *47*(1), pp.316-320.

Bindoff, N.L. and McDougall, T.J., 2000. Decadal changes along an Indian Ocean section at 32 S and their interpretation. *Journal of Physical Oceanography*, *30*(6), pp.1207-1222.

Blanquart, F., Kaltz, O., Nuismer, S.L. and Gandon, S., 2013. A practical guide to measuring local adaptation. *Ecology Letters*, *16*(9), pp.1195-1205.

Block, B.A., 2005. Physiological ecology in the 21st century: advancements in biologging science. *Integrative and Comparative Biology*, *45*(2), pp.305-320.

Blunier, T. and Brook, E.J., 2001. Timing of millennial-scale climate change in Antarctica and Greenland during the last glacial period. *Science*,*291*(5501), pp.109-112.

Bøhle, B., 1977. Vekst og utvikling av larver og postlarver av dypvannreke (Pandalus borealis KRØYER) ved eksperimentelle forhold.

Boitsov, V.D., Karsakov, A.L. and Trofimov, A.G., 2012. Atlantic water temperature and climate in the Barents Sea, 2000–2009. *ICES Journal of Marine Science: Journal du Conseil*, *69*(5), pp.833-840.

Bondad-Reantaso, M.G., McGladdery, S.E., East, L. and Subasinghe, R., 2001. *Asia diagnostic guide to aquatic animal diseases*. FAO.

Booke, H.E., 1981. The conundrum of the stock concept-are nature and nurture definable in fishery science?. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*(12), pp.1479-1480.

Bourdeau, P.E., 2009. Prioritized phenotypic responses to combined predators in a marine snail. *Ecology*, *90*(6), pp.1659-1669.

Bowring, S.A., Erwin, D.H. and Isozaki, Y., 1999. The tempo of mass extinction and recovery: The end-Permian example. *Proceedings of the National Academy of Sciences*, *96*(16), pp.8827-8828.

Bozinovic, F., Calosi, P. and Spicer, J.I., 2011. Physiological correlates of geographic range in animals. *Annual Review of Ecology, Evolution, and Systematics*, *42*, pp.155-179.

Bridges, C.R. and Brand, A.R., 1980. The effect of hypoxia on oxygen consumption and blood lactate levels of some marine Crustacea.*Comparative Biochemistry and Physiology Part A: Physiology*, *65*(4), pp.399-409

Brierley, A.S. and Kingsford, M.J., 2009. Impacts of climate change on marine organisms and ecosystems. *Current biology*, *19*(14), pp.R602-R614.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B., 2004. Toward a metabolic theory of ecology. *Ecology*, *85*(7), pp.1771-1789.

Bryan, G.W. and Hummerstone, L.G., 1971. Adaptation of the polychaete Nereis diversicolor to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. *Journal of the Marine Biological Association of the United Kingdom*, *51*(04), pp.845-863.

Bundy, J.G., Davey, M.P. and Viant, M.R., 2009. Environmental metabolomics: a critical review and future perspectives. *Metabolomics*, *5*(1), pp.3-21.

Burke, E.M., 1979. Aerobic and anaerobic metabolism during activity and hypoxia in two species of intertidal crabs. *The Biological Bulletin*, *156*(2), pp.157-168.

Burnett, L.E. and Stickle, W.B., 2001. Physiological responses to hypoxia.*Coastal hypoxia: consequences for living resources and ecosystems*, pp.101-114.

Burrows, M.T., Schoeman, D.S., Buckley, L.B., Moore, P., Poloczanska, E.S., Brander, K.M., Brown, C., Bruno, J.F., Duarte, C.M., Halpern, B.S. and Holding, J., 2011. The pace of shifting climate in marine and terrestrial ecosystems. *Science*, *334*(6056), pp.652-655.

Bushnell, P.G. and Brill, R.W., 1992. Oxygen transport and cardiovascular responses in skipjack tuna (Katsuwonus pelamis) and yellowfin tuna (Thunnus albacares) exposed to acute hypoxia.*Journal of Comparative Physiology B*, *162*(2), pp.131-143.

Bustamante, R.H., Branch, G.M., Eekhout, S., Robertson, B., Zoutendyk, P., Schleyer, M., Dye, A., Hanekom, N., Keats, D., Jurd, M. and McQuaid, C., 1995. Gradients of intertidal primary productivity around the coast of South Africa and their relationships with consumer biomass. *Oecologia*,*102*(2), pp.189-201.

Butler IV, M.J., Paris, C.B., Goldstein, J.S., Matsuda, H. and Cowen, R.K., 2011. Behavior constrains the dispersal of long-lived spiny lobster larvae.*Marine Ecology Progress Series*, *422*, pp.223-237.

Caicedo, A.L. and Schaal, B.A., 2004. Population structure and phylogeography of *Solanum pimpinellifolium* inferred from a nuclear gene. *Molecular Ecology*, *13*(7), pp.1871-1882.

Calosi, P., Rastrick, S.P., Lombardi, C., de Guzman, H.J., Davidson, L., Jahnke, M., Giangrande, A., Hardege, J.D., Schulze, A., Spicer, J.I. and Gambi, M.C., 2013. Adaptation and acclimatization to ocean acidification in marine ectotherms: an in situ transplant experiment with polychaetes at a shallow CO2 vent system. *Phil. Trans. R. Soc. B*, *368*(1627), p.20120444.

Calow, P., 1991. Physiological costs of combating chemical toxicants: ecological implications. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, *100*(1-2), pp.3-6.

Capossela, K.M., Brill, R.W., Fabrizio, M.C. and Bushnell, P.G., 2012. Metabolic and cardiorespiratory responses of summer flounder Paralichthys dentatus to hypoxia at two temperatures.*Journal of fish biology*, *81*(3), pp.1043-1058.

Carr, S.M. and Marshall, H.D., 2008. Intraspecific phylogeographic genomics from multiple complete mtDNA genomes in Atlantic cod (*Gadus morhua*): origins of the “codmother,” transatlantic vicariance and midglacial population expansion. *Genetics*, *180*(1), pp.381-389.

Cerrano, C., Bavestrello, G., Bianchi, C.N., Cattaneo-Vietti, R., Bava, S., Morganti, C., Morri, C., Picco, P., Sara, G., Schiaparelli, S. and Siccardi, A., 2000. A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999.*Ecology letters*, *3*(4), pp.284-293.

Chabot, D. and DUTIL, J.D., 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *Journal of Fish Biology*, *55*(3), pp.472-491.

Chan, F., Barth, J.A., Lubchenco, J., Kirincich, A., Weeks, H., Peterson, W.T. and Menge, B.A., 2008. Emergence of anoxia in the California Current large marine ecosystem. *Science*, *319*(5865), pp.920-920.

Chapman, L.G., Galis, F. and Shinn, J., 2000. Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters*, *3*(5), pp.387-393.

Charnov, E.L. and Anderson, P.J., 1989. Sex change and population fluctuations in pandalid shrimp. *The American Naturalist*, *134*(5), pp.824-827

Chen, Z., Anttila, K., Wu, J., Whitney, C.K., Hinch, S.G. and Farrell, A.P., 2013. Optimum and maximum temperatures of sockeye salmon (Oncorhynchus nerka) populations hatched at different temperatures.*Canadian Journal of Zoology*, *91*(5), pp.265-274.

Chien, Y.H., Liao, H.H. and Wang, C.C., 1991. Coloration and Growth of Artemia under Various Diets. *臺灣水產學會刊*, *18*(4), pp.295-299.

Chow, S. and Sandifer, P.A., 1991. Differences in growth, morphometric traits, and male sexual maturity among Pacific white shrimp, Penaeus vannamei, from different commercial hatcheries. *Aquaculture*, *92*, pp.165-178.

Cisterna, J.A., Saldías, G.S. and Cáceres, C.W., 2008. Hypoxia effect on foraging behavior of *Cancer setosus* (Molina, 1782)(Crustacea: Decapoda) feeding on *Mytilus chilensis* (Hupé, 1854). *Revista de biología marina y oceanografía*, *43*(2), pp.419-423.

Clark, M.S., Husmann, G., Thorne, M.A., Burns, G., Truebano, M., Peck, L.S., Abele, D. and Philipp, E.E., 2013. Hypoxia impacts large adults first: consequences in a warming world. *Global change biology*, *19*(7), pp.2251-2263.

Clark, T.D., Sandblom, E. and Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology*, *216*(15), pp.2771-2782.

Clarke L., K. Jiang, K. Akimoto, M. Babiker, G. Blanford, K. Fisher-Vanden, J.-C. Hourcade, V. Krey, E. Kriegler, A. Löschel, D. McCollum, S. Paltsev, S. Rose, P.R. Shukla, M. Tavoni, B. van der Zwaan, and D. P. van Vuuren, 2014: Assessing Transformation Pathways. In: *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Conover, D.O., 1998. Local adaptation in marine fishes: evidence and implications for stock enhancement. *Bulletin of Marine Science*, *62*(2), pp.477-493.

Conover, D.O., Clarke, L.M., Munch, S.B. and Wagner, G.N., 2006. Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of Fish Biology*, *69*(sc), pp.21-47.

Cooper, S.R. and Brush, G.S., 1993. A 2,500-year history of anoxia and eutrophication in Chesapeake Bay. *Estuaries*, *16*(3), pp.617-626.

Cornwallis, C.K. and Birkhead, T.R., 2008. Plasticity in reproductive phenotypes reveals status-specific correlations between behavioral, morphological, and physiological sexual traits. *Evolution*, *62*(5), pp.1149-1161.

Correll, D.L., Jordan, T.E. and Weller, D.E., 1992. Nutrient flux in a landscape: effects of coastal land use and terrestrial community mosaic on nutrient transport to coastal waters. *Estuaries*, *15*(4), pp.431-442.

Cowen, R.K. and Castro, L.R., 1994. Relation of coral reef fish larval distributions to island scale circulation around Barbados, West Indies.*Bulletin of Marine Science*, *54*(1), pp.228-244.

Cowen, R.K., Lwiza, K.M., Sponaugle, S., Paris, C.B. and Olson, D.B., 2000. Connectivity of marine populations: open or closed?. *Science*,*287*(5454), pp.857-859

Coyne, J.A. and Orr, H.A., 2004. *Speciation* (Vol. 37). Sunderland, MA: Sinauer Associates.

Crain, C.M., Kroeker, K. and Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology letters*,*11*(12), pp.1304-1315.

Crispo, E. and Chapman, L.J., 2011. Hypoxia drives plastic divergence in cichlid body shape. *Evolutionary Ecology*, *25*(4), pp.949-964.

Crowley, T.J., 2000. Causes of climate change over the past 1000 years. *Science*, *289*(5477), pp.270-277.

D'Croz, L. and Maté, J.L., 2004. Experimental responses to elevated water temperature in genotypes of the reef coral Pocillopora damicornis from upwelling and non-upwelling environments in Panama. *Coral Reefs*, *23*(4), pp.473-483.

Daniel, R.M. and Danson, M.J., 2010. A new understanding of how temperature affects the catalytic activity of enzymes. *Trends in biochemical sciences*, *35*(10), pp.584-591.

Daoud, D., Chabot, D., Audet, C. and Lambert, Y., 2007. Temperature induced variation in oxygen consumption of juvenile and adult stages of the northern shrimp, Pandalus borealis. *Journal of Experimental Marine Biology and Ecology*, *347*(1), pp.30-40.

Darling, E.S. and Côté, I.M., 2008. Quantifying the evidence for ecological synergies. *Ecology letters*, *11*(12), pp.1278-1286.

De Wit, P. and Palumbi, S.R., 2013. Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and local adaptation. *Molecular Ecology*, *22*(11), pp.2884-2897.

Debuse, V.J., Addison, J.T. and Reynolds, J.D., 2001. Morphometric variability in UK populations of the European lobster. *Journal of the Marine Biological Association of the UK*, *81*(03), pp.469-474.

Decker, M.B., Breitburg, D.L. and Marcus, N.H., 2003. Geographical differences in behavioral responses to hypoxia: local adaptation to an anthropogenic stressor?. *Ecological Applications*, *13*(4), pp.1104-1109.

DeFur, P.L. and Pease, A.L., 1988. Metabolic and respiratory compensation during long term hypoxia in blue crabs, Callinectes sapidus. *Understanding the estuary, advances in chesapeake bay research, Chesapeake Research Consortium Publication*, *129*.

Depledge, M.H., 1994. The rational basis for the use of biomarkers as ecotoxicological tools. *Nondestructive biomarkers in vertebrates*, pp.271-295.

Wit, P. and Palumbi, S.R., 2013. Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and local adaptation. *Molecular Ecology*, *22*(11), pp.2884-2897.

DeWitt, T.J. and Scheiner, S.M. eds., 2004. *Phenotypic plasticity: functional and conceptual approaches*. Oxford University Press.

Diaz, R.J., 2001. Overview of hypoxia around the world. *Journal of environmental quality*, *30*(2), pp.275-281.

Diaz, R.J. and Breitburg, D.L., 2009. The hypoxic environment. *Fish physiology*, *27*, pp.1-23.

Diaz, R.J. and Rosenberg, R., 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna.*Oceanography and marine biology. An annual review*, *33*, pp.245-03.

Diaz, R.J. and Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. *science*, *321*(5891), pp.926-929.

Dimmock, A., Williamson, I. and Mather, P.B., 2004. The influence of environment on the morphology of Macrobrachium australiense (Decapoda: Palaemonidae). *Aquaculture International*, *12*(4-5), pp.435-456.

Dittman, D.E., 1997. Latitudinal compensation in oyster ciliary activity.*Functional Ecology*, *11*(5), pp.573-578.

Dittman, D.E., Ford, S.E. and Haskin, H.H., 1998. Growth patterns in oysters, Crassostrea virginica, from different estuaries. *Marine Biology*,*132*(3), pp.461-469.

Dixon, D.G. and Sprague, J.B., 1981a. Acclimation to copper by rainbow trout (Salmo gairdneri)-a modifying factor in toxicity. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*(8), pp.880-888.

Dixon, D.G. and Sprague, J.B., 1981b. Acclimation-induced changes in toxicity of arsenic and cyanide to rainbow trout, Salmo gairdneri Richardson.*Journal of Fish Biology*, *18*(5), pp.579-589.

Dlugokencky, E. & Pieter Tans, P., 2016. NOAA/ESRL Global Greenhouse Gas Reference Network (www.esrl.noaa.gov/gmd/ccgg/trends/)

Domenici, P., Claireaux, G. and McKenzie, D.J., 2007. Environmental constraints upon locomotion and predator–prey interactions in aquatic organisms: an introduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1487), pp.1929-1936.

Drengstig, A., Fevolden, S.E., Galand, P.E. and Aschan, M.M., 2000. Population structure of the deep-sea shrimp (Pandalus borealis) in the north-east Atlantic based on allozyme variation. *Aquatic Living Resources*, *13*(2), pp.121-128.

Drew, M.C., 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual review of plant biology*, *48*(1), pp.223-250.

Dunlap, K.D. and Wingfield, J.C., 1995. External and internal influences on indices of physiological stress. I. Seasonal and population variation in adrenocortical secretion of free-living lizards, Sceloporus occidentalis.*Journal of Experimental Zoology*, *271*(1), pp.36-46.

Dupont, S. and Thorndyke, M., 2013. Direct impacts of near-future ocean acidification on sea urchins. *Climate change perspective from the Atlantic: past, present and future*, pp.461-485.

Dupont-Prinet, A., Pillet, M., Chabot, D., Hansen, T., Tremblay, R. and Audet, C., 2013. Northern shrimp (Pandalus borealis) oxygen consumption and metabolic enzyme activities are severely constrained by hypoxia in the Estuary and Gulf of St. Lawrence. *Journal of Experimental Marine Biology and Ecology*, *448*, pp.298-307.

Dybdahl, M.F., 1989. *Evolutionary consequences of limited dispersal and environment heterogeneity in Tigriopus californicus life-histories* (Doctoral dissertation, PhD thesis. Univ. of California, Davis).

Ebert, T.A. and Russell, M.P., 1988. Latitudinal variation in size structure of the west coast purple sea urchin: a correlation with headlands. *Limnology and Oceanography*,

Edwards, M. and Richardson, A.J., 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*(7002), pp.881-884.

Ehrhardt, N.M., 1991. Potential impact of a seasonal migratory jumbo squid (Dosidicus gigas) stock on a Gulf of California sardine (Sardinops sagax caerulea) population. *Bulletin of Marine Science*, *49*(1-2), pp.325-332.

El Mousadik, A. and Petit, R.J., 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [Argania spinosa (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics*, *92*(7), pp.832-839.

Eldon, B. and Wakeley, J., 2008. Linkage disequilibrium under skewed offspring distribution among individuals in a population. *Genetics*, *178*(3), pp.1517-1532.

Enebjörk, L. and Fränne, I., 2006. Characterization of Gullmarn according to the Water Framework Directive. *Länsstyrelsen Västra Götalands län, Göteborg*, pp.42-44.

Eriksson, S.P., Nabbing, M. and Sjöman, E., 2006. Is brood care in Nephrops norvegicus during hypoxia adaptive or a waste of energy?.*Functional Ecology*, *20*(6), pp.1097-1104.

Erwin, D.H., 1993. *The great Paleozoic crisis: life and death in the Permian*. Columbia University Press.

Espinosa, F., Nakano, T., Guerra-García, J.M. and García-Gómez, J.C., 2011. Population genetic structure of the endangered limpet *Cymbula nigra* in a temperate Northern hemisphere region: influence of palaeoclimatic events?. *Marine Ecology*, *32*(1), pp.1-5.

Excoffier, L., Laval, G. and Schneider, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis.*Evolutionary bioinformatics online*, *1*, p.47.

Excoffier, L., Smouse, P.E. and Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), pp.479-491.

Fangue, N.A., Hofmeister, M. and Schulte, P.M., 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, Fundulus heteroclitus. *Journal of Experimental Biology*, *209*(15), pp.2859-2872.

FAO (2016). FAO Fisheries & Aquaculture – Gobal Statistical Collections http://www.fao.org/fishery/statistics/en. Accessed 28/03/16

FAO, 2014. The State of World Fisheries and Aquaculture: opportunities and challenges. Rome, 233p.

Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D. and Hales, B., 2008. Evidence for upwelling of corrosive" acidified" water onto the continental shelf. *science*, *320*(5882), pp.1490-1492.

Fevolden, S.E., Westgaard, J.I., Pedersen, T. and Præbel, K., 2012. Settling-depth vs. genotype and size vs. genotype correlations at the Pan I locus in 0-group Atlantic cod *Gadus morhua. Marine Ecology Progress series, 468,* pp. 267-278

Filipsson, H.L. and Nordberg, K., 2004. Climate variations, an overlooked factor influencing the recent marine environment. An example from Gullmar Fjord, Sweden, illustrated by benthic foraminifera and hydrographic data.*Estuaries*, *27*(5), pp.867-881.

Finke, E., Pörtner, H.O., Lee, P.G. and Webber, D.M., 1996. Squid (Lolliguncula brevis) life in shallow waters: oxygen limitation of metabolism and swimming performance. *Journal of experimental biology*, *199*(4), pp.911-921.

Floreto, E.A.T., Brown, P.B. and Bayer, R.C., 2001. The effects of krill hydrolysate-supplemented soya-bean based diets on the growth, colouration, amino and fatty acid profiles of juvenile American lobster, Homarus americanus. *Aquaculture Nutrition*, *7*(1), pp.33-43.

Foo, S.A., Dworjanyn, S.A., Poore, A.G. and Byrne, M., 2012. Adaptive capacity of the habitat modifying sea urchin Centrostephanus rodgersii to ocean warming and ocean acidification: performance of early embryos. *PLoS One*, *7*(8), p.e42497.

Frankham, R., 2005. Genetics and extinction. *Biological conservation*,*126*(2), pp.131-140.

Fraser, J., de Mello, L.V., Ward, D., Rees, H.H., Williams, D.R., Fang, Y., Brass, A., Gracey, A.Y. and Cossins, A.R., 2006. Hypoxia-inducible myoglobin expression in nonmuscle tissues. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(8), pp.2977-2981.

Frederich, M. and Pörtner, H.O., 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, Maja squinado. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *279*(5), pp.R1531-R1538.

Fritsche, R. and Nilsson, S., 1990. Autonomic nervous control of blood pressure and heart rate during hypoxia in the cod, Gadus morhua. *Journal of Comparative Physiology B*, *160*(3), pp.287-292.

Fry, F. E. J. 1947. *Effects of the environment on animal activity*. Toronto, University of Toronto Press.

Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. In: Fish Physiology, pp. 1–98. Ed. by W. S. Hoar, and D. J. Randall. Academic Press, New York

FRY, F. and Hart, J.S., 1948. The relation of temperature to oxygen consumption in the goldfish. *The Biological Bulletin*, *94*(1), pp.66-77.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, *147*(2), pp.915-925.

Furevik, T., 2001. Annual and interannual variability of Atlantic Water temperatures in the Norwegian and Barents Seas: 1980–1996. *Deep Sea Research Part I: Oceanographic Research Papers*, *48*(2), pp.383-404.

Garcia, E.G. and Sims, D. W., 2007. The northern shrimp (Pandalus borealis) offshore fishery in the Northeast Atlantic. *Advances in marine biology*, *52*, pp.147-266.

Gaston, K.J. and Spicer, J.I., 1998. Do upper thermal tolerances differ in geographically separated populations of the beachflea Orchestia gammarellus (Crustacea: Amphipoda)?. *Journal of Experimental Marine Biology and Ecology*, *229*(2), pp.265-276.

Gaston, K.J. and Spicer, J.I., 1998. Do upper thermal tolerances differ in geographically separated populations of the beachflea Orchestia gammarellus (Crustacea: Amphipoda)?. *Journal of Experimental Marine Biology and Ecology*, *229*(2), pp.265-276.

Geber, M.A. and Griffen, L.R., 2003. Inheritance and natural selection on functional traits. *International Journal of Plant Sciences*, *164*(S3), pp.S21-S42.

Gerhardt, L. and Baden, S.P., 1998. Gender-and oxygen-related irrigation behaviour of the decapod Nephrops norvegicus. *Marine Biology*, *131*(3), pp.553-558.

Gibson, R.N. and Atkinson, R.J.A., 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanography and Marine Biology: An Annual Review*, *41*, pp.1-45.

Glover, K.A., Skaala, Ø., Limborg, M., Kvamme, C. and Torstensen, E., 2011. Microsatellite DNA reveals population genetic differentiation among sprat (Sprattus sprattus) sampled throughout the Northeast Atlantic, including Norwegian fjords. *ICES Journal of Marine Science: Journal du Conseil*, *68*(10), pp.2145-2151.

González-Ortegón, E., Pascual, E. and Drake, P., 2013. Respiratory responses to salinity, temperature and hypoxia of six caridean shrimps from different aquatic habitats. *Journal of Experimental Marine Biology and Ecology*, *445*, pp.108-115.

Goodall-Copestake, W.P., Tarling, G.A. and Murphy, E.J., 2012. On the comparison of population-level estimates of haplotype and nucleotide diversity: a case study using the gene cox1 in animals. *Heredity*, *109*(1), pp.50-56.

Google Maps Version 3.24, 2016. 58°11’51.4”N 11°10’20.8 https://www.google.co.uk/maps/place/58%C2%B011'51.4%22N+11%C2%B010'20.8%22E/@58.1976218,11.1702543,17z/data=!3m1!4b1!4m5!3m4!1s0x0:0x0!8m2!3d58.197619!4d11.172443 Accessed July 2016

Goudet, J., 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of heredity*, *86*(6), pp.485-486.

Gracey, A.Y., 2007. Interpreting physiological responses to environmental change through gene expression profiling. *Journal of Experimental Biology*,*210*(9), pp.1584-1592.

Graham, J.B., Dudley, R., Aguilar, N.M. and Gans, C., 1995. Implications of the late Palaeozoic oxygen pulse for physiology and evolution. *Nature*,*375*(6527), pp.117-120.

Grant, P.R. and Grant, B.R., 1995. Predicting microevolutionary responses to directional selection on heritable variation. *Evolution*, pp.241-251.

Grant, P.R. and Grant, B.R., 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *science*, *296*(5568), pp.707-711.

Grant, W.A.S. and Bowen, B.W., 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, *89*(5), pp.415-426.

Grant, W.S., 1995, June. Genetic effects of straying of non-native hatchery fish into natural populations. In *Proceedings of the Workshop*. NOAA Technical Memorandum NMFS-NWFSC-30

Gray, J. S., Wu, R. S., Or, Y. Y. Effects of hypoxia and organic enrichment on the coastal marine environment. 2002. *Marine Ecology Progress Series*, 238: 249-27

Green, E.J. and Carritt, D.E., 1967. New tables for oxygen saturation of seawater. *Journal of marine research, vol 25, p 140-147, Feb 1967. 1 fig, 3 tab, 14 ref.*

Greenberg, A.J., Moran, J.R., Coyne, J.A. and Wu, C.I., 2003. Ecological adaptation during incipient speciation revealed by precise gene replacement.*Science*, *302*(5651), pp.1754-1757.

Grieshaber, M.K., Hardewig, I., Kreutzer, U. and Pörtner, H.O., 1993. Physiological and metabolic responses to hypoxia in invertebrates. In*Reviews of Physiology, Biochemistry and Pharmacology, Volume 125* (pp. 43-147). Springer Berlin Heidelberg.

Griggs, D.J. and Noguer, M., 2002. Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change. *Weather*, *57*(8), pp.267-269.

Groeneveld, J.C. and Branch, G.M., 2002. Long-distance migration of South African deep-water rock lobster *Palinurus gilchristi*. *Marine Ecology Progress Series*, *232*, pp.225-238.

Gustafsson, M. and Nordberg, K., 1999. Benthic foraminifera and their response to hydrography, periodic hypoxic conditions and primary production in the Koljö fjord on the Swedish west coast. *Journal of Sea Research*, *41*(3), pp.163-178.

Gustafsson, M. and Nordberg, K., 2000. Living (stained) benthic foraminifera and their response to the seasonal hydrographic cycle, periodic hypoxia and to primary production in Havstens Fjord on the Swedish west coast. *Estuarine, Coastal and Shelf Science*, *51*(6), pp.743-761.

Hagerman, L. and Uglow, R.F., 1985. Effects of hypoxia on the respiratory and circulatory regulation of Nephrops norvegicus.*Marine Biology*, *87*(3), pp.273-278.

Hagerman, L., 1998. Physiological flexibility; a necessity for life in anoxic and sulphidic habitats. *Hydrobiologia*, *375*, pp.241-254.

Hall, T.A., 1999, January. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* (Vol. 41, pp. 95-98).

Halpern, B.S., Selkoe, K.A., Micheli, F. and Kappel, C.V., 2007. Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. *Conservation Biology*, *21*(5), pp.1301-1315.

Hammer, K.M. and Pedersen, S.A., 2013. Deep-water prawn Pandalus borealis displays a relatively high pH regulatory capacity in response to CO2-induced acidosis. *Marine Ecology Progress Series*, *492*, pp.139-151.

Harpending, H.C., 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human biology*, pp.591-600.

Harvey, B.P., Al-Janabi, B., Broszeit, S., Cioffi, R., Kumar, A., Aranguren-Gassis, M., Bailey, A., Green, L., Gsottbauer, C.M., Hall, E.F. and Lechler, M., 2014. Evolution of marine organisms under climate change at different levels of biological organisation. *Water*, *6*(11), pp.3545-3574.

Haynes, E.B. and Wigley, R.L., 1969. Biology of the northern shrimp, Pandalus borealis, in the Gulf of Maine. *Transactions of the American Fisheries Society*, *98*(1), pp.60-76.

Hedgecock, D. and Pudovkin, A.I., 2011. Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. *Bulletin of Marine Science*, *87*(4), pp.971-1002.

Hedgecock, D., 1994. 2.5 does variance in reproductive success limit effective population sizes of marine organisms. *Genetics and evolution of aquatic organisms*, p.122.

Hellberg, M.E., Burton, R.S., Neigel, J.E. and Palumbi, S.R., 2002. Genetic assessment of connectivity among marine populations. *Bulletin of marine science*, *70*(Supplement 1), pp.273-290.

Helmuth, B., Broitman, B.R., Blanchette, C.A., Gilman, S., Halpin, P., Harley, C.D., O'Donnell, M.J., Hofmann, G.E., Menge, B. and Strickland, D., 2006. Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecological Monographs*, *76*(4), pp.461-479.

Henry, W., 1803. Experiments on the quantity of gases absorbed by water, at different temperatures, and under different pressures. *Philosophical Transactions of the Royal Society of London*, *93*, pp.29-276.

Hereford J. 2009. A quantitative survey of local adaptation and fitness trade-offs. American Naturalist 173: 579–588

Herreid, C.F., 1980. Hypoxia in invertebrates. *Comparative Biochemistry and Physiology Part A: Physiology*, *67*(3), pp.311-320.

Hilborn, R., Branch, T.A., Ernst, B., Magnusson, A., Minte-Vera, C.V., Scheuerell, M.D. and Valero, J.L., 2003. State of the world's fisheries.*Annual review of Environment and Resources*, *28*(1), p.359.

Hines, A.H., 1986. Larval patterns in the life histories of brachyuran crabs (Crustacea, Decapoda, Brachyura). *Bulletin of marine science*, *39*(2), pp.444-466.

Hiscock, K., Southward, A., Tittley, I. and Hawkins, S., 2004. Effects of changing temperature on benthic marine life in Britain and Ireland. *Aquatic Conservation: marine and freshwater Ecosystems*, *14*(4), pp.333-362.

Hjerpe, L. Bergström, U. Florin, A-B. & Grip, K. 2004. Bakgrundsmaterial för utredning av möjligheten att införa fiskestopp i ett skyddat marint område. Fiskeriverket informerar 2004:4.

Hjort J (1914) Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapports et Proce`s-Verbaux des Reunions, Conseil Permanent International pour l'Exploration de la Mer, 20, 1–228.

Hochachka, P.W. and Somero, G.N., 2002. Water-solute adaptations: the evolution and regulation of the internal milieu. *Biochemical adaptation: Mechanism and process in physiological evolution*, pp.217-289.

Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N.,Hoang, A., Hill, C.E. & Beerli, P. 2001. Strength and of tempo of directional selection in the wild. *Proceedings of the National Academy of Science USA*98: 9157–9160.

Hoffmann, A.A. & Parsons, P.A. 1997. Extreme Environmental Change and Evolution. Cambridge University Press, Cambridge.

Holeton, G.F. and Randall, D.J., 1967. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *Journal of Experimental Biology*, *46*(2), pp.317-327.

Hollander, J., 2008. Testing the grain-size model for the evolution of phenotypic plasticity. *Evolution*, *62*(6), pp.1381-1389.

Huey, R.B. and Ward, P.D., 2005. Hypoxia, global warming, and terrestrial Late Permian extinctions. *Science*, *308*(5720), pp.398-401.

Hughes, T.P., Baird, A.H., Bellwood, D.R., Card, M., Connolly, S.R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J.B.C., Kleypas, J. and Lough, J.M., 2003. Climate change, human impacts, and the resilience of coral reefs. *science*, *301*(5635), pp.929-933.

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp

IPCC. 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T. F., D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. http://www.climatechange2013.org/images/report/WG1AR5_TS_FINAL.pdf

Ishibashi, Y., Kato, K., Ikeda, S., Murata, O., Nasu, T. and Kumai, H., 1992 Effects of dietary ascorbic acid on tolerance to intermittent hypoxic stress in Japanese parrot fish. *Nippon Suisan Gakkaishi, 58*(11), pp.2147-2152.

Janas, U. and Spicer, J.I., 2010. Seasonal and temperature effects on osmoregulation by the invasive prawn Palaemon elegans Rathke, 1837 in the Baltic Sea. *Marine Biology Research*, *6*(3), pp.333-337.

Jin, P., Gao, K. and Beardall, J., 2013. Evolutionary responses of a coccolithophorid Gephyrocapsa oceanica to ocean acidification. *Evolution*,*67*(7), pp.1869-1878.

Johannesson, K., Smolarz, K., Grahn, M. and André, C., 2011. The future of Baltic Sea populations: local extinction or evolutionary rescue?. *Ambio*,*40*(2), pp.179-190.

Jónsdóttir, Ó.D., Imsland, A.K. and Nævdal, G., 1998. Population genetic studies of northern shrimp, Pandalus borealis, in Icelandic waters and the Denmark Strait. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*(3), pp.770-780.

Joos, F., Plattner, G.K., Stocker, T.F., Marchal, O. and Schmittner, A., 1999. Global warming and marine carbon cycle feedbacks on future atmospheric CO2. *Science*, *284*(5413), pp.464-467.

Jorde, P.E., Knutsen, H., Espeland, S.H. and Stenseth, N.C., 2007. Spatial scale of genetic structuring in coastal cod Gadus morhua and geographic extent of local populations.

Jorde, P.E., Søvik, G., Westgaard, J.I., Albretsen, J., André, C., Hvingel, C., Johansen, T., Sandvik, A.D., Kingsley, M. and Jørstad, K.E., 2015. Genetically distinct populations of northern shrimp, Pandalus borealis, in the North Atlantic: adaptation to different temperatures as an isolation factor.*Molecular ecology*, *24*(8), pp.1742-1757.

Jormalainen, V., Honkanen, T. and Vesakoski, O., 2008. Geographical divergence in host use ability of a marine herbivore in alga–grazer interaction. *Evolutionary Ecology*, *22*(4), pp.545-559.

Josefson, A.B. and Widbom, B., 1988. Differential response of benthic macrofauna and meiofauna to hypoxia in the Gullmar Fjord basin. *Marine Biology*, *100*(1), pp.31-40.

Jueterbock, A., Kollias, S., Smolina, I., Fernandes, J.M., Coyer, J.A., Olsen, J.L. and Hoarau, G., 2014. Thermal stress resistance of the brown alga Fucus serratus along the North-Atlantic coast: acclimatization potential to climate change. *Marine genomics*, *13*, pp.27-36.

Jump, A.S. and Penuelas, J., 2005. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters*, *8*(9), pp.1010-1020.

Jump, A.S., Marchant, R. and Peñuelas, J., 2009. Environmental change and the option value of genetic diversity. *Trends in plant science*, *14*(1), pp.51-58.

Kanciruk, P. and Herrnkind, W., 1978. Mass migration of spiny lobster, Panulirus argus (Crustacea: Palinuridae): behavior and environmental correlates. *Bulletin of Marine Science*, *28*(4), pp.601-623.

Kartavtsev, Y. P., Berenboim, B. I., and Zgurovsky, K. I. 1991. Population genetic differentiation of the pink shrimp, *Pandalus borealis* Krøyer, 1838, from the Barents and Bering Seas. Journal of Shellfish Research, 10: 333-339.

Kartavtsev, Y.P., Zgurovsky, K.A. and Fedina, Z.M., 1993. Spatial structure of the pink shrimp *Pandalus borealis* Kroyer, 1838 from the far-eastern seas as proved by methods of population-genetics and morphometrics. *Journal of Shellfish Research*, *12*(1), pp.81-87.

Kawahara-Miki, R., Wada, K., Azuma, N. and Chiba, S., 2011. Expression profiling without genome sequence information in a non-model species, pandalid shrimp (Pandalus latirostris), by next-generation sequencing. *PLoS One*, *6*(10), p.e26043.

Kawecki, T.J. and Ebert, D., 2004. Conceptual issues in local adaptation.*Ecology letters*, *7*(12), pp.1225-1241.

Keeling, R.F. and Garcia, H.E., 2002. The change in oceanic O2 inventory associated with recent global warming. *Proceedings of the National Academy of Sciences*, *99*(12), pp.7848-7853.

Keeling, R.F., Körtzinger, A. and Gruber, N., 2010. Ocean deoxygenation in a warming world. *Marine Science*, *2*.

Keller, K., Slater, R.D., Bender, M. and Key, R.M., 2001. Possible biological or physical explanations for decadal scale trends in North Pacific nutrient concentrations and oxygen utilization. *Deep Sea Research Part II: Topical Studies in Oceanography*, *49*(1), pp.345-362.

Kelly, M.W., Sanford, E. and Grosberg, R.K., 2012, January. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. In *Proc. R. Soc. B* (Vol. 279, No. 1727, pp. 349-356). The Royal Society.

Keys, A.B., 1930. The measurement of the respiratory exchange of aquatic animals. *The Biological Bulletin*, *59*(2), pp.187-198.

Kidder, D.L. and Worsley, T.R., 2004. Causes and consequences of extreme Permo-Triassic warming to globally equable climate and relation to the Permo-Triassic extinction and recovery. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *203*(3), pp.207-237.

Kirkpatrick, M. and Barton, N.H., 1997. Evolution of a species' range. *The American Naturalist*, *150*(1), pp.1-23.

Knight, C.A., Vogel, H., Kroymann, J., Shumate, A., Witsenboer, H. and Mitchell-Olds, T., 2006. Expression profiling and local adaptation of Boechera holboellii populations for water use efficiency across a naturally occurring water stress gradient. *Molecular Ecology*, *15*(5), pp.1229-1237.

Knoll, A. H., Bambach, R. K., Canfield, D. E., & Grotzinger, J. P. (1996). Comparative Earth history and Late Permian mass extinction. *Science - New York then Washington-*, 452-457.

Knutsen, H., Jorde, P.E., Gonzalez, E.B., Eigaard, O.R., Pereyra, R.T., Sannæs, H., Dahl, M., André, C. and Søvik, G., 2015. Does population genetic structure support present management regulations of the northern shrimp (*Pandalus borealis*) in Skagerrak and the North Sea?. *ICES Journal of Marine Science: Journal du Conseil*, *72*(3), pp.863-871.

Knutsen, H., Jorde, P.E., Gonzalez, E.B., Robalo, J., Albretsen, J. and Almada, V., 2013. Climate change and genetic structure of leading edge and rear end populations in a northwards shifting marine fish species, the corkwing wrasse (Symphodus melops).*PloS one*, *8*(6), p.e67492.

Koeller, P.A., 2000. Relative importance of abiotic and biotic factors to the management of the northern shrimp (Pandalus borealis) fishery on the Scotian Shelf. *Journal of Northwest Atlantic Fishery Science*, *27*, pp.21-34.

Kritzer, J.P. and Sale, P.F., 2006. The metapopulation ecology of coral reef fishes. *Marine metapopulations*, pp.31-67.

Kuo, E.S. and Sanford, E., 2009. Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. *Marine Ecology Progress Series*, *388*, pp.137-146.

Lallier, F. and Truchot, J.P., 1989. Hemolymph oxygen transport during environmental hypoxia in the shore crab, Carcinus maenas.*Respiration physiology*, *77*(3), pp.323-336.

Lamont, P.A. and Gage, J.D., 2000. Morphological responses of macrobenthic polychaetes to low oxygen on the Oman continental slope, NW Arabian Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*,*47*(1), pp.9-24.

Lande, R., 1994. Risk of population extinction from fixation of new deleterious mutations. *Evolution*, pp.1460-1469.

Lande, R., 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of evolutionary biology*, *22*(7), pp.1435-1446.

Lannig, G., Cherkasov, A. S., Pörtner, H. O., Bock, C., & Sokolova, I. M. (2008). Cadmium-dependent oxygen limitation affects temperature tolerance in eastern oysters (*Crassostrea virginica* Gmelin). *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *294*(4), R1338-R1346.

Larade, K. and Storey, K.B., 2002. A Profile of the Metabolic Responses to Anoxia in Marine. *Sensing, Signaling and Cell Adaptation*, *3*, p.27.

Lasaga, A.C. and Ohmoto, H., 2002. The oxygen geochemical cycle: dynamics and stability. *Geochimica et Cosmochimica Acta*, *66*(3), pp.361-381.

Lascelles, B., Notarbartolo Di Sciara, G., Agardy, T., Cuttelod, A., Eckert, S., Glowka, L., Hoyt, E., Llewellyn, F., Louzao, M., Ridoux, V. and Tetley, M.J., 2014. Migratory marine species: their status,

threats and conservation management needs. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(S2), pp.111-127.

Le Treut, H., R. Somerville, U. Cubasch, Y. Ding, C. Mauritzen, A. Mokssit, T. Peterson and M. Prather, 2007: Historical Overview of Climate Change. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Leary, R.F. and Allendorf, F.W., 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology & Evolution*, *4*(7), pp.214-217.

Leimu, R. and Fischer, M., 2008. A meta-analysis of local adaptation in plants. *PLoS One*, *3*(12), p.e4010.

Leis, J.M., 1991. The pelagic stage of reef fishes: the larval biology of coral reef fishes. *The ecology of fishes on coral reefs*, pp.183-230.

Leiva, F.P., Urbina, M.A., Cumillaf, J.P., Gebauer, P. and Paschke, K., 2015. Physiological responses of the ghost shrimp Neotrypaea uncinata (Milne Edwards 1837)(Decapoda: Thalassinidea) to oxygen availability and recovery after severe environmental hypoxia. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *189*, pp.30-37.

Leroi, A.M., Bennett, A.F. and Lenski, R.E., 1994. Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proceedings of the National Academy of Sciences*, *91*(5), pp.1917-1921.

Levin, L.A., 2006. Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and comparative biology*, *46*(3), pp.282-297.

Gibson, R.N. and Atkinson, R.J.A., 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanography and Marine Biology: An Annual Review*, *41*, pp.1-45.

Levin, L. A., Dayton, P. K. 2009 Ecological theory and continental margins: where shallow meets deep. *Trends in Ecology & Evolution*, 24 (11): 606-617

Levins, R., 1968. *Evolution in changing environments: some theoretical explorations* (No. 2). Princeton University Press.

Levitus, S., Antonov, J.I., Boyer, T.P., Locarnini, R.A., Garcia, H.E. and Mishonov, A.V., 2009. Global ocean heat content 1955–2008 in light of recently revealed instrumentation problems. *Geophysical Research Letters*,*36*(7).

Lexer, C. and Fay, M.F., 2005. Adaptation to environmental stress: a rare or frequent driver of speciation?. *Journal of evolutionary biology*, *18*(4), pp.893-900.

Li, F., Luan, W., Zhang, C., Zhang, J., Wang, B., Xie, Y., Li, S. and Xiang, J., 2009. Cloning of cytoplasmic heat shock protein 90 (FcHSP90) from Fenneropenaeus chinensis and its expression response to heat shock and hypoxia. *Cell Stress and Chaperones*, *14*(2), pp.161-172.

Li, T. and Brouwer, M., 2009. Bioinformatic analysis of expressed sequence tags from grass shrimp Palaemonetes pugio exposed to environmental stressors. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *4*(3), pp.187-195.

Lindahl, O. and Hernroth, L., 1988. Large-scale and long-term variations in the zooplankton community of the Gullmar fjord, Sweden, in relation to advective processes. *Marine ecology progress series. Oldendorf*, *43*(1), pp.161-171.

Lindahl, O., 1987. Plankton community dynamics in relation to water exchange in the Gullmar fjord. *Sweden. Diss., Department of Zoology, University of Stockholm*.

Lonsdale, D.J. and Levinton, J.S., 1985. Latitudinal differentiation in copepod growth: an adaptation to temperature. *Ecology*, *66*(5), pp.1397-1407.

Lu, J., Shi, Y., Wang, S., Chen, H., Cai, S. and Feng, J., 2016. NMR-based metabolomic analysis of Haliotis diversicolor exposed to thermal and hypoxic stresses. *Science of The Total Environment*, *545*, pp.280-288.

Lymbery, R.A. and Evans, J.P., 2013. Genetic variation underlies temperature tolerance of embryos in the sea urchin Heliocidaris erythrogramma armigera. *Journal of evolutionary biology*, *26*(10), pp.2271-2282.

Lynch, M. and Gabriel, W., 1987. Environmental tolerance. *American Naturalist*, pp.283-303.

MacFarling Meure, C., Etheridge, D., Trudinger, C., Steele, P., Langenfelds, R., Van Ommen, T., Smith, A. and Elkins, J., 2006. Law Dome CO2, CH4 and N2O ice core records extended to 2000 years BP. *Geophysical Research Letters*, *33*(14).

Maggs, C.A., Castilho, R., Foltz, D., Henzler, C., Jolly, M.T., Kelly, J., Olsen, J., Perez, K.E., Stam, W., Väinölä, R. and Viard, F., 2008. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology*,*89*(sp11).

Magnum, C. & W. van Winkle, 1973. Responses of aquatic invertebrates to declining oxygen tensions. Am. Zool., Vol. 13, pp. 529-541.

Magozzi, S. and Calosi, P., 2015. Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Global change biology*, *21*(1), pp.181-194.

Main, K.L. and Laramore, R., 1999. Shrimp health management. *Farming marine shrimp in recirculating freshwater systems. Florida Department of Agriculture and Consumer Services—Harbor Branch Oceanic Institute, Florida*.

Mandic, M., Sloman, K.A. and Richards, J.G., 2009. Escaping to the Surface: A Phylogenetically Independent Analysis of Hypoxia-Induced Respiratory Behaviors in Sculpins. *Physiological and Biochemical Zoology*,*82*(6), pp.730-738.

Marcil, J., Swain, D.P. and Hutchings, J.A., 2006 b. Countergradient variation in body shape between two populations of Atlantic cod (Gadus morhua).*Proceedings of the Royal Society of London B: Biological Sciences*,*273*(1583), pp.217-223.

Martinez, I., Aschan, M., Skjerdal, T., & Aljanabi, S. M. (2006). The genetic structure of *Pandalus borealis* in the Northeast Atlantic determined by RAPD analysis. *ICES Journal of Marine Science: Journal du Conseil*, *63*(5), 840-850.

Martinez, I., Aschan, M., Skjerdal, T. and Aljanabi, S.M., 2006. The genetic structure of Pandalus borealis in the Northeast Atlantic determined by RAPD analysis. *ICES Journal of Marine Science: Journal du Conseil*, *63*(5), pp.840-850.

Marvin, D.E. and Burton, D.T., 1973. Cardiac and respiratory responses of rainbow trout, bluegills and brown bullhead catfish during rapid hypoxia and recovery under normoxic conditions.*Comparative Biochemistry and Physiology Part A: Physiology*,*46*(4), pp.755-765.

Matear, R.J., Hirst, A.C. and McNeil, B.I., 2000. Changes in dissolved oxygen in the Southern Ocean with climate change. *Geochemistry, Geophysics, Geosystems*, *1*(11).

McBryan, T.L., Anttila, K., Healy, T.M. and Schulte, P.M., 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integrative and comparative biology*,*53*(4), pp.648-659.

McKenzie, D.J., 2001. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *128*(3), pp.605-619.

McKenzie, D.J., Lund, I. and Pedersen, P.B., 2008. Essential fatty acids influence metabolic rate and tolerance of hypoxia in Dover sole (Solea solea) larvae and juveniles. *Marine Biology*, *154*(6), pp.1041-1051.

McMahon, B.R., 2001. Respiratory and circulatory compensation to hypoxia in crustaceans. *Respiration physiology*, *128*(3), pp.349-364.

McNatt, R.A. and Rice, J.A., 2004. Hypoxia-induced growth rate reduction in two juvenile estuary-dependent fishes. *Journal of Experimental Marine Biology and Ecology*, *311*(1), pp.147-156.

McWilliams, J. P., Côté, I. M., Gill, J. A., Sutherland, W. J., & Watkinson, A. R. (2005). Accelerating impacts of temperature-induced coral bleaching in the Caribbean. *Ecology*, *86*(8), 2055-2060.

Melzner, F., Bock, C. and Pörtner, H.O., 2006. Temperature-dependent oxygen extraction from the ventilatory current and the costs of ventilation in the cephalopod Sepia officinalis. *Journal of Comparative Physiology B*,*176*(7), pp.607-621.

Mee, L.D., 2001. Eutrophication in the Black Sea and a basin wide approach to control it. In: Von Bodungen, B., Turner, R.K. (Eds.), Science and Integrated Coastal Management. Dahlem University Press, Berlin, pp. 71–91.

Menge, B.A., 1992. Community Regulation: Under What Conditions Are Bottom-Up Factors Important on Rocky Shores?. *Ecology*, *73*(3), pp.755-765.

Menge, B.A., 2000. Top-down and bottom-up community regulation in marine rocky intertidal habitats. *Journal of Experimental Marine Biology and Ecology*, *250*(1), pp.257-289.

Menge, B.A., Daley, B.A., Wheeler, P.A., Dahlhoff, E., Sanford, E. and Strub, P.T., 1997. Benthic–pelagic links and rocky intertidal communities: Bottom-up effects on top-down control?. *Proceedings of the National Academy of Sciences*, *94*(26), pp.14530-14535.

Merilä, J. and Crnokrak, P., 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, *14*(6), pp.892-903.

Merilä, J., Söderman, F., O'hara, R., Räsänen, K. and Laurila, A., 2004. Local adaptation and genetics of acid-stress tolerance in the moor frog, Rana arvalis. *Conservation Genetics*, *5*(4), pp.513-527.

Meyerhof, O. and Lohmann, K., 1928. Über die natürlichen Guanidinophosphorsäuren (Phosphagene) in der quergestreiften Muskulatur. II. Die physikalisch-chemischen Eigenschaften der Guanidinophosphorsäuren. *Biochem. Z.*, *196*, pp.49-72.

Michalak, P., Minkov, I., Helin, A., Lerman, D.N., Bettencourt, B.R., Feder, M.E., Korol, A.B. and Nevo, E., 2001. Genetic evidence for adaptation-driven incipient speciation of Drosophila melanogaster along a microclimatic contrast in “Evolution Canyon,” Israel. *Proceedings of the National Academy of Sciences*, *98*(23), pp.13195-13200.

Mitchell-Olds T, Schmitt J. 2006. Genetic mechanisms and evolutionary significance of natural variation in Arabidopsis. Nature 441: 947–952.

Moran, N.A., 1992. The evolutionary maintenance of alternative phenotypes.*American Naturalist*, pp.971-989.

Mousseau, T.A. and Roff, D.A., 1987. Natural selection and the heritability of fitness components. *Heredity*, *59*(Pt 2), pp.181-197.

Myksvoll, M.S., Jung, K.M., Albretsen, J. and Sundby, S., 2014. Modelling dispersal of eggs and quantifying connectivity among Norwegian coastal cod subpopulations. *ICES Journal of Marine Science: Journal du Conseil*, *71*(4), pp.957-969.

Narum, S.R., Campbell, N.R., Meyer, K.A., Miller, M.R. and Hardy, R.W., 2013. Thermal adaptation and acclimation of ectotherms from differing aquatic climates. *Molecular Ecology*, *22*(11), pp.3090-3097.

Nei, M. and Tajima, F., 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics*, *97*(1), pp.145-163.

Nei, M., 1987. *Molecular evolutionary genetics*. Columbia university press.

Newell, R.C. ed., 2013. *Adaptation to environment: essays on the physiology of marine animals*. Elsevier.

Newman, R.A., 1992. Adaptive plasticity in amphibian metamorphosis. *BioScience*, *42*(9), pp.671-678.

Nicastro, K.R., Zardi, G.I., McQuaid, C.D., Teske, P.R. and Barker, N.P., 2008. Coastal topography drives genetic structure in marine mussels. *Marine Ecology Progress Series*, *368*, pp.189-195.

Nonnotte, G., Maxime, V., Truchot, J.P., Williot, P. and Peyraud, C., 1993. Respiratory responses to progressive ambient hypoxia in the sturgeon, Acipenser baeri. *Respiration physiology*, *91*(1), pp.71-82.

Nordberg, K., Filipsson, H.L., Gustafsson, M., Harland, R. and Roos, P., 2001. Climate, hydrographic variations and marine benthic hypoxia in Koljö Fjord, Sweden. *Journal of Sea Research*, *46*(3), pp.187-200.

Norin, T., Malte, H. and Clark, T.D., 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures.*Journal of Experimental Biology*, *217*(2), pp.244-251.

Nosil, P., Funk, D.J. and Ortiz-Barrientos, D.., 2009. Divergent selection and heterogeneous genomic divergence. *Molecular ecology*, *18*(3), pp.375-402.

Nuismer, S.L. and Gandon, S., 2008. Moving beyond common-garden and transplant designs: insight into the causes of local adaptation in species interactions. *The American Naturalist*, *171*(5), pp.658-668.

Obernier, J.A. and Baldwin, R.L., 2006. Establishing an appropriate period of acclimatization following transportation of laboratory animals. *ILAR journal*,*47*(4), pp.364-369.

O'Dor, R.K., 1992. Big squid in big currents. *South African Journal of Marine Science*, *12*(1), pp.225-235.

Oellermann, M., Pörtner, H.O. and Mark, F.C., 2012. Mitochondrial dynamics underlying thermal plasticity of cuttlefish (Sepia officinalis) hearts. *Journal of Experimental Biology*, *215*(17), pp.2992-3000.

Oliver, P.G., 2001. Functional morphology and description of a new species of Amygdalum (Mytiloidea) from the oxygen minimum zone of the Arabian Sea. *Journal of Molluscan Studies*, *67*(2), pp.225-241.

Ono, T., Midorikawaz, T., Watanabe, Y.W., Tadokoro, K. and Saino, T., 2001. Temporal increases of phosphate and apparent oxygen utilization in the subsurface waters of western subarctic Pacific. *Geophysical Research Letters*, *28*(17), pp.3285-3288.

Oostermeijer, J. G. B., S. H. Luijten, and J. C. M. Den Nijs. "Integrating demographic and genetic approaches in plant conservation." *Biological conservation* 113, no. 3 (2003): 389-398.

Orti, G., Bell, M.A., Reimchen, T.E. and Meyer, A., 1994. Global survey of mitochondrial DNA sequences in the threespine stickleback: evidence for recent migrations. *Evolution*, pp.608-622.

Osovitz, C.J. and Hofmann, G.E., 2005. Thermal history-dependent expression of the hsp70 gene in purple sea urchins: biogeographic patterns and the effect of temperature acclimation. *Journal of Experimental Marine Biology and Ecology*, *327*(2), pp.134-143.

Ouellet, P. and Chabot, D., 2005. Rearing Pandalus borealis (Krøyer) larvae in the laboratory. *Marine biology*, *147*(4), pp.869-880.

Ouellet, P., and Allard, J. P. 2006. Vertical distribution and behavior of shrimp Pandalus borealis larval stages in thermally stratified water columns: laboratory experiment and field observations. Fisheries Oceanography, 15: 373–389

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., Thomas, M. B. 2013. Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19: 2373-2380

Padilla, D.K. and Adolph, S.C., 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evolutionary Ecology*, *10*(1), pp.105-117.

Palmer, A.R., 1994. 15 Temperature Sensitivity, Rate of Development, and Time to Maturity: Geographic Variation inLaboratory-Reared Nucella and a Cross-Phyletic Overview.

Pampoulie, C., Stefánsson, M.Ö., JörundsdóttiR, T.D., Danilowicz, B.S. and Danielsdottir, A.K., 2008. Recolonization history and large-scale dispersal in the open sea: the case study of the North Atlantic cod, Gadus morhua L. *Biological Journal of the Linnean Society*, *94*(2), pp.315-329.

Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, pp.637-669.

Parmesan, Camille, Michael T. Burrows, Carlos M. Duarte, Elvira S. Poloczanska, Anthony J. Richardson, David S. Schoeman, and Michael C. Singer. "Beyond climate change attribution in conservation and ecological research." *Ecology letters* 16, no. s1 (2013): 58-71.

Pascoe, D. and Beattie, J.H., 1979. Resistance to cadmium by pretreated rainbow trout alevins. *Journal of Fish Biology*, *14*(3), pp.303-308.

Paul, A.J. and Nunes, P., 1983. Temperature modification of respiratory metabolism and caloric intake of Pandalus borealis (Krøyer) first zoeae. *Journal of Experimental Marine Biology and Ecology*, *66*(2), pp.163-168.

Pauly, D., Watson, R. and Alder, J., 2005. Global trends in world fisheries: impacts on marine ecosystems and food security. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1453), pp.5-12.

Pearson, G.A., Lago-Leston, A. and Mota, C., 2009. Frayed at the edges: selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations. *Journal of Ecology*, *97*(3), pp.450-462.

Pechenik, J.A., Eyster, L.S., Widdows, J. and Bayne, B.L., 1990. The influence of food concentration and temperature on growth and morphological differentiation of blue mussel Mytilus edulis L. larvae. *Journal of Experimental Marine Biology and Ecology*, *136*(1), pp.47-64.

Peck, L.S., Convey, P. and Barnes, D.K., 2006. Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability.*Biological reviews*, *81*(1), pp.75-109.

Pecorino, D., Barker, M.F., Dworjanyn, S.A., Byrne, M. and Lamare, M.D., 2014. Impacts of near future sea surface pH and temperature conditions on fertilisation and embryonic development in Centrostephanus rodgersii from northern New Zealand and northern New South Wales, Australia. *Marine biology*, *161*(1), pp.101-110.

Pedersen, O.P., Aschan, M., Rasmussen, T., Tande, K.S. and Slagstad, D., 2003. Larval dispersal and mother populations of Pandalus borealis investigated by a Lagrangian particle-tracking model. *Fisheries Research*,*65*(1), pp.173-190.

Pereyra, R.T., Westgaard, J.I., Dahl, M., Johansen, T., Knutsen, H., Ring, A.K., Søvik, G. and André, C., 2012. Isolation and characterization of nuclear microsatellite loci in the northern shrimp, Pandalus borealis.*Conservation Genetics Resources*, *4*(1), pp.109-112.

Perry, A.L., Low, P.J., Ellis, J.R. and Reynolds, J.D., 2005. Climate change and distribution shifts in marine fishes. *science*, *308*(5730), pp.1912-1915.

Pespeni, M.H., Barney, B.T. and Palumbi, S.R., 2013. Differences in the regulation of growth and biomineralization genes revealed through long-term common-garden acclimation and experimental genomics in the purple sea urchin. *Evolution*, *67*(7), pp.1901-1914.

Petit, R.J., El Mousadik, A. and Pons, O., 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation biology*, *12*(4), pp.844-855.

Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. and Moczek, A.P., 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends in ecology & evolution*, *25*(8), pp.459-467.

Pigliucci, M., 2001. *Phenotypic plasticity: beyond nature and nurture*. JHU Press.

Pihl, L., 1989. Effects of oxygen depletion on demersal fish in coastal areas of the south-east Kattegat.

Pillet, M., Dupont-Prinet, A., Chabot, D., Tremblay, R. and Audet, C., 2016. Effects of exposure to hypoxia on metabolic pathways in northern shrimp (Pandalus borealis) and Greenland halibut (Reinhardtius hippoglossoides).*Journal of Experimental Marine Biology and Ecology*, *483*, pp.88-96.

Pittman, S.J. and McAlpine, C.A., 2003. Movements of marine fish and decapod crustaceans: process, theory and application. *Advances in marine biology*, *44*(1), pp.205-294.

Pogson, G.H., Mesa, K.A. and Boutilier, R.G., 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics*, *139*(1), pp.375-385.

Poloczanska, E.S., Brown, C.J., Sydeman, W.J., Kiessling, W., Schoeman, D.S., Moore, P.J., Brander, K., Bruno, J.F., Buckley, L.B., Burrows, M.T. and Duarte, C.M., 2013. Global imprint of climate change on marine life. *Nature Climate Change*, *3*(10), pp.919-925.

Pörtner, H., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*,*88*(4), pp.137-146.

Pörtner, H.O., 2002. Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. *Journal of Experimental Biology*, *205*(15), pp.2217-2230.

Pörtner, H.O. and Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *science*, *315*(5808), pp.95-97.

Pörtner, H.O., Langenbuch, M. and Michaelidis, B., 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO2 on marine animals: From Earth history to global change. *Journal of Geophysical Research: Oceans*, *110*(C9).

Pörtner, H.O. and Farrell, A.P., 2008. Physiology and climate change. *Science*, *322*(5902), pp.690-692.

Pörtner, H.O., Langenbuch, M. and Michaelidis, B., 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO2 on marine animals: From Earth history to global change. *Journal of Geophysical Research: Oceans*, *110*(C9).

Pörtner, H.O., Mark, F.C. and Bock, C., 2004. Oxygen limited thermal tolerance in fish?: Answers obtained by nuclear magnetic resonance techniques. *Respiratory physiology & neurobiology*, *141*(3), pp.243-260.

Price, T.D., Qvarnström, A. and Irwin, D.E., 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*(1523), pp.1433-1440.

Pritchard, J.K., Stephens, M. and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), pp.945-959.

Rand, P.S., Hinch, S.G., Morrison, J., Foreman, M.G.G., MacNutt, M.J., Macdonald, J.S., Healey, M.C., Farrell, A.P. and Higgs, D.A., 2006. Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River sockeye salmon. *Transactions of the American Fisheries Society*, *135*(3), pp.655-667.

Rasmussen, T., Thollesson, M. and Nilssen, E.M., 1993. Preliminary investigations on the population genetic differentiation of the deep water prawn, Pandalus borealis KRØYER 1838, from Northern Norway and the Barents Sea. *ICES CM*.

Rasmussen, T. and Aschan, M., 2011. Larval stages of Pandalus borealis.*Marine Biology Research*, *7*(2), pp.109-121.

Rasmussen, T. and Tande, K., 1995. Temperature-dependent development, growth and mortality in larvae of the deep-water prawn Pandalus borealis reared in the laboratory. *Marine ecology progress series. Oldendorf*, *118*(1), pp.149-157.

Raymond, M. and Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of heredity*,*86*(3), pp.248-249.

Regoli, F., Frenzilli, G., Bocchetti, R., Annarumma, F., Scarcelli, V., Fattorini, D. and Nigro, M., 2004. Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, Mytilus galloprovincialis, during a field translocation experiment. *Aquatic toxicology*,*68*(2), pp.167-178.

Reid, W.V., 1994. Setting objectives for conservation evaluation. *Systematics association special volume*, *50*, pp.1-1.

Remerie, T., Bourgois, T., Peelaers, D., Vierstraete, A., Vanfleteren, J. and Vanreusel, A., 2006. Phylogeographic patterns of the mysid *Mesopodopsis slabberi* (Crustacea, Mysida) in Western

Europe: evidence for high molecular diversity and cryptic speciation. *Marine Biology*, *149*(3), pp.465-481.

Reusch, T.B., 2014. Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. *Evolutionary Applications*, *7*(1), pp.104-122.

Reusch, T.B., Ehlers, A., Hämmerli, A. and Worm, B., 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity.*Proceedings of the National Academy of Sciences of the United States of America*, *102*(8), pp.2826-2831.

Riedel, B., Pados, T., Pretterebner, K., Schiemer, L., Steckbauer, A., Haselmair, A., ... & Stachowitsch, M. (2014). Effect of hypoxia and anoxia on invertebrate behaviour: ecological perspectives from species to community level. *Biogeosciences (BG)*, *11*(6), 1491-1518.

Rieseberg, L.H., Widmer, A., Arntz, A.M. and Burke, J.M., 2002. Directional selection is the primary cause of phenotypic diversification. *Proceedings of the National Academy of Sciences*, *99*(19), pp.12242-12245.

Riginos, C. and Henzler, C.M., 2008. Patterns of mtDNA diversity in North Atlantic populations of the mussel *Mytilus edulis*. *Marine Biology*, *155*(4), pp.399-412.

Romano, J.C., Bensoussan, N., Younes, W.A. and Arlhac, D., 2000. Anomalie thermique dans les eaux du golfe de Marseille durant l'été 1999. Une explication partielle de la mortalité d'invertébrés fixés?. *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie*, *323*(4), pp.415-427.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. and Pounds, J.A., 2003. Fingerprints of global warming on wild animals and plants. *Nature*, *421*(6918), pp.57-60.

Rosas, C., Martinez, E., Gaxiola, G., Brito, R., Sánchez, A., & Soto, L. A. (1999). The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* (Linnaeus) juveniles. *Journal of Experimental Marine Biology and Ecology*,*234*(1), 41-57.

Rosenberg, R. 1990. Negative oxygen trends in Swedish coastal bottom waters. Marine Pollution Bulletin, 21(7): 335-339

Rosenberg, R., 1985. Eutrophication—the future marine coastal nuisance?.*Marine pollution bulletin*, *16*(6), pp.227-231.

Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., Menzel, A., Root, T.L., Estrella, N., Seguin, B. and Tryjanowski, P., 2008. Attributing physical and biological impacts to anthropogenic climate change.*Nature*, *453*(7193), pp.353-357.

Rundle, H.D. and Nosil, P., 2005. Ecological speciation. *Ecology letters*,*8*(3), pp.336-352.

Rydberg, L., 1977. Circulation in the Gullmaren-a sill fjord with externally maintained stratification. Göteborgs Univ., Oceanografiska Institutionen.

Sanford, E. and Kelly, M.W., 2011. Local adaptation in marine invertebrates. *Annual Review of Marine Science*, *3*, pp.509-535.

Sanford, E., Holzman, S.B., Haney, R.A., Rand, D.M. and Bertness, M.D., 2006. Larval tolerance, gene flow, and the northern geographic range limit of fiddler crabs. *Ecology*, *87*(11), pp.2882-2894.

Schaack, S. and Chapman, L.J., 2003. Interdemic variation in the African cyprinid *Barbus neumayeri*: correlations among hypoxia, morphology, and feeding performance. *Canadian journal of zoology*, *81*(3), pp.430-440.

Schaefer, J. and Ryan, A., 2006. Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. *Journal of Fish Biology*,*69*(3), pp.722-734.

Scheltema, R.S., 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *The biological bulletin*, *140*(2), pp.284-322.

Schluter, D., 2000. *The ecology of adaptive radiation*. OUP Oxford.

Schlüter, M. and Jerosch, K., 2009. Digital atlas of the North Sea. *Alfred Wegener Institute for Polar and Marine Research et al*.

Schmidt, C., Heinz, P., Kucera, M. and Uthicke, S., 2011. Temperature-induced stress leads to bleaching in larger benthic foraminifera hosting endosymbiotic diatoms. *Limnology and Oceanography*, *56*(5), pp.1587-1602.

Schmidt, C., Heinz, P., Kucera, M. and Uthicke, S., 2011. Temperature-induced stress leads to bleaching in larger benthic foraminifera hosting endosymbiotic diatoms. *Limnology and Oceanography*, *56*(5), pp.1587-1602.

Schoolfield, R.M., Sharpe, P.J.H. and Magnuson, C.E., 1981. Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. *Journal of theoretical biology*, *88*(4), pp.719-731.

Schulte, P.M., Healy, T.M. and Fangue, N.A., 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure.*Integrative and comparative biology*, p.icr097.

Schurmann, H. and Steffensen, J.F., 1992. Lethal oxygen levels at different temperatures and the preferred temperature during hypoxia of the Atlantic cod, Gadus morhua L. *Journal of Fish Biology*, *41*(6), pp.927-934.

Schweiger, O., Settele, J., Kudrna, O., Klotz, S. and Kühn, I., 2008. Climate change can cause spatial mismatch of trophically interacting species. *Ecology*, *89*(12), pp.3472-3479.

Scicurve.com publication search, 2014. Retrieved 10th October 2014. http://scicurve.com/search/

Sharpe, P.J. and DeMichele, D.W., 1977. Reaction kinetics of poikilotherm development. *Journal of Theoretical Biology*, *64*(4), pp.649-670.

Sheldon, N.D. and Retallack, G.J., 2002. Low oxygen levels in earliest Triassic soils. *Geology*, *30*(10), pp.919-922.

Shumway, S.E., Perkins, H.C., Schick, D.F. and Stickney, A.P., 1985. Synopsis of biological data on the pink shrimp, Pandalus borealis Kroyer, 1838.

Skuladottir, U., Pétursson, G. and Brynjólfsson, S.H., 2005. The biology of northern shrimp (Pandalus borealis) on the Flemish Cap. *J Northw Atl Fish Sci*, *37*, pp.147-164.

Slatkin M (1993) Isolation by distance in equilibrium and nonequilibrium populations. Evolution, 47, 264–279.

SMHI, 2016. Swedish Meteorological and Hydrological Institute http://www.smhi.se/#ws=wpt-a,proxy=wpt-a,geonameid=2673730 Accessed 16/05/16

Smith, L.W., Barshis, D. and Birkeland, C., 2007. Phenotypic plasticity for skeletal growth, density and calcification of Porites lobata in response to habitat type. *Coral Reefs*, *26*(3), pp.559-567.

Smith-Keune, C. and van Oppen, M., 2006. Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef.*Coral Reefs*, *25*(3), pp.493-502.

Snell-Rood, E. C., Van Dyken, J. D., Cruickshank, T., Wade, M. J., & Moczek, A. P. (2010). Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *Bioessays*, *32*(1), 71-81.

Sokal RR, Rohlf FJ. 1995. Biometry. New York, NY, USA: W.H. Freeman.

Sokolova, I. and Pörtner, H.O., 2001. Temperature effects on key metabolic enzymes in Littorina saxatilis and L. obtusata from different latitudes and shore levels. *Marine Biology*, *139*(1), pp.113-126.

Sokolova, I.M. and Boulding, E.G., 2004. A neutral DNA marker suggests that parallel physiological adaptations to open shore and salt marsh habitats have evolved more than once within two different species of gastropods.*Marine Biology*, *145*(1), pp.133-147.

Solomon, S. (Ed.). 2007. *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC* (Vol. 4). Cambridge University Press.

Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'.*Journal of Experimental Biology*, *213*(6), pp.912-920.

Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integrative and comparative biology*, *42*(4), pp.780-789.

Sparnocchia, S., Schiano, M.E., Picco, P., Bozzano, R. and Cappelletti, A., 2006, March. The anomalous warming of summer 2003 in the surface layer of the Central Ligurian Sea (Western Mediterranean). In *Annales Geophysicae* (Vol. 24, No. 2, pp. 443-452).

Spicer, J.I., 2014. What can an ecophysiological approach tell us about the physiological responses of marine invertebrates to hypoxia?. *Journal of Experimental Biology*, *217*(1), pp.46-56.

Spicer, J.I. and El-Gamal, M.M., 1999. Hypoxia accelerates the development of respiratory regulation in brine shrimp-but at a cost. *Journal of Experimental Biology*, *202*(24), pp.3637-3646.

Spicer, J.I. and Eriksson, S.P., 2003. Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial postlarvae in the Norway lobster Nephrops norvegicus (L.)?. *Journal of Experimental Marine Biology and Ecology*, *295*(2), pp.219-243.

Spicer, J. and Baden, S., 2001. Environmental hypoxia and haemocyanin between-individual variability in Norway lobsters Nephrops norvegicus (L.).*Marine Biology*, *139*(4), pp.727-734.

St-Pierre, J., Tattersall, G.J. and Boutilier, R.G., 2000. Metabolic depression and enhanced O2 affinity of mitochondria in hypoxic hypometabolism. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *279*(4), pp.R1205-R1214.

Stanton, M.L., Galen, C. and Shore, J., 1997. Population structure along a steep environmental gradient: consequences of flowering time and habitat variation in the snow buttercup, Ranunculus adoneus. *Evolution*, pp.79-94.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Stickle, W.B., Kapper, M.A., Liu, L.L., Gnaiger, E. and Wang, S.Y., 1989. Metabolic adaptations of several species of crustaceans and molluscs to hypoxia: tolerance and microcalorimetric studies. *The Biological Bulletin*,*177*(2), pp.303-312.

Stillman, J.H., 2002. Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus Petrolisthes. *Integrative and comparative biology*, *42*(4), pp.790-796.

Stillman, J.H. and Tagmount, A., 2009. Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in porcelain crabs, Petrolisthes cinctipes. *Molecular ecology*, *18*(20), pp.4206-4226.

Stillman, J.H. and Hurt, D.A., 2015. Crustacean genomics and functional genomic responses to environmental stress and infection. *Physiology*, *4*, p.420.

Stillman, J.H. and Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus Petrolisthes: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology*, *73*(2), pp.200-208.

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., ... & Vasconcellos de Menezes, V., 2013. *Climate Change 2013. The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change-Abstract for decision-makers*. Groupe d'experts intergouvernemental sur l'evolution du climat/Intergovernmental Panel on Climate Change-IPCC, C/O World Meteorological Organization, 7bis Avenue de la Paix, CP 2300 CH-1211 Geneva 2 (Switzerland).

Stramma, L., Schmidtko, S., Levin, L.A. and Johnson, G.C., 2010. Ocean oxygen minima expansions and their biological impacts. *Deep Sea Research Part I: Oceanographic Research Papers*, *57*(4), pp.587-595.

Strasser, C.A. and Barber, P.H., 2009. Limited genetic variation and structure in softshell clams (*Mya arenaria*) across their native and introduced range.*Conservation Genetics*, *10*(4), pp.803-814.

Strömberg, J.O. and Spicer, J.I., 2000. Cold comfort for krill? Respiratory consequences of diel vertical migration by Meganyctiphanes norvegica into deep hypoxic waters. *Ophelia*, *53*(3), pp.213-217.

Struhsaker, J.W., 1968. Selection mechanisms associated with intraspecific shell variation in Littorina picta (Prosobranchia: Mesogastropoda). *Evolution*, pp.459-480.

Stumpp, M., Hu, M.Y., Melzner, F., Gutowska, M.A., Dorey, N., Himmerkus, N., Holtmann, W.C., Dupont, S.T., Thorndyke, M.C. and Bleich, M., 2012. Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. *Proceedings of the National Academy of Sciences*, *109*(44), pp.18192-18197.

Sultan, S.E. and Spencer, H.G., 2002. Metapopulation structure favors plasticity over local adaptation. *The American Naturalist*, *160*(2), pp.271-283.

Sultan, S.E., 1995. Phenotypic plasticity and plant adaptation.*Acta Botanica Neerlandica*, *44*(4), pp.363-383.

Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., & Reusch, T. B. (2014). Evolution in an acidifying ocean. *Trends in ecology & evolution*, *29*(2), 117-125.

Svendsen, J.C., Steffensen, J.F., Aarestrup, K., Frisk, M., Etzerodt, A. and Jyde, M., 2011. Excess posthypoxic oxygen consumption in rainbow trout (Oncorhynchus mykiss): recovery in normoxia and hypoxia. *Canadian journal of zoology*, *90*(1), pp.1-11.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*(3), pp.585-595.

Taylor, A.C. and Spicer, J.I., 1987. Metabolic responses of the prawns Palaemon elegans and P. serratus (Crustacea: Decapoda) to acute hypoxia and anoxia. *Marine Biology*, *95*(4), pp.521-530.

Taylor, A.C. and Spicer, J.I., 1987. Metabolic responses of the prawns Palaemon elegans and P. serratus (Crustacea: Decapoda) to acute hypoxia and anoxia. *Marine Biology*, *95*(4), pp.521-530.

Taylor, A.C. and Spicer, J.I., 1991. Acid-base disturbances in the haemolymph of the prawns, Palaemon elegans (Rathke) and P. serratus (Pennant)(Crustacea: Decapoda) during exposure to hypoxia. *Comparative Biochemistry and Physiology Part A: Physiology*, *98*(3), pp.445-452.

Taylor, E.B., 1991. A review of local adaptation in Salmonidac, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, *98*(1), pp.185-207.

Taylor, E.W., Butler, P.J. and Al-Wassia, A., 1977. Some responses of the shore crab, Carcinus maenas (L.) to progressive hypoxia at different acclimation temperatures and salinities. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *122*(3), pp.391-402.

Taylor, J.C. and Miller, J.M., 2001. Physiological performance of juvenile southern flounder, Paralichthys lethostigma (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. *Journal of Experimental Marine Biology and Ecology*, *258*(2), pp.195-214.

Tepolt, C.K. and Somero, G.N., 2014. Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, Carcinus maenas. *Journal of Experimental Biology*, *217*(7), pp.1129-1138.

Teske, P.R., Papadopoulos, I., Barker, N.P. and McQuaid, C.D., 2012. Mitochondrial DNA paradox: sex-specific genetic structure in a marine mussel–despite maternal inheritance and passive dispersal. *BMC genetics*,*13*(1), p.1.

Thompson, D.B., 1999. Different spatial scales of natural selection and gene flow: the evolution of behavioral geographic variation and phenotypic plasticity. *Geographic variation in behavior: perspectives on evolutionary mechanisms. Oxford University Press, Oxford*, pp.33-51.

Thompson, J.N. and Cunningham, B.M., 2002. Geographic structure and dynamics of coevolutionary selection. *Nature*, *417*(6890), pp.735-738.

Tiffin, P. and Ross-Ibarra, J., 2014. Advances and limits of using population genetics to understand local adaptation. *Trends in ecology & evolution*,*29*(12), pp.673-680.

Tishkoff, S.A., Pakstis, A.J., Ruano, G. and Kidd, K.K., 2000. The accuracy of statistical methods for estimation of haplotype frequencies: an example from the CD4 locus. *The American Journal of Human Genetics*, *67*(2), pp.518-522.

Tomanek, L. and Somero, G.N., 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus Tegula) from different thermal habitats: implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology*, *202*(21), pp.2925-2936.

Trussell, G.C., 2000. Phenotypic clines, plasticity, and morphological trade-offs in an intertidal snail. *Evolution*, *54*(1), pp.151-166.

Tsoi, K.H., Wang, Z.Y. and Chu, K.H., 2005. Genetic divergence between two morphologically similar varieties of the kuruma shrimp Penaeus japonicus. *Marine Biology*, *147*(2), pp.367-379.

Ulstrup, K.E., Ralph, P.J., Larkum, A.W.D. and Kühl, M., 2006. Intra-colonial variability in light acclimation of zooxanthellae in coral tissues of Pocillopora damicornis. *Marine Biology*, *149*(6), pp.1325-1335.

Untersee, S. and Pechenik, J.A., 2007. Local adaptation and maternal effects in two species of marine gastropod (genus Crepidula) that differ in dispersal potential. *Marine Ecology Progress Series*, *347*, pp.79-85.

Van der Putten, W.H., Macel, M. and Visser, M.E., 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), pp.2025-2034.

Vandenthillart, G., Dalla Via, J., Vitali, G. and Cortesi, P., 1994. Influence of long-term hypoxia exposure on the energy-metabolism of solea-solea. 1. Critical o-2 levels for aerobic and anaerobic metabolism. *Marine Ecology Progress Series*, *104*(1-2), pp.109-117.

Vanderplancke, G., Claireaux, G., Quazuguel, P., Huelvan, C., Corporeau, C., Mazurais, D. and Zambonino-Infante, J.L., 2015b. Exposure to chronic moderate hypoxia impacts physiological and developmental traits of European sea bass (Dicentrarchus labrax) larvae. *Fish physiology and biochemistry*, *41*(1), pp.233-242.

Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferraresso, S., Sévère, A., Zambonino-Infante, J.L. and Mazurais, D., 2015a. Hypoxic episode during the larval period has long-term effects on European sea bass juveniles (Dicentrarchus labrax). *Marine Biology*, *162*(2), pp.367-376.

Vaquer-Sunyer, R. and Duarte, C.M., 2008. Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences*, *105*(40), pp.15452-15457.

Verberk, W.C., Bilton, D.T., Calosi, P. and Spicer, J.I., 2011. Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. *Ecology*, *92*(8), pp.1565-1572.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S.M., Schlichting, C.D. and Van Tienderen, P.H., 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology & Evolution*, *10*(5), pp.212-217.

Virani, N.A. and Rees, B.B., 2000. Oxygen consumption, blood lactate and inter-individual variation in the gulf killifish, Fundulus grandis, during hypoxia and recovery. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *126*(3), pp.397-405.

Volis, S., Ormanbekova, D., Yermekbayev, K., Song, M. and Shulgina, I., 2015. Multi-approaches analysis reveals local adaptation in the emmer wheat (*Triticum dicoccoides*) at macro-but not micro-geographical scale. *PloS one*,*10*(3), p.e0121153.

Walsh, P.J. and Somero, G.N., 1981. Temperature adaptation in sea anemones: physiological and biochemical variability in geographically separate populations of Metridium senile. *Marine Biology*, *62*(1), pp.25-34.

Weibel, E.R. and Hoppeler, H., 2005. Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. *Journal of Experimental Biology*,*208*(9), pp.1635-1644.

Weir, B.S. and Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. *evolution*, pp.1358-1370.

Weiss, R.F., 1970, August. The solubility of nitrogen, oxygen and argon in water and seawater. In *Deep Sea Research and Oceanographic Abstracts*(Vol. 17, No. 4, pp. 721-735). Elsevier.

West-Eberhard, M.J., 1989. Phenotypic plasticity and the origins of diversity.*Annual review of Ecology and Systematics*, pp.249-278.

West-Eberhard, M.J., 2003. *Developmental plasticity and evolution*. Oxford University Press.

Westgaard, J.I. and Fevolden, S.E., 2007. Atlantic cod (Gadus morhua L.) in inner and outer coastal zones of northern Norway display divergent genetic signature at non-neutral loci. *Fisheries research*, *85*(3), pp.306-315.

Wetson, A.M., Zörb, C., John, E.A. and Flowers, T.J., 2012. High phenotypic plasticity of Suaeda maritima observed under hypoxic conditions in relation to its physiological basis. *Annals of botany*, *109*(5), pp.1027-1036.

Whitlock, M.C., 2000. Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution*,*54*(6), pp.1855-1861.

Whitman, D.W. and Agrawal, A.A., 2009. What is phenotypic plasticity and why is it important. *Phenotypic plasticity of insects*, *10*, pp.1-63.

Whitney, F.A., Freeland, H.J. and Robert, M., 2007. Persistently declining oxygen levels in the interior waters of the eastern subarctic Pacific. *Progress in Oceanography*, *75*(2), pp.179-199.

Wieland, K., 2004. Length at sex transition in northern shrimp (Pandalus borealis) off West Greenland in relation to changes in temperature and stock size. *Fisheries Research*, *69*(1), pp.49-56.

Wienberg, R., 1982. Studies on the influence of temperature, salinity, light, and feeding rate on laboratory reared larvae of deep sea shrimp Pandalus borealis Kröyer 1838. *Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung*, (3).

Wigginton, J.E., Cutler, D.J. and Abecasis, G.R., 2005. A note on exact tests of Hardy-Weinberg equilibrium. *The American Journal of Human Genetics*, *76*(5), pp.887-893.

Willi, Y., Van Buskirk, J. and Hoffmann, A.A., 2006. Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics*, pp.433-458.

Williams, G.C. and Burt, A., 1997. *Adaptation and natural selection* (p. 93). na.

Wilson, G.M., Thomas, W.K. and Beckenbach, A.T., 1987. Mitochondrial DNA analysis of Pacific Northwest populations of *Oncorhynchus tshawytscha*. *Canadian Journal of Fisheries and Aquatic Sciences*, *44*(7), pp.1301-1305.

Wilson, R.S. and Franklin, C.E., 2002. Testing the beneficial acclimation hypothesis. *Trends in Ecology & Evolution*, *17*(2), pp.66-70.

Winnepenninckx, B., Backeljau, T. and De Wachter, R., 1993. Complete small ribosomal subunit RNA sequence of the chiton Acanthopleura japonica (Lischke, 1873)(Mollusca, Polyplacophora). *Nucleic acids research*, *21*(7), p.1670.

Woods, H.A. and Harrison, J.F., 2002. Interpreting rejections of the beneficial acclimation hypothesis: when is physiological plasticity adaptive?. *Evolution*,*56*(9), pp.1863-1866.

Wu, R.S., 2002. Hypoxia: from molecular responses to ecosystem responses. *Marine Pollution Bulletin*, *45*(1), pp.35-45.

Wu, R.S.S., Lam, P.K.S. and Wan, K.L., 2002. Tolerance to, and avoidance of, hypoxia by the penaeid shrimp (Metapenaeus ensis). *Environmental Pollution*, *118*(3), pp.351-355.

Yamada, S.B., 1989. Are direct developers more locally adapted than planktonic developers?. *Marine Biology*, *103*(3), pp.403-411.

Yampolsky, L.Y., Schaer, T.M. and Ebert, D., 2014. Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1776), p.20132744.

Yanick, J.F., Heath, J.W. and Heath, D.D., 2003. Survival and growth of local and transplanted blue mussels (Mytilus trossulus, Lamark). *Aquaculture Research*, *34*(10), pp.869-875.

Zambonino-Infante, J.L., Claireaux, G., Ernande, B., Jolivet, A., Quazuguel, P., Sévère, A., Huelvan, C. and Mazurais, D., 2013. Hypoxia tolerance of common sole juveniles depends on dietary regime and temperature at the larval stage: evidence for environmental conditioning. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1758), p.20123022.

Zhang, J., Gilbert, D., Gooday, A., Levin, L., Naqvi, S.W.A., Middelburg, J.J., Scranton, M., Ekau, W., Pena, A., Dewitte, B. and Oguz, T., 2010. Natural and human-induced hypoxia and consequences for coastal areas: synthesis and future development. *Biogeosciences*, *7*, pp.1443-1467.

Appendices

i. Local inner fjord oxygen regime

The thermocline in Gullmarfjord ranges between 35 – 50 m (Engström pers. comms. 2014). Insufficient data for Gullmarfjord itself was available beyond 40 m and on a monthly time scale as to reliably plot the oxygen regimes. Two adjacent fjords, Havstensfjord and Kalvefjord, with similar freeze and water residence timings have been used as a proxy.

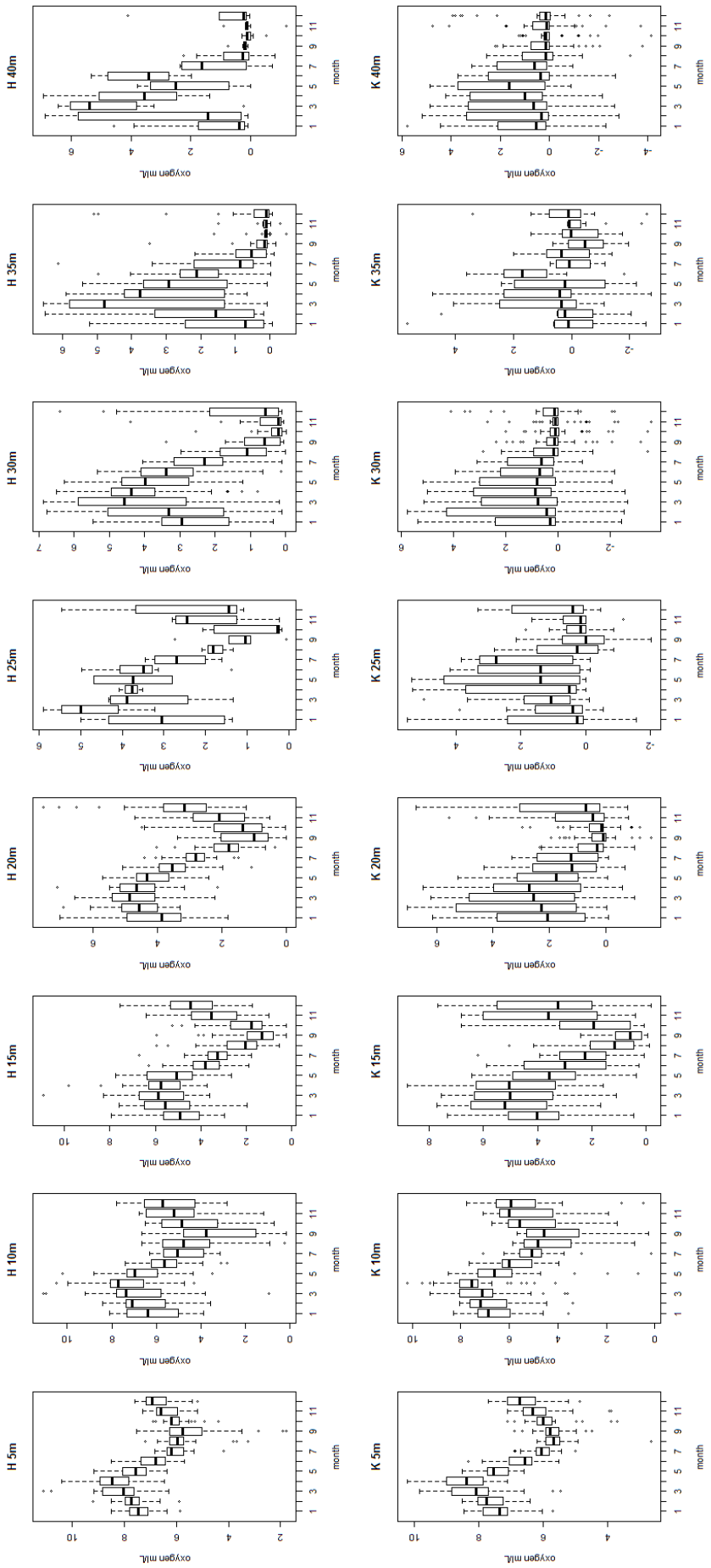


Figure i Annual oxygen regimes of fjords adjacent to Gullmarfjord (Havstensfjord - H and Kalvefjord - K) with similar seasonal freezing and water residence times across different depth profiles down to the

thermocline. Dissolved oxygen values (mg L-1) from 1958 – 2014 were gathered from the Swedish Meteorological and Hydrological Institute.

ii. Mesocosm design

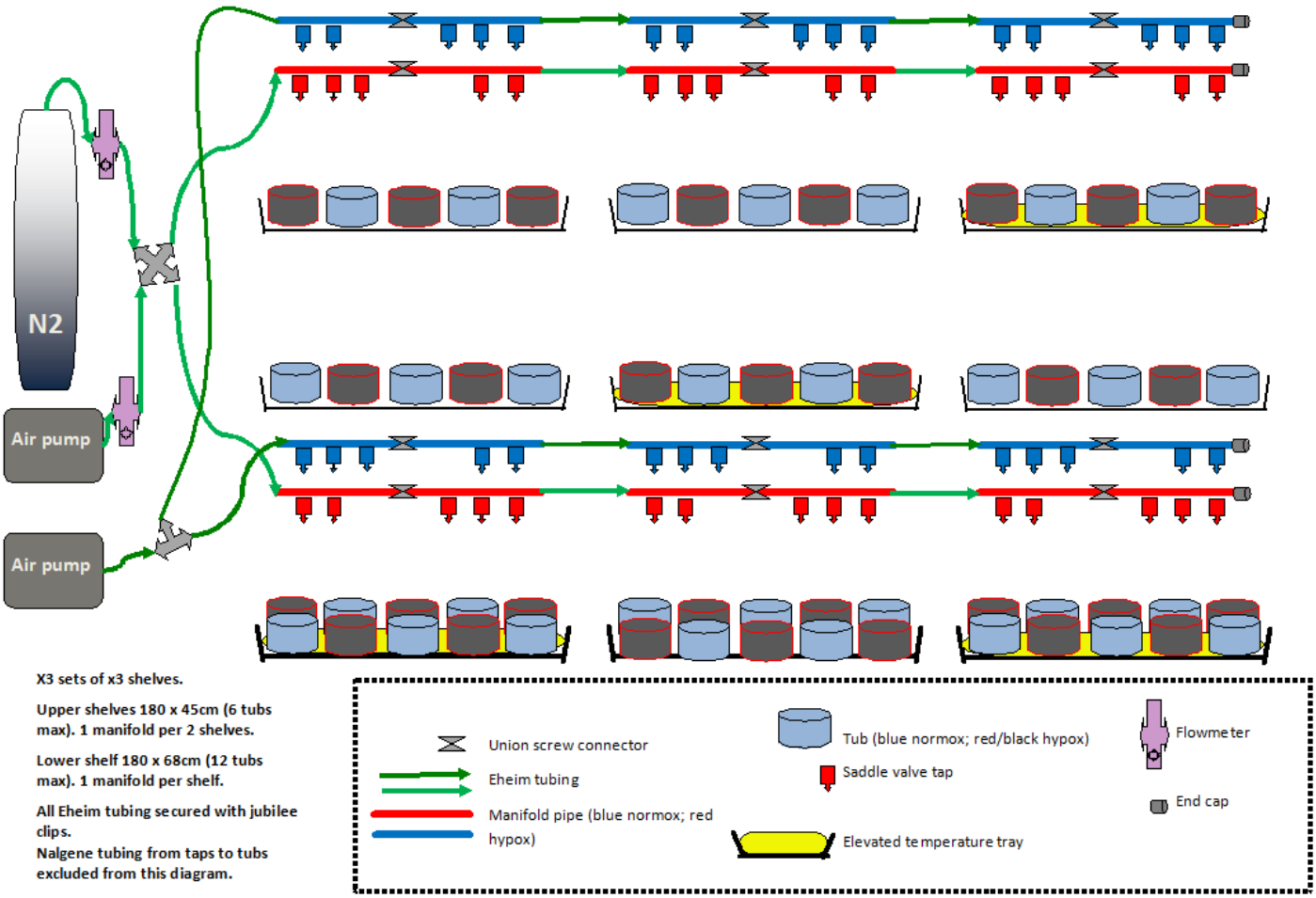


Figure ii Mesocom array used in the common garden experiment detailed in Chapter 3.

iii. MO2 calculations

i. A list of conversions and calculations used for the respirometry data in Chapter 3.

Calculation abbreviations:

AV = Volume of shrimp

cCV = Corrected respirometry chamber volume

EqV = Volume of barrier, petri dish and flea used to restrain shrimp and maintain circulation within respirometry chamber

G = Solubility coefficient according to Green & Carritt (1967) as determined by water temperature and salinity

O_2C = Total O_2 concentration in the respirometry chamber

OPr = O_2C corrected for vapour pressure

P = Barometric pressure

T0 = start point of the respirometry experiment

Te = end point of the respiromentry experiment when shrimp is extracted from the chamber

Vw = O_2 vapour coefficient according to Weiss (1974) as determined by temperature and barometric pressure

ΔO_2 or MO_2 = difference in O_2C from the beginning of the respirometry experiment to the end. Equivalent

1. **cCV = CV – (AV + EqV)**
2. **O_2C = G * (cCV / 1000)**
3. **OPr = (((mmol O_2 * P * 760) / 1000) – Vw) / 760**
4. **$\Delta O_2 = OPr_{T0} - OPr_{Te} = MO_2$ mmol L^{-1}**

ΔO_2 may then be corrected for units of time and individual weight of shrimp by dividing the ΔO_2 by these values.

N.B. 760 mmhg is the standard value for air pressure at sea level.

iv. Common garden experiment shrimp biometrics

ii. Mass data and its relationship with MO_2. The mass and MO2 of shrimp used in the common garden experiment detailed in Chapter 3.

Table iv. Common garden mass and RMR data.

Treatment	Mass (g)	RMR (μmol O_2 L^{-1} h^{-1})
NAB1	5.3	NA
NAB1	5.17	39.67
NAB1	6.59	35.37
NAB1	4.99	27.37
NAB1	5.33	26.97
NAG1	5.18	28.47
NAG1	4.97	NA
NAG1	6	29.17
NAG1	6.41	14.77
NAG1	6.63	NA
HAB1	4.99	NA
HAB1	7.94	22.15
HAB1	7.92	64.75
HAB1	6.8	57.05
HAG1	7.32	55.05
HAG1	6.55	3.15
HAG1	6.29	8.45
HAG1	5.99	29.85
HAG1	7.19	46.95
NAB4	4.8	0
NAB4	6.55	NA
NAB4	3.72	15.67
NAB4	4.32	14.47
NAG4	4.03	0.87
NAG4	4.37	NA
NAG4	5.63	10.77
NAG4	5.08	NA
NAG4	5.77	23.07
HAG4	4.36	0
HAG4	3.46	25.75
HAG4	5.25	5.05
HAG4	4.81	5.85
NEB1	5.64	16.42
NEB1	4.83	24.12
NEB1	6.37	0
NEB1	6.98	36.32
NEB1	5.55	21.72
NEB1	8	13.32
NEB1	5.08	21.52
NEG1	6.73	32.02
NEG1	5.51	18.72
NEG1	8.15	NA
NEG1	7.86	38.72
NEG1	5.59	10.82
NEG1	5.52	21.32
NEG1	7.29	10.32
HEB1	5.66	NA
HEB1	6.1	24.03

HEB1	6.63		41.83
HEG1	4.92		20.03
HEG1	6.07		31.43
HEG1	5.58		41.33
NEB4	5.23		7.62
NEB4	5.35		16.92
NEB4	3.7		15.82
NEB4	6.58		37.62
NEB4	5.8	NA	
NEB4	4.12	NA	
NEB4	6		34.82
NEG4	3.77		43.02
NEG4	4.68		19.62
NEG4	5.54		17.32
NEG4	5.35		8.42
NEG4	5.19		27.22
HEB4	6.05		35.03
HEB4	3.67		0
HEB4	5.56		0
HEG4	7.35		39.43
HEG4	6.22		12.03
HEG4	3.56		36.43
HEG4	4.83		0

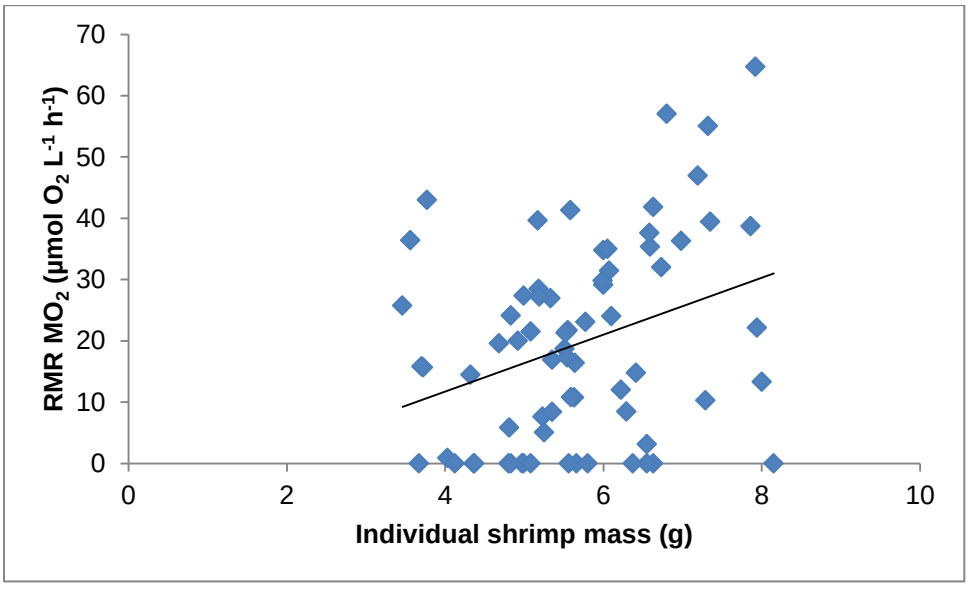


Figure iv Plot of the positive relationship between oxygen consumption (MO2) and individual shrimp mass. This relationship is particularly pronounced in the RMR data.

v. Metabolomics figures and tables

iii. Development work of the computational models conducted on the metabolomics data detailed in Chapter 4 and tables of the most expressed metabolites and their putative annotations.

Site (p = 0.0000) Population (p = 0.0000) Interaction (p = 0.0030)

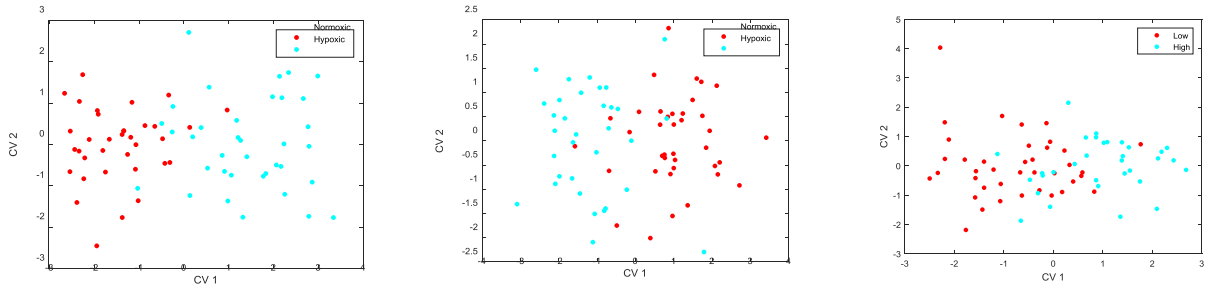


Figure v.i. MANOVA score plots of metabolomic peak data showing the significant interaction between exposure site and population origin.

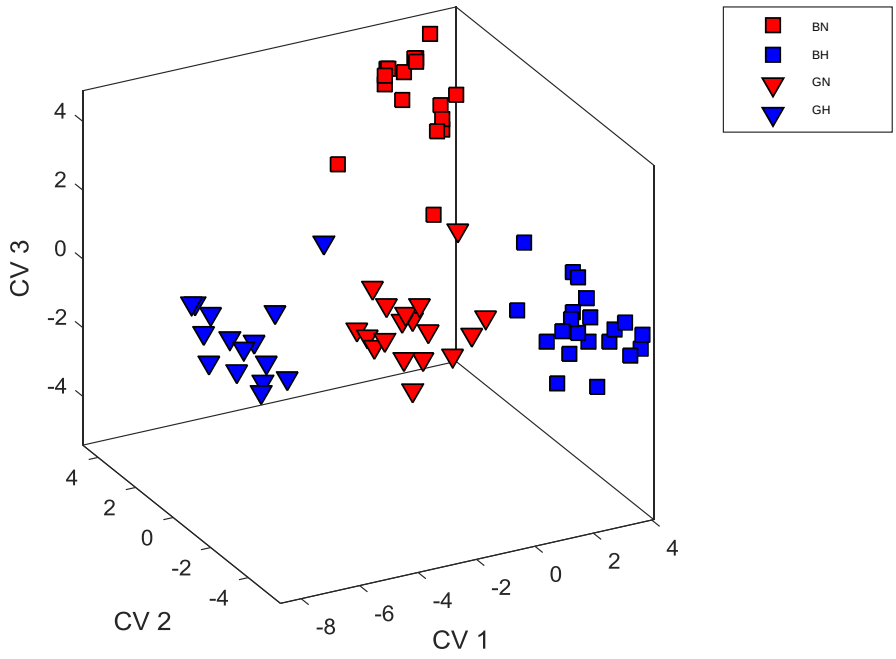


Figure v.ii.: MANOVA score for the direct comparison of the four groups of samples. The labels BN, BH, GN, and GH refer to samples from Brofjorden (B) and Gullmarfjord (G) translocated to either a normoxic site (N) or a hypoxic site (H). The p-value for the observed group separation was 9e-4.

Table v.i: Top 10 peaks for the population and site effect. For each effect the significant peaks with the highest absolute fold change are reported.

m/z	Intensity	q-value[b]	locfdr[c]	FC[a]	Putative annotation	Annotation description
POPULATION						
234.12096	7167874	1.73E-05	5.30E-05	1.608191	'Isouron'	$C_{10}H_{17}N_3O_2$: pesticide cholinesterase inhibitor, causes respiratory arrest in mammals, preventing hydrolysis and inactivation of acetylcholine, leading to malfunction of the PNS and CNS.
215.08469	7222221	5.66E-05	9.77E-05	-1.4152	0	NA
288.13543	3160630	3.21E-08	1.53E-07	1.329582	0	NA
217.15450	7649504	0.031292	0.065419	1.270296	0	NA
186.05530	8967798	0.000211	0.000676	1.129799	0	NA
287.13282	19837187	9.27E-08	5.17E-05	1.162839	0	NA
272.86399	2318263	0.001602	0.010149	-1.11727	0	NA
234.01725	14198793	5.74E-05	0.000563	-1.11786	0	NA
236.20420	8869100	0.012214	0.022891	-1.05498	0	NA
142.06257	9610901	0.025113	0.065419	1.006532	0	NA
SITE						
553.38880	59988989	0.004181	0.022099	-2.42337	0	NA
554.39216	29190839	0.001944	0.011954	-1.79846	0	NA
204.06284	17995128	1.21E-08	5.99E-07	-1.76152	'3-Amino-3-(4-hydroxyphenyl)propanoate / D-Tyrosine / D L-Tyrosine / L-threo-3-Phenylserine'	$C_9H_{11}NO_3$: beta amino acid used in protein synthesis, amino acid biosynthesis and cell signalling. Hydroxyphenyllactic acid prevalent in individuals with a deficiency of oxidase enzymes or indicative of unbalanced gut flora. / Tyrosine is a hydrophobic amino acid and neurotransmitter precursor. Component of aminoacyl-tRNA, phenylalanine, tyrosine, tryptophan, stilbene, coumarine and lignin biosynthesis; of tyrosine, riboflavin and phenylalanine metabolism. / phenylpropanoic acid involved in protein and amino acid synthesis.
741.98728	5493370	1.55E-06	3.02E-05	-1.58945	0	NA
363.15449	9985947	2.71E-05	0.000134	-1.58496	0	NA
130.15892	47422109	0.040988	0.112986	-1.4639	'Octylamine'	$C_8H_{19}N$: amine, causes an increase in cytochrome b_5 (ubiquitous electron transport haemoproteins) reduction and delays its reoxidation in the presence of limiting quantities of NAD+
183.08435	1.39E+08	9.15E-10	9.15E-10	-1.462	'Crotono-betaine'	$C_7H_{13}NO_2$: Tropane alkaloid, involved in ligase enzymes, carnitine metabolism, and transporter activity.
182.08100	1.4E+09	9.15E-10	9.15E-10	-1.456	'3-Amino-3-(4-hydroxyphenyl)propanoate / D-Tyrosine / D L-Tyrosine / L-threo-3-Phenylserine'	See above
616.15007	12676306	8.48E-05	0.000134	1.45565	0	NA
165.05444	3.55E+08	9.15E-10	5.70E-08	-1.4449	"'2-Hydroxy-3-phenylpropenoate3-Coumaric acid / 4-CoumarateBenzoyl acetate /Caffeic aldehyde / Phenylpyruvatecis-2-Hydroxycinnamatecis-p-C...'' <Preview truncated at 128 characters>'	$C_9H_8O_3$: nutrient / acetate involved in the degradation of aromatic compounds / involved in phenylpropanoid biosynthesis / involved in tyrosine biosynthesis.

[a] *The fold change between groups A and B is reported as log2(b/a), where a and b correspond to the average peak intensity for samples in group A and B, respectively.*

[b] *The q-value is defined as Pr(null|Z≥z). In other words it corresponds to the probability that peaks with a test-statistics with a value of z or larger are a "null" (not significant). By only marking peaks with a q-value* ≤ 0.05 *as significant we control the False Discovery Rate at this level.Therefore we expect that at the most 5% of the peaks flagged as significant are false positives.*

[c] *the local fdr-value is defined as Pr(null|z). It corresponds to the probability that a peak with a test-statistic value of z is a "null".*

Table v.ii. Top 10 peaks with putative annotation for the population and site effect. For each effect the significant peaks with the highest absolute fold change are reported.

m/z	Intensity	q-value	locfdr	FC	Putative annotation	Annotation description
POPULATION						
234.12096	7167874	1.73E-05	5.30E-05	1.608191	'Isouron'	$C_{10}H_{17}N_3O_2$: pesticide cholinesterase inhibitor, causes respiratory arrest in mammals, preventing hydrolysis and inactivation of acetylcholine, leading to malfunction of the PNS and CNS.
538.17178	26644881	0.034842	0.065419	-1.08116	'Validamycin A'	$C_{20}H_{35}NO_{13}$: antibiotic and inhibitor of trehalase (glycoside hydrolase enzyme that catalyzes the conversion of trehalose to glucose).
SITE						
204.06284	17995128	1.21E-08	5.99E-07	-1.76152	'3-Amino-3-(4-hydroxyphenyl)propanoateD-TyrosineDL-TyrosineL-TyrosineL-threo-3-Phenylserine'	See Table 4
130.15892	47422109	0.040988	0.112986	-1.4639	'Octylamine'	See Table 4
183.08435	1.39E+08	9.15E-10	9.15E-10	-1.462	'Crotono-betaine'	See Table 4
182.08100	1.4E+09	9.15E-10	9.15E-10	-1.456	'3-Amino-3-(4-hydroxyphenyl)propanoateD-TyrosineDL-TyrosineL-TyrosineL-threo-3-Phenylserine'	See Table 4
165.05444	3.55E+08	9.15E-10	5.70E-08	-1.4449	'"2-Hydroxy-3-phenylpropenoate3-Coumaric acid4-CoumarateBenzoyl acetateCaffeic aldehydePhenylpyruvatecis-2-Hydroxycinnamatecis-p-C..." <Preview truncated at 128 characters>'	See Table 4
447.20206	8791991	0.00192	0.010374	-1.44012	'Estrone glucuronide / Yangambin'	$C_{24}H_{30}O_8$: Sterol lipid hormone used to detect ovulation. Has a role in membrane stability, an energy source and storage, waste product, cell signalling; component of sulphur, androgen and oestrogen metabolism. / Phenylpropanoid antagonist that selectively blocks Platelet Activating Factor receptors on platelets.
147.04387	73966018	9.15E-10	9.15E-10	-1.41895	'Coumarin'	$C_9H_6O_2$: involved in phenylpropanoid biosynthesis High levels cause cell toxicity and acts as a carcinogen.
263.13891	8685930	1.20E-05	0.00011	-1.32589	'Methohexital / Physovenine'	$C_{14}H_{18}N_2O_3$: barbituric acid derivative and indole, binds at a distinct binding site associated with a Cl^- ionopore at the $GABA_A$ receptor (neurotransmitter). / Alkaloid acting on the PNS and CNS.
166.08606	2.04E+09	8.48E-05	0.000134	-1.18764	'4-Hydroxy-1-(3-pyridinyl)-1-butanone Benzocaine / D-PhenylalanineL-Phenylalanine / Metolcarb Phenylalanine /Tricaine'	$C_9H_{11}NO_2$: ester inhibits neurotransmission / precursor to tyrosine and a component in aminoacyl-trna, phenylalanine,

						tyrosine and tryptophan, and novobiocin; and phenylalanine and tyrosine metabolism/ crystalline carbamate insecticide / involved in protein synthesis.
189.12322	48578659	0.000279	0.000966	-1.16669	'6-Acetamido-3-aminohexanoate / Glycyl-leucineN / 6-Acetyl-L-lysine'	$C_8H_{16}N_2O_3$: Aminocaproic acid inhibits plasminogen activators (prevents plasmin activation – plasmin dissolves blood clots). Reacts to form glutamate for energy release. Involved in cell signalling, fuel or energy storage, membrane stability, protein synthesis. / eptide derived from amino carboxylic acid. / acetylated amino acid / acetylhistone, role in transcriptional activation, cell-cycle progression, apoptosis, DNA repair, and cytoskeletal organisation.

Table v.iii: Top 10 peaks for the interaction effect. The ranking was based on the canonical variance (CV) weights from the MGSDA model.

Top 10 peaks						
m/z	Intensity	FC	CV weight	Rank	Putative annotation	Annotation description
344.34695	1811246	-0.46917	2.390194	1	0	NA
272.86399	2318263	-1.11164	1.594132	2	0	NA
530.93153	644923.5	0.363084	-1.46125	3	0	NA
780.48770	1741944	0.515079	-1.31963	4	0	NA
907.57426	3522528	-0.48495	1.168758	5	0	NA
440.20310	1167150	-0.15509	0.938763	6	'Butroxydim / Spiramine A'	$C_{24}H_{33}NO_4$: Cyclohexenone derivative, pesticide / Terpenoid alkaloid.
247.19865	2413435	-0.56635	0.933362	7	0	NA
377.15487	2233753	-0.41477	0.793064	8	0	NA
350.16649	1792610	-0.37821	0.790205	9	0	NA
280.22117	769494.6	-0.23375	0.772949	10	0	NA
Top 10 peaks with putative annotation						
440.20310	1167150	-0.15509	0.938763	6	'Butroxydim / Spiramine A'	See above
128.07060	1.86E+09	0.221031	-0.47316	16	'''(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate / Guvacine / Isoguvacine / L-Baikiain / alpha-(Methylenecyclopropyl)glycinedelta1-Piperideine-2...'' <Preview truncated at 128 characters>'	$C_6H_9NO_2$: part of the glycine, serine and threonine metabolism, lysine degradation, and peroxisome pathways / alkaloid, gaba (neurotransmitter) reuptake inhibitor / tetrahydropyridine, $gaba_a$ receptor agonist / metabolite of tryptophan catabolism, inflammatory reactions, protein synthesis / lysine degradation, tropane, piperidine and pyridine alkaloid biosynthesis.
547.29003	2144162	0.315514	-0.3591	19	'Decoside'	$C_{30}H_{42}O_9$: steroid saponin, nutrient.
343.22290	79772973	-0.32091	0.192322	31	'(15:1)-Cardanol / 3beta-Hydroxypregn-5-ene / Pregnan-21-al'	$C_{21}H_{34}O$: phenolic lipid / steroid involved in cell signalling, fuel and energy source and storage, membrane

						stability and as a nutrient/ steroid derivative
133.03162	2936858	-0.62417	0.109963	37	'Psicofuranin'	$C_{11}H_{15}N_5O_5$: involved in synthesis of proteins, muscle contraction and intracellular signal transduction.
298.11424	1.97E+08	0.289112	-0.10763	38	'THTC'	$C_5H_8O_2S$: Carboxylic acid used for building amino acid chains.
208.13306	18276069	-0.51322	0.077064	40	'3-(Dimethylamino)propyl benzoate / Fenobucarb / Promecarb'	$C_{12}H_{17}NO_2$: ester plant derivative, antimicrobial properties / carbamate insecticide (reversibly inactivating the enzyme acetylcholinesterase).
553.45863	30854953	0.147701	-0.00155	50	'Epoxymurin-A'	$C_{35}H_{62}O_3$: annonaceous acetogenin, waxy derivatives of fatty acids, nutrient.

In figure 3a the canonical variate for the interaction effect is presented. Peaks with a weight outside of the black dotted cutoff lines were flagged as important. Subsequently, the remaining peaks were ranked based on their absolute weight. Table 5 presents the top 10 ranked peaks for the interaction effect.

(a) (b)

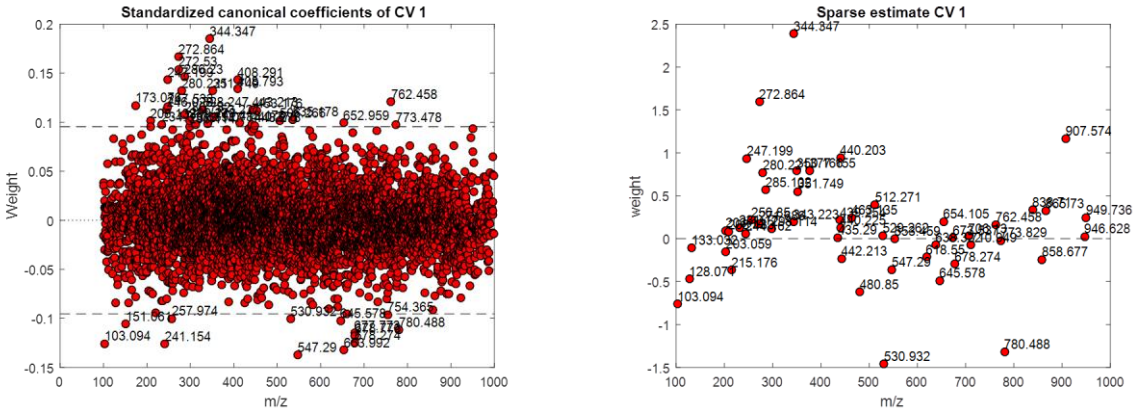


Figure v.iii. Variable importance for interaction population x site estimated by (a) rMANOVA, and (b) MGSDA. The canonical variate for the interaction effect is presented. Peaks with a weight outside of the black dotted cutoff lines were flagged as important. Subsequently, the remaining peaks were ranked based on their absolute weight. Table 5 presents the top 10 ranked peaks for the interaction effect.

vi. Body index analysis

iv. Analysis of the body lengths, length/mass (body index), and mortality for all the shrimp used in laboratory work confirming that there was no significant difference between the superficial body conditions of the two populations.

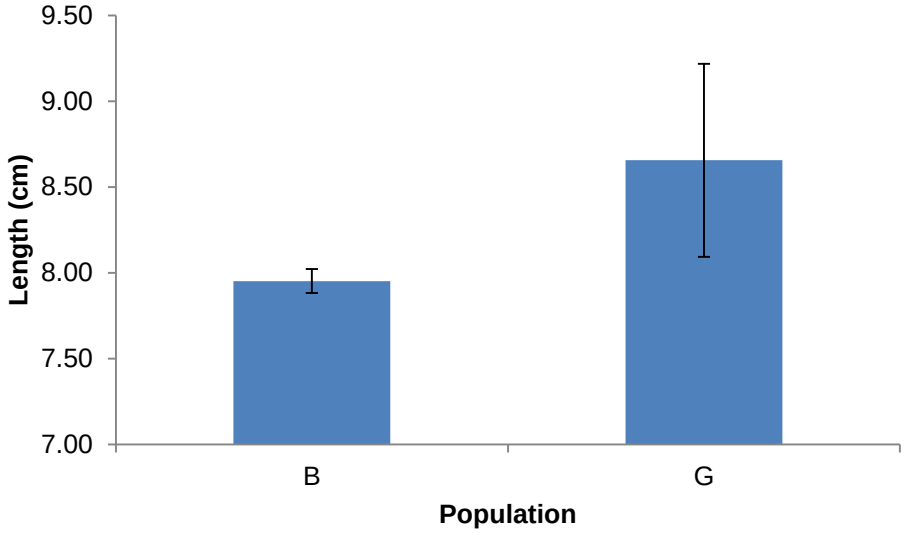


Figure v.i. Body length from base of the eye stalk to the end of the telson (cm, mean ± s.e.) of *Pandalus borealis* collected from Brofjorden (B, n = 140) and Gullmarfjord (G, n =142) that were used for laboratory study. Welch two sample t-test, 145.49 df, p > 0.05.

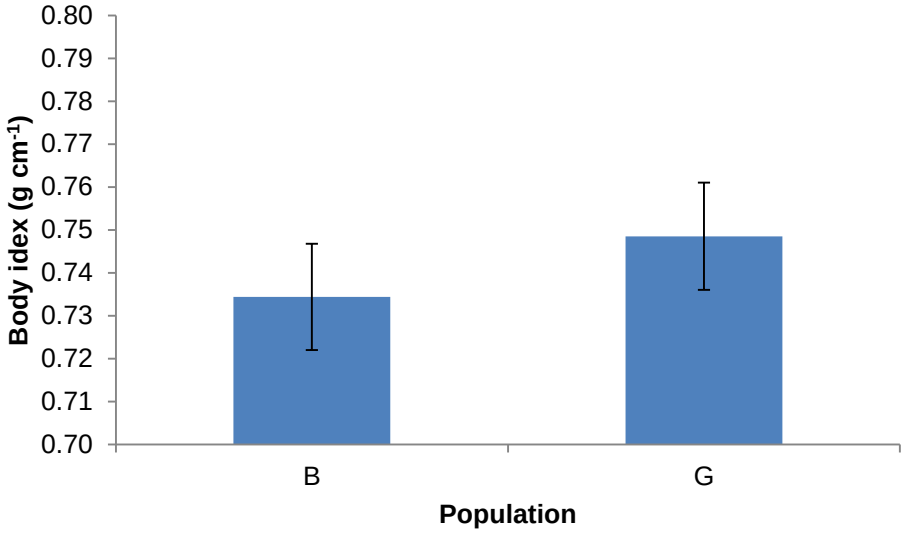


Figure v.ii. Body index (mass per unit length, mean ± s.e.) of *Pandalus borealis* collected from Brofjorden (B, n = 140) and Gullmarfjord (G, n =142) that were used for laboratory study. Two sample t-test, 280 df, p > 0.05.

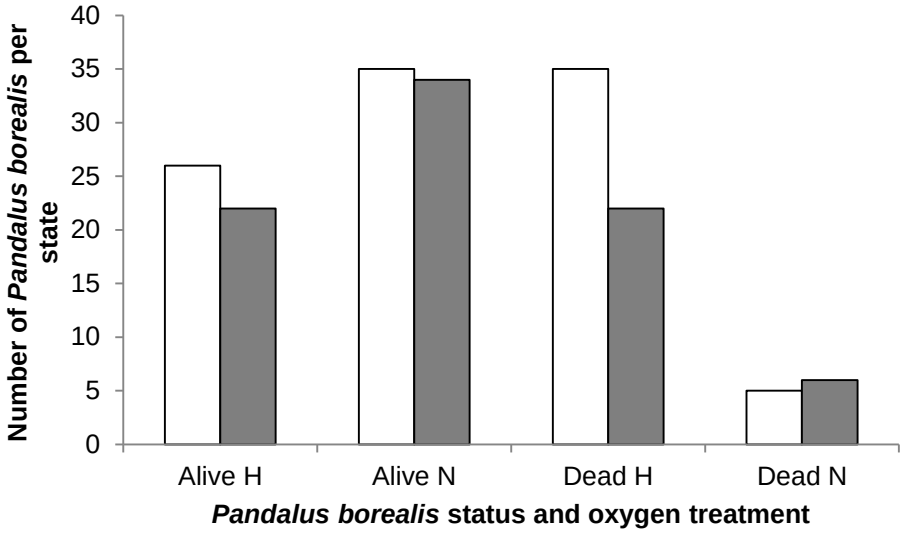


Figure v.iii Survivorship of *Pandalus borealis* of *Pandalus borealis* collected from Brofjorden (white bars, n = 140) and Gullmarfjord (grey bars, n =142) that were used for laboratory study. Differences in survivorship were not significant, $\chi^2 = 1.86$, $p > 0.005$.

vii. Photographs of *Pandalus borealis* morphological comparison

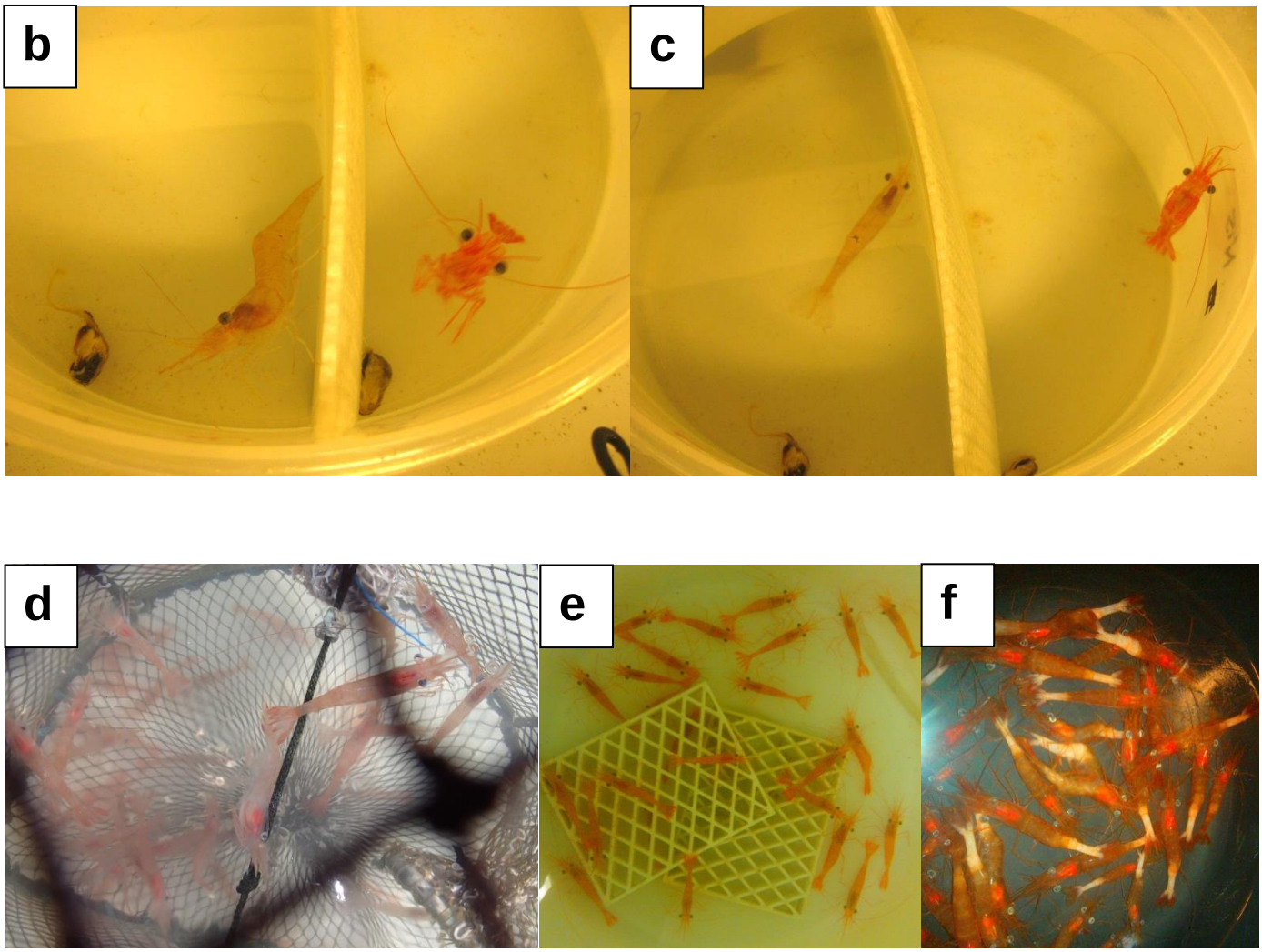


Figure vii. Shrimp pictured on the left side of the barrier in images a-c and in the cage in image d were collected in Brofjorden. The more pigmented shrimp pictured on the right in images a-c and pictured in images e and f were collected in Gullmarfjord. Shrimp in image f had sustained some injuries post-trawl, but were included due to the lack of clarity in other available images.

viii. Salinity experiment

v. Preliminary trials of the translocation experiment in an adjacent fjord to Gullmarfjord resulted in a mass die off of the shrimp. I was hypothesised that this was due to a low salinity event. The was a small difference in the salinity levels of the sites ultimately used for the translocation experiment as described in Chapter 4. To ensure that this would not confound the results, a short mesocosm-based experiment was conducted, exposing shrimp to 26, 27, and 28 psu under normoxic and hypoxic conditions. 27 psu was found to represent the tolerance threshold for shrimp exposed for one week. This was lower than the level in the newly selected field site, so we proceeded with the translocation experiment.

Preliminary experiments

A series of 24 h trials were used to ascertain an appropriate salinity range and gas mix for the mesocosm. Initially single shrimp were kept under normoxic conditions at field temperature at a

series of salinity concentrations. 100 % mortality was observed at salinities below 25 under normoxic conditions. Trials were then run using the mesocosm as described in Chapter 3 at 45 % a.s. $O_{2.}$ 100 % mortality was observed below salinity 27 psu within 24 h. Ultimately, 60 % a.s. O_2 levels, reflecting the conditions that season in Havstensfjord where the hypoxic treatment animals exposed in Chapter 3, and 26, 27 and 28 psu were selected as the final mesocosm parameters.

Experimental design for mesocosm salinity experiment

The shrimp were haphazardly allocated, two *per* tank, one from each population, across 30 closed-system tanks (5 L volume) filled with 4.5 L of aerated deep fjord sea water. The shrimp were separated by a perforated plastic barrier to aid identification and prevent aggression or competition for food. Animals were fed *ad libitum* throughout the experimental period on blue mussel *Mytilus edulis* and chopped herring (*Clupea harengus)*. Residual food was regularly removed to prevent water fouling. Nitrogen and air were mafipulated through the water to achieve normoxic or 60 % a.s. hypoxic conditions to mirror field conditions. We recorded the body condition of shrimp at salinity 26 (n 7), 27 (n 26) and 28 (n 30) daily for one week. All treatments were run at ambient field temperature (8.11 ± 0.02 °C).

A 100 % water change was carried out every 48 h to maintain salinity levels and prevent fouling. Consistent gas mixing was achieved by bubbling nitrogen gas (1-3 ± 0.4 L min^{-1}, depending on the experiment, manipulated using flow meter (Omega FLD 3000 series, Manchester, UK) mixed with 5 ± 0.4 L min^{-1} normal air through the treatment tanks. Percentage air saturation levels of dissolved oxygen and water temperature were monitored throughout the system set up and experiments using an optical oxygen probe (Fibox 4, PreSens Precision Sensing GmbH, Regensburg, Germany) connected to an oxygen meter calibrated daily. pH and salinity were measured daily across the mesocosm using a pH meter (Seven Go SG2) and a hand held refractometer (H2Ocean Precision Instrument Refractometer, DD The Aquarium Solution, Ilford, UK) respectively. Nutrient levels (API Aquarium Pharmaceuticals Saltwater Master Test Kit, Chalfont, USA) were measure three times *per* week.

A rapid visual assessment of body condition was conducted after twelve hours and daily thereafter for each shrimp. Body condition was broadly divided into four categories and recorded. Any individuals exhibiting no signs of tissue deterioration or impaired movement were recorded as being in “normal” body condition. Tissue deterioration generally began either at the tail segment closest to the telson or at sites of carapace damage. The celephalothorax was without exception the final segment to undergo clear deterioration. Those individuals undergoing tissue deterioration – here assumed to be apoptosis – were grouped into two categories based on the number of body segments affected; “partially apoptotic” individuals had three or fewer segments affected, “fully

apoptotic" individuals were those with minimum four body segments undergoing cell death, but still with beating gills. The final category was "dead", established when gill beating ceased.

Statistical analyses

A two-way ANOVA was run to analyse the differences in numbers of shrimp from each population that exhibited either normal body condition or deterioration in conjunction with low salinity exposure.

Results

Pandalus borealis experiences a significant decline in body condition and survivorship from salinity 28 to 26 regardless of oxygen availability (see Table 1 and Figures 1 and 2). The results of the preliminary investigations indicate that reduced oxygen availability beyond a certain threshold does interact deleteriously with hyposalinity; shrimp exposed to 27 psu at 45 % a.s. O_2 all died within 24 h. At 28 and 27 psu at 60 % a.s., however, body condition and survivorship were better under hypoxia than normoxia.

Table 2 Results of ANOVA test the effect of hypoxia and reduced salinity on four categories of body condition in *Pandalus borealis*. df: degrees of freedom, MS: mean of square, F: F-ratio, p: probability level

Trait	Source	df	MS	F	p
Body condition	Salinity levels	1	11929.6	42.918	< 0.001
	Population origin	1	313.2	1.127	< 0.05

Shrimp body condition deteriorated at salinity concentrations below 28 but when kept at 28 they suffer little if any irreversible damage regardless of oxygen treatment. Differences in body condition and mortality between the three salinity concentrations was highly significant (see Figures 1 and 2 and Table 1 salinity levels $p < 0.001$), suggesting that 28 represents a salinity tolerance threshold. Populations differed significantly in vulnerability to low salinity levels. Brofjorden individuals exposed to 60 % a.s. O_2 appeared to tolerate low salinity levels better than any other group including their counterparts under fully aerated conditions (see Figures 1 and 2).

Further investigation revealed that neither oxygen levels nor any interactions between oxygen levels, population origin nor length of exposure were statistically significant in explaining body condition.

1.

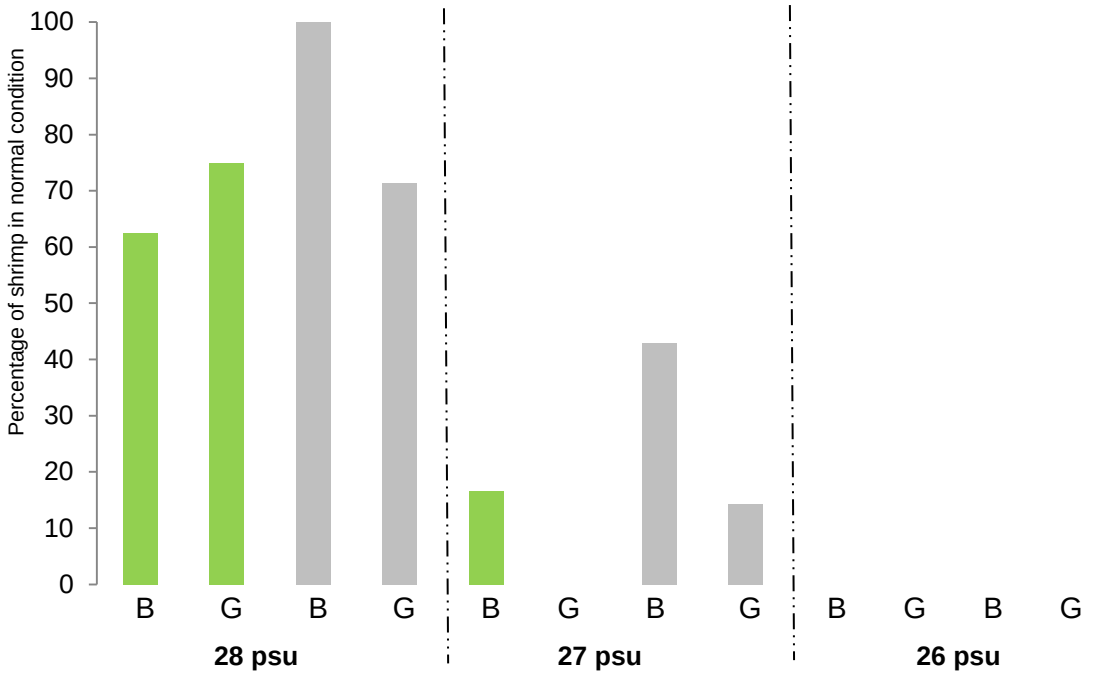


Figure 1 Percentage of *Pandalus borealis* in normal body condition after 144 h of exposure to salinity 28**,** 27 and 26 psu under normoxic and hypoxic conditions. The population origin of each treatment group in indicated under each histogram; "B" for Brofjorden individuals, "G" for Gullmarsfjord. *P. borealis* were exposed to normoxic (93.146 ± 0.258 % air saturation O_2 : green bars) and hypoxic (46.030 ± 0.523 % air saturation O_2: grey bars) oxygen concentrations.

ix. A review of common garden and translocation population comparison studies investigating differential responses to environmental stressors.

Table ix. Studies that have identified either physiological differentiation or local adaptation using common garden (CG) or translocation (TE) experimental techniques. Drivers: temperature (T), ocean acidification (OA), salinity (S), hypoxia (H), pollution (P), habitat characteristics known to contribute towards natural selection (NS – including, but not limited to predation, nutrient level, light exposure, disturbance, wave action and desiccation), and unknown (U). Outcomes: local adaptation (LA), phenotypic plasticity (PP)

Taxon	Experimental approach	Driver	Response variable	Outcome	Authors
Actinopterygii					
Danio rerio	CG	T, NS	Growth, thermal tolerance	PP	Schaefer & Ryan 2006
Lepomis macrochirus	CG	NS	Morphology	PP, LA	Robinson & Wilson 1996
Poecilia reticulata	CG	NS	Growth, morphology – head characteristics, size at maturity	PP	Torres-Dowdall et al. 2012

Pseudocrenilabrus multicolor victoriae	CG	H	Hematocrit and enzyme activity	LA	Martinez et al. 2009
Amphibia					
Rana sylvatica	CG	NS	Behaviour, life history traits, morphology, growth	PP, LA in one of eight population studied	Relyea 2002
Amphipoda					
Eogammarus confervicolus	CG	NS	Habitat selection	LA	Stanhope et al. 1992
Ampithoe longimana	CG	NS; NS	Growth, survival, reproduction; growth, survival, reproduction	LA	Sotka & Hay 2002; Sotka et al. 2003
Paracalliope novizealandiae	CG	NS	Immuno-competence	LA	Bryan-Walker et al. 2007
Anthozoa					
Acropora millepora	CG	T	Coral bleaching	LA	Smith-Keune & van Oppen 2006 ; Smith 2005
Metridium senile	CG	T	Respiration, enzyme activities	LA	Walsh & Somero 1981
Pocillopora damicornis	CG	T	Zooxanthellae density (coral bleaching), polyp behaviour	LA or acclimation PP; LA	D'Croz & Maté 2004; Ulstrup et al. 2006
Bivalvia					
Crassostrea virginica	CG	T	Growth, ciliary activity	LA	Dittman 1997, Dittman et al. 1998
Mytilus edulis	CG	P; NS	Survival, reproduction; morphology – shell thickness	LA	Hoare et al. 1995 Freeman & Byers 2006
Macoma balthica	CG	NS	Morphology – gill : palp mass ratio	PP	Drent et al. 2004
Bryozoa					
Bugula neritina	CG	P	Growth, survival	LA	Piola & Johnston 2006
Copepoda					
Acartia hudsonica	CG	NS	Growth, survival, reproduction	LA	Colin & Dam 2004
Acartia tonsa	CG	H	Habitat selection	LA	Decker et al. 2003
Eurytemora affinis	CG	S	Growth, survival	LA	Lee & Petersen 2006
Scottolana canadensis	CG	T	Morphology – body size, growth, development rate	LA	Lonsdale & Levinton 1985
Tigriopus californicus	CG	S, T; T	Reproduction, life history; thermal tolerance	LA	Dybdahl 1989
Dictyledonae					
Cynoglossum officinale	CG	NS	Morphology – size, life history trait – fecundity, flowering phenology	PP	Williams et al. 2008
Gastropoda					

Elysia viridis	CG	NS	Growth	LA	Trowbridge & Todd 2001
Littorina obtusata	CG	T; NS	Enzyme activity; growth	LA	Sokolova & Portner 2001; Trussell 2002
Littorina saxatilis	CG	P; NS; NS	Survival; growth; morphology, habitat selection; enzyme activity	LA	Daka & Hawkins 2004; Janson 1982; Johannesson & Johannesson 1996; Sokolova & Portner 2001
Littorina subrotundata	CG	S, NS	Salinity and dessication tolerance, survival	LA	Sokolova & Boulding 2004
Monetaria annulus	CG	U	Morphology – body size	PP	Irie & Morimoto 2008
Nucella canaliculata	CG	NS; T	Behaviour – feeding ability; heat tolerance, survival	LA	Sanford & Worth 2009; Kuo & Sanford 2009
Nucella lapillus	CG	NS	Behaviour – foraging activity	LA	Hughes & Taylor 1997
Nucella ostrina	CG	T	Development rates	LA	Palmer 1994
Isopoda					
Idotea balthica	CG	NS	Growth, survival, reproduction; growth, reproduction	LA	Vesakoski et al. 2009; Jormalainen et al. 2008
Polychaeta					
Nereis diversicolor	CG	T; P	Survival; survival	LA	Bryan & Hummerstone 1971; Grant et al. 1989
Spirorbis borealis	CG	NS	Habitat selection	LA	MacKay & Doyle 1978
Anthozoa					
Porites lobata	TE	NS; T	Growth, calcification; Growth, stress proteins	PP; LA	Smith et al. 2007; Barshis et al. 2010 ; Smith et al. 2007
Porites sillimaniani	TE	NS	Morphology	PP	Muko et al. 2000
Bivalvia					
Cerastoderma edule	TE	NS	Growth	PP, LA	De Montaudouin 1996
Cirripedia					
Semibalanus balanoides	TE	T	Survival; genetic polymorphism	LA	Bertness & Gaines 1993; Schmidt et. al 2011
Gastropoda					
Bembicium vittatum	TE	U	Growth	LA	Parsons 1998
Littorina obtusata	TE	T, NS	Growth	LA	Trussell 2000
Littorina picta (Nodilittorina hawaiiensis)	TE	NS	Survival	LA	Struhsaker 1968
Nucella	TE				

canaliculata					
Nucella lapillus	TE	NS	Morphology, survival	LA	Kitching et al. 1966
		NS	Growth, survival	LA	Sanford & Worth 2010
Magnoliopsida					
Suaeda maritima	TE	H	Metabolite levels, protein expression	PP	Weston et al. 2012
Liliopsida					
Zostera marina	TE	NS	Growth	LA	Hämmerli & Reusch 2002

x. Shellfish sensory quality paper

A paper produced with collaborations established during my field season.

Can't stomach ocean acidification – First evidence of altered sensory quality in a shellfish exposed to decreased pH

Sam Dupont[1,*], Emilie Hall[2], Piero Calosi[2,3] and Bengt Lundve[4]

[1]Department of Biological and Environmental Sciences, University of Gothenburg, Fiskebäckskil, 45178, Sweden

[2]School of Marine Science and Engineering, Plymouth University, Plymouth, PL4 8AA, United Kingdom

[3]Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, Rimouski, QC G5L 3A1, Canada

[4]The Sven Lovén Centre for Marine Sciences – Kristineberg, University of Gothenburg, Fiskebäckskil, 45178, Sweden

*Corresponding author. Email: sam.dupont@bioenv.gu.se

Running title: Ocean acidification alters shellfish sensory quality

Abstract

Understanding how seafood will be influenced by coming environmental changes such as ocean acidification is a research priority. One major gap in knowledge relates to the fact that many experiments are not considering relevant endpoints directly related to production (e.g. survival) and product quality (e.g. sensory quality) which can have important repercussion on the consumers and the fish market. The aim of this experiment was to compare the survival and sensory quality (appearance, texture and taste) of the adult northern shrimp (*Pandalus borealis*) exposed for three weeks to a temperature at the extreme of its thermal tolerance (11 ºC) and two pH treatments: pH 8.0, the present average pH at the sampling site and, pH 7.5, which is out of the present natural variability and relevant for near-future ocean acidification. Our results showed that decreased pH significantly increased mortality by 63 %. Sensory quality was assessed through a semi-qualitative scoring by a panel of local connoisseurs. Decreased pH significantly reduced the score for *appearance* and *taste* but not *texture*. As a consequence, shrimps maintained in pH 8.0 had a 3.4 times increased probability to be scored as the best shrimp on the plate while shrimps from the pH 7.5 treatment had 2.6 times more chance to be scored as the least desirable shrimp on the plate. These results are proving the concept that ocean acidification can modulate sensory quality of the northern shrimp *P. borealis*. More research is now needed to evaluate impacts on other seafood species, socio-economic consequences and potential options.

Key words: pH, acidification, taste, texture, appearance, northern shrimp, *Pandalus borealis*

Introduction

The Ocean contributes significantly to society. Dependence on marine protein is expected to continue to rise with the increasing human population, as world capture fisheries and aquaculture are estimated to provide food to 4.3 billion people with at least 15% of their animal protein. Over the last 30 years world food production by aquaculture has expanded 12-fold; nearly half of the human seafood production. Understanding how seafood production and quality will be influenced by coming environmental changes is critical. The economic consequences of a collapsing fishery and aquaculture industry would be dire indeed.

Ocean acidification (OA) is a rapid and unprecedented increase in ocean acidity. Intensive fossil-fuel burning and deforestation over the last two centuries has increased atmospheric CO_2 to almost 40 % above preindustrial values. The global ocean currently absorbs around 30 % of released anthropogenic CO_2, fundamentally altering ocean chemistry by acidifying sea water. It is predicted that average oceanic pH will decrease by 0.4 pH units by 2100, a rate of change 100 times faster than anything seen in the past hundreds of millennia (Caldeira & Wickett 2003). Together with other stressors such as global warming, it challenges marine species and ecosystems (Wittmann & Pörtner 2013; Dupont & Pörtner 2013). Despite a growing body of evidence demonstrating that OA is a major threat for seafood security (availability of sustainable and good quality seafood), and recommendation that species and endpoints with direct economic importance should be prioritized (Hilmi et al. 2014), consequences for seafood remain unclear. One exception is the impact of OA on oyster production in hatcheries located on the Northern west coast of the US, a US$ 270 million industry. A significant decline in the survival of oyster larvae since 2005 appeared to be connected to near-shore OA (Barton et al. 2010).

One major gap in knowledge relates to the fact that many experiments are not considering relevant endpoints. To make any projection on future impacts of OA and other environmental challenges on seafood, it is critical to consider parameters that are directly related to production (e.g. survival) and quality (e.g. sensory quality). The aim of this experiment was to test on the northern shrimp (*Pandalus borealis*) the impact on survival and sensory quality of three weeks exposure to a temperature at the extreme of its thermal tolerance (11 ºC) and pH conditions within (pH 8.0) and outside (pH 7.5) of the present range of pH variability.

The northern shrimp is a keystone species in the marine ecosystem of the continental shelves in the North-Atlantic and is an important prey for demersal fish species, most notably cod. It also has substantial economic (e.g. 100 million euros for Norway alone; Søvik & Thangstad 2013) as well as high cultural value as seafood for the public. Shrimps are famous for their sensory appeal such as their appearance, texture and flavor.

The future of shrimp as a harvested resource is unsure (Wieland et al. 2012). Shrimp landings have declined in the Northwest-Atlantic. The decline may be caused by recruitment failure caused by mismatch between timing of phytoplankton bloom and hatching of larvae (Koeller et al. 2009), as well as by increased predator abundance. Previous work on *P. borealis* demonstrated that the species is relatively tolerant to OA (Arnberg et al. 2013; Bechmann et al. 2011; Hammer and Pedersen 2013). Decreased pH (ΔpH = 0.5) induced a delay in development but had no impact on mortality (Bechmann et al. 2011; Arnberg et al. 2013). As adult, the northern shrimp was able to compensate for extreme acidification (ΔpH = 1.2) for up to 16 days and without any documented consequence for mortality (Hammer & Pedersen 2013). All these studies were performed at temperature ranging between 5 and 9.5ºC. *Pandalus borealis* are mostly found at low temperatures (0–5ºC), but show a wide temperature range as adults and can live at temperatures as high as 12 ºC which is experienced in the North Sea (Allen 1959; Bergstrom 2003). Organisms specialize within certain temperature ranges and are sensitive to extremes. It can be predicted that exposure to temperature at the limit of the thermal tolerance will enhance sensitivity to stress including OA (Pörtner & Farrell 2008; Dupont & Pörtner 2013). It is also well established that environmental factors causing stress can affect the quality of meat. For example, stressed fish can have a metallic aftertaste (Borderıas & Sanchez-Alonso 2011). As a consequence, our hypothesis was that OA will increase shrimps stress level with negative consequences for their survival and sensory eating quality.

Materials and Methods

Animal collection

Adult females of the Northern shrimp (*Pandalus borealis*) were sampled on the 24th and 25th April and the 13th May from central Gullmarsfjord (58°20'26.1"N 11°33'31.7"E) and close to Brofjorden (58°18'12.4"N 11°17'59.7"E) using an otter trawl at 85 – 110 m for 30 – 60 min. As soon as they were on board, the shrimp were transferred to chilled, aerated tanks for transportation to minimise exposure to thermal fluctuations. At The Sven Lovén Centre for Marine Sciences – Kristineberg, they were kept in

basins with flowing deep sea water (temperature 8 °C, salinity 33) until the beginning of the experiment (11th June) and were fed *ad libitum* on chopped herring (*Clupea harengus)*, blue mussel (*Mytilus edulis)*, *Artemia nauplii* and Marine Flake (New Era®, Thorne, UK). Residual food was regularly removed to avoid contaminations.

Experimental design and seawater chemistry

Shrimps were maintained in 4 x 100 L basins (two replicated basins *per* pH treatment; 42 to 57 shrimps *per* basin) with natural flowing seawater with a replacement of 1 L min^{-1}. They were kept at 11 ºC, salinity 33 and fed *ad libitum* with chopped herring every 4th day. Number of living shrimps was noted every 4th day and dead individuals were removed. pH was maintained in each experimental basin using a computerized feedback system (AquaMedic) that regulated pH by addition of pure gaseous CO_2 directly into the sea water. pH was measured twice a week on the total scale (pH_T) after calibration using TRIS (Tris/HCl) and AMP (2-aminopyridine/HCl) buffer solutions (provided by Unité d'Océanographie Chimique, Université de Liège, Belgium) and used for adjustment of the pH-system settings. Total alkalinity (A_T) was assessed once a week on filtered samples (0.2 μm) by titration (TitroLine Alpha Plus, SI Analytics). pCO_2 was calculated from pH_T and A_T using CO2SYS (Lewis and Wallace 1998) with dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Two nominal pH treatments were compared: (i) pH 8.0, the present average pH and, (ii) pH 7.5, outside of the present range of pH variability and relevant scenario for near-future ocean acidification (Dorey et al. 2013). pH and pCO_2 were significantly different between treatments with no significant difference between the two replicates (Table 1).

Table 1 – Seawater chemistry in the two pH treatments (measured pH_T and calculated pCO_2 from pH_T and A_T = 2260 ± 56).

Nominal pH		**ANOVA 2**		
8.0	**7.5**	**Model**	**pH**	**Replicate**

				treatment	
			$F_{3,79}$ = 3484	F=10452	F=0.24
pH_T	7.99 ± 0.01	7.55 ± 0.01			
			p < 0.0001	p<0.0001	p=0.78
***p*CO_2**			$F_{3,79}$=3864	F=10902	F=0.20
	459 ± 5	1368 ± 7			
(μatm)			p<0.0001	p<0.0001	p=0.82

Sensory evaluation

After three weeks of exposure, shrimps were sampled and prepared for the tasting assessment. More than 30 shrimps were collected from each basin and prepared under the supervision of a professional chef (Kirsten Johannsen). Shrimps from each basin were cooked by immersion for 3 min into 2 L of boiling water made from the same stock of surface sea water with 60 g of sea? salt *per* L (as per local industry standard). Shrimps were then rinsed in cold surface sea water and cooled down at room temperature for 1 h. Thirty plates were prepared with shrimp from the four basins randomly assigned to four different positions on each plate (#1 to #4) so tasters could not influence each other. Shrimp lengths varied between 8.6 and 13.9 cm and were visually selected to minimize size difference within each plate (maximum of 11.6 % size variation).

A panel of 30 voluntary local voluntary connoisseurs was gathered at the Sven Lovén Centre for Marine Sciences – Kristineberg (Sex ratio 1:1; age: 42 ± 2 years old (between 24 and 56)). All of them confirmed liking shrimp and eat shrimp 1.76 ± 0.21 times a month (between four times a month and four times a year). The sensory evaluation was blind and tasters were not informed of the design of the experiment. Each received a plate and was asked to score the 4 shrimps (1=bad to 5=excellent) based on 3 criteria: appearance, texture and taste. They were also asked if they would be willing to pay more for better tasting shrimp.

Statistical analyses

Relative mortality rate expressed in % day^{-1} was calculated for each replicate and pH treatment as the coefficient of the significant linear relationship between relative density (%) and time (day). Differences between treatments and replicates were tested using an ANCOVA model.

ANOVA 2 models were used to test differences between pH treatments and replicates for the sensory evaluation parameters (appearance, texture, taste). These parameters were not based on a standardized scale. As a consequence, differences in scoring between tasters can be expected. To account for these differences, shrimps on every plate were ranked based on the sum of scores for appearance, texture and taste between 1 (best shrimp, highest score) and 4 (worst shrimp, lowest score). For this ranking, difference between pH treatments and replicates were tested using chi-square statistics.

All statistical analyses were performed using the SAS/STAT software. The normality of data distributions was checked with a Shapiro-Wilk test and the homoscedasticity was tested using the Bartlett test. Each mean value is expressed with its standard error of mean (mean ± SEM). The significance level applied was 5%.

Results

Relative mortality rate was on average 2.05 ± 0.23 % day^{-1} at pH 7.5 and 1.27 ± 0.24 % day^{-1} at pH *.0, this difference being statistically significant (ANCOVA, model: $F_{1,15} = 16.9$, $p < 0.0001$, pH: $F = 5.32$, $p = 0.042$). There was no significant differences between replicates ($F=0.08$, $p=0.79$) or interactions between pH and replicates ($F=0.01$, $p=0.97$).

On average, shrimps from the pH 8.0 treatment had significantly higher scores for *appearance* and *taste* compared to shrimps from the pH 7.5 treatment, with no significant differences between replicates or interactions between replicates and pH treatments. No significant difference was observed for the *texture* (see Table 3 for statistics; Figure 1).

Figure 1 – Average scores of the 3 sensory evaluation parameters (appearance, texture, taste) in the two pH treatments.

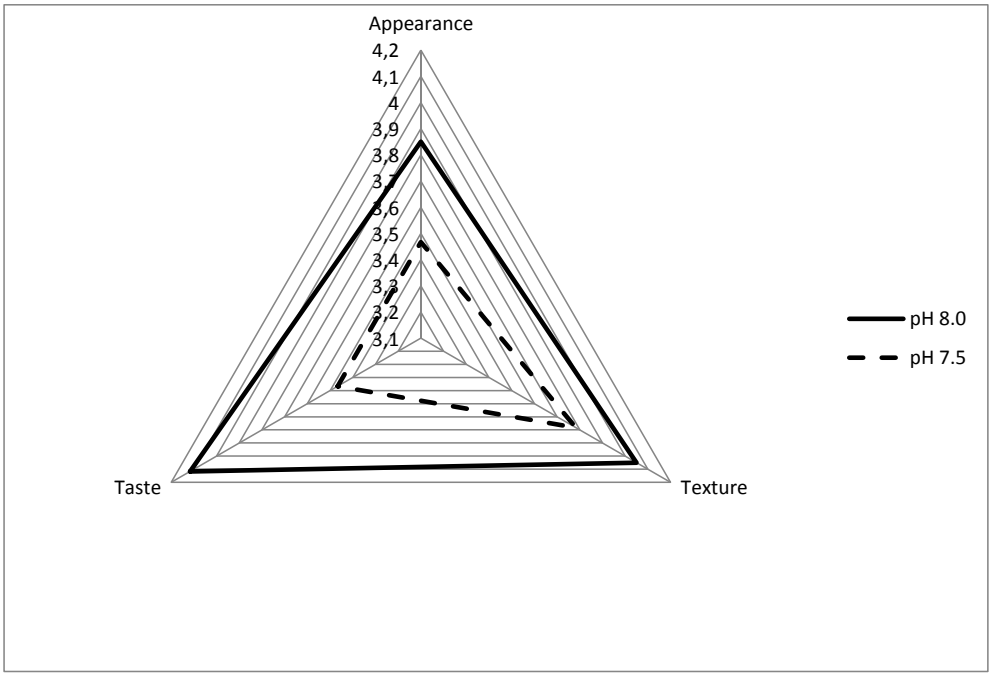
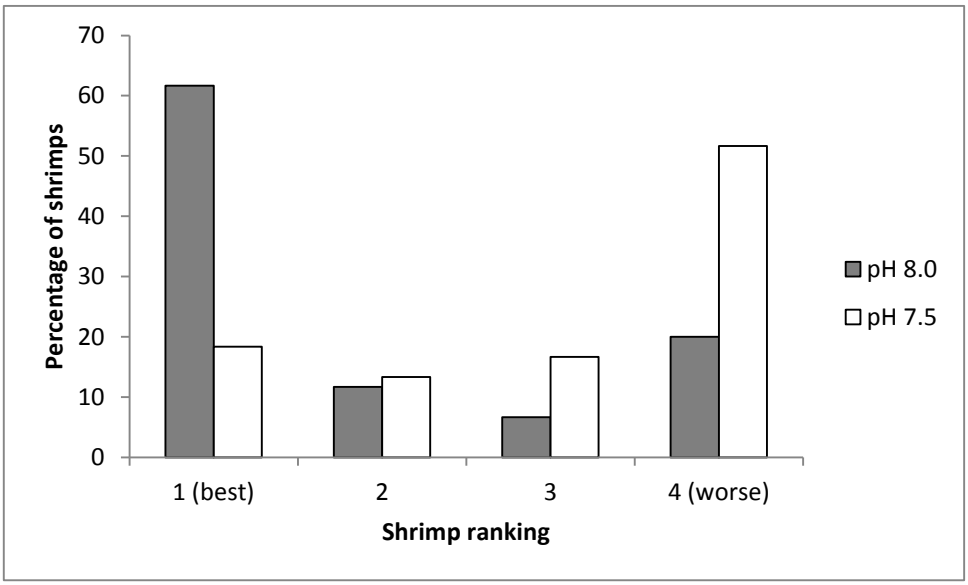


Table 3 – Statistical analyses of the sensory evaluation parameters using an ANOVA 2 model.

	Model	pH treatment	Replicate	pHxreplicate
Appearance	$F_{3,119}$=2.85	F=4.67	F=0.11	F=0.72
	p=0.042	p=0.03	p=0.11	p=0.40
Texture	$F_{3,119}$=1.63	/	/	/
	p=0.19			
Taste	$F_{3,119}$=6.08	F=17.05	F=0.91	F=0.28
	p=0.0007	p<0.0001	p=0.34	p=0.60

Significant differences were observed between pH treatments for the ranking of the shrimp on each plate (χ^2=25.12, p<0.0001), with no differences between replicates within each tested pH (pH 8.1: χ^2=6.33, p=0.10; pH 7.5: χ^2=4.71, p=0.19). Shrimps from the pH 8.0 treatment had 3.4 times more chance to be scored as the best shrimp of the plate (rank 1) while the shrimps from the pH 7.5 treatment had 2.6 times more chance to be scored as the least desirable shrimp on the plate (rank 4; Figure 2).

Figure 2 – Percentage of shrimp ranking from best (1) to worse (4) on individual plates in both pH treatments



All but one of the 30 voluntary tasters claimed that they would be willing to pay more for better quality shrimps.

Discussion

Our results demonstrate that a three week exposure to decreased pH relevant in the context of OA (ΔpH=0.5) can lead to a 1.6 times increase in adult shrimp mortality. This is somewhat contrasting with the conclusions of Hammer & Pedersen (2013) showing tolerance toward extreme acidosis (ΔpH=1.2) in the same species. The main difference between the two studies is the temperature used. Hammer & Pedersen (2013) made their experiment at an optimal temperature (7 ºC) while we used a temperature at the extreme of their thermal tolerance (11 ºC). Response to OA can be highly dependent upon thermal conditions. Pörtner & Farrell (2008) developed a theoretical framework to predict the combined impact of temperature and OA. All organisms live within a limited range of body temperatures, due to optimized structural and kinetic coordination of molecular, cellular, and systemic processes, and functional constraints result at temperature extremes. It is hypothesized that additional stressors like OA have the potential to narrow these thermal windows. This theoretical framework highlights the fact that response to ocean acidification can be highly dependent upon thermal conditions. For example, temperature modulated the impact of decreasing

pH on sea urchin larvae leading to a positive effect of low pH temperature, a neutral effect at optimal temperature and a negative effect at high temperature (Gianguzza et al. 2014). Our results on *P. borealis* confirm the importance of considering a range of temperatures covering today and the future environmental variability in any experiment aiming at studying the impact of OA.

The exposure to decreased pH also negatively impacted the sensory quality of the exposed shrimps with a significantly lower score for appearance and taste. This led to a 3.4 times increased probability to be scored as the best shrimp of the plate for the ones cultured in pH 8.0 while the shrimp from the pH 7.5 treatment had 2.6 times more chance to be scored as the worst shrimp on the plate. These results should be considered as a proof-of-concept that OA can have an impact on seafood sensory quality that can be detected by local connoisseurs rather than an attempt to make any prediction for future shrimp quality. First, our experimental design is only considering short term exposure (three weeks) and simplistic scenarios (two stable pH treatments). More realistic perturbation experiments should consider longer exposure time (ideally across life-history stages and through multiple generations), take into account natural variability in pH and other relevant local environmental parameters, including food quality and quantity, allow for adaptation and acclimation, etc. (e.g. Hilmi et al. 2013, Sunday et al. 2014, Gaylor et al. 2014). Other seafood species should also be considered, as species- and phyla-specific sensitivities have been shown to be extremely different (Melzner et al. 2009, Christen et al. 2013, Wittman and Poertner 2013). Secondly, sensory quality is relative to consumer preference and this parameter can vary between regions and evolve through time. Finally, other assessment methods (e.g. trained panelist, electronic nose, Quantitative Descriptive Analysis; e.g. Mejholm et al. 2005, Zeng et al. 2005 for *P. borealis*) and endpoints (e.g. stress markets, lipid content) should be considered. Research should also focus on potential options including consequences of different aquaculture practices and selection of strains resilient to OA on sensory quality should be investigated. More work is needed to evaluate the impact of OA on seafood sensory quality and potential economic consequences. For example, taste is one of the key factors influencing consumer behavior (Shyam 2012). This is consistent with the 97% of our panel list willingness to pay more for better quality shrimp.

The fact that OA can have a detectable impact on shrimp sensory quality can be a unique opportunity to efficiently communicate about global changes. In Scandinavian countries, the *Pandalus* shrimp is extremely popular and an integral part of local folklore and culture. It is then an ideal model to attract citizen's interest ("*Can you imagine Scandinavia without shrimp sandwich?*", "*Can you taste climate change?*") and initiate the discussion about future threats on their favorite seafood and potential actions (e.g. reduction of CO_2 emissions) to engage them also on the broader marine biodiversity crisis we may be facing.

Acknowledgements

Authors are grateful to the chef Kirsten Johannsen who provided her professional expertise and help for the cooking the shrimps; many thanks to Manuela Truebano and Lotti Alvord for their invaluable help during shrimp collection and husbandry. Shrimp collection was funded by the ASSEMBLE, KVA and TOTAL Foundation grants. SD is funded by was financially supported by the Linnaeus Centre for Marine Evolutionary Biology at the University of Gothenburg (http://www.cemeb.science.gu.se/) and a Linnaeus grant from the Swedish Research Councils VR and Formas.

Literature cited

Allen, J. A. 1959. On the biology of *Pandalus borealis* Kroyer, with reference to a population off the Northumberland coast. J. Mar. Biol. Assoc. UK. 38:189-220.

Arnberg, M., P. Calosi, J. I. Spicer, A. H. S. Tandberg, M. Nilsen, S. Westerlund & R. K. Bechmann. 2013. Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (*Pandalus borealis*) larvae. Mar. Biol. 160:2037-2048.

Barton, A., B. Hales, G. G. Waldbusser, C. Langdon & R. A. Feely. 2012. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. Limnol. Oceanogr., 57:698-710.

Bechmann, R. K., B. K. Larsen, I. C. Taban, L. I. Hellgren, P. Møller & S. Sanni. 2010. Chronic exposure of adults and embryos of *Pandalus borealis* to oil causes PAH accumulation, initiation of biomarker responses and an increase in larval mortality. Mar. Pollut. Bull. 60:2087-2098.

Bergstrom, B. 2000. The biology of Pandalus. Adv. Mar. Biol. 38:55–245.

Borderías, A. J. & I Sánchez-Alonso. 2011. First Processing Steps and the Quality of Wild and Farmed Fish. J. Food Sci. 76:R1-R5.

Caldeira, K. & M. E. Wickett. 2003. Anthropogenic carbon and ocean pH. Nature 425, 365.

Dickson, A.G. & F. J. Millero. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep. Sea Res. 34:1733–1743.

Gianguzza, P., G. Visconti, F. Gianguzza, S. Vizzini, S. Gianluca & S. Dupont. 2014. Temperature modulates the response of the thermophilous sea urchin *Arbacia lixula* early life stages to CO_2-driven acidification. Mar. Environ. Res. 93:70-77.

Dupont, S. & H. Pörtner (2013) Get ready for ocean acidification. Nature 498:429.

Gaylor, B., K. J. Kroeker, J. M. Sunday, K. M. Anderson, J. P. Barry, N. E. Brown, S. D. Connell, S. Dupont, K. E. Fabricius, J. M. Hall-Spencer, T. Klinger, M. Milazzo, P. L. Munday, B. D. Russell, E. Sanford, S. J. Schreiber, V. Thiyagarajan, M. L. H. Vaughan, S. Widdicombe & C. D. G. Harley. 2014. Ocean acidification through the lens of ecological theory. Ecology. DOI: 10.1890/14-0802.1.

Hammer, K.M. & S.A. Pedersen. 2013 Deep-water prawn *Pandalus borealis* displays a relatively high pH regulatory capacity in response to CO_2-induced acidosis. Mar. Ecol. Prog. Ser. 492:139-151.

Hilmi, N., D. Allemand, S. Dupont, A. Safa, G. Haraldsson, P. L. D. Nunes, C. Moore, C. Hattam, S. Reynaud, J. M. Hall-Spencer, M. Fine, C. Turley, R. Jeffree, J. Orr, P. L. Munday & S. R. Cooley. 2013. Towards improved socio-economic assessments of ocean acidification's impacts. Mar. Biol. 160:1773-1787.

Hilmi, N., D. Allemand, M. Cinar, S. Cooley, J. Hall-Spencer, G. Haraldsson, C. Hattam, J. A. Jeffree, J. C. Orr, K. Rehdanz, S. Reynaud, A. Safa & S. Dupont. 2014 Exposure of Mediterranean Countries to Ocean Acidification. Water. 6, doi:10.3390/w60x000x.

Koeller, P., C. Fuentes-Yaco, T. Platt, S. Sathyendranath, A. Richards, P. Ouellet, D. Orr, U. Skúladóttir, K. Wieland, L. Savard & M. Aschan. 2009. Basin-Scale Coherence in Phenology of Shrimps and Phytoplankton in the North Atlantic Ocean. Science. 324:791-793.

Lewis, E. & D. Wallace D. 1998. Program developed for CO_2 system calculations. ORNL/CDIAC. Oak Ridge, Tennessee: Carbon Dioxide Information Analysis Center.

Mehrbach, C., C. H. Culberson, J. E. Hawley & R. M. Pytkowicz. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18:897-907.

Mejlholm, O., N. Bøknæs & P. Dalgaard. 2005. Shelf life and safety aspects of chilled cooked and peeled shrimps (*Pandalus borealis*) in modified atmosphere packaging. J. App. Microbiol. 99:66–76.

Pörtner, H. O. & A. P. Farrell. 2008. Physiology and climate change. Science 322:690-692.

Shyam, S. S. 2013. Demand pattern and willingness to pay for high value fishes in India. J. Mar. Biol. Ass. India. 55:48-54.

Sunday, J. M., P. Calosi, S. Dupont, P. L. Munday, J. H. Stillman & T. B. H. Reusch. 2014. Evolution in an acidifying ocean. Trends Ecol. Evol. 29:117-125.

Søvik, G. & T. Thangstad. 2013. The Norwegian Fishery for Northern Shrimp (*Pandalus borealis*) in Skagerrak and the Norwegian Deep (ICES Divisions IIIa and IVa east), 1970-2013. NAFO SCR Doc.13/066. Pp 33.

Wieland, K., H. Siegstad, J. M. Casas Sanchez & D. Orr. 2012. Recent decline of northern shrimp stocks in the Northwest Atlantic – Coincidence, multiple causes or response to synchronous changes in the environment? The Ecosystem Studies of Sub-Arctic Seas (ESSAS) Program, Featured article (www.imr.no/essas).

Wittmann, A. & H. O. Pörtner HO. 2013. Sensitivities of extant animal taxa to ocean acidification. Nature Clim. Change. 3: 995-1001.

Zeng, Q. Z., K. A. Thorarinsdottir & G. Olafsdottir. 2005. Quality changes of Shrimp (*Pandalus borealis*) stored under different cooling conditions. J. Food Sci. 70:s459–s466.

xi. Evolution paper

A paper produced as a result of collaborations established during the CeMEB Marine Evolution under Climate Change course I attended in 2013.

Water **2014**, *6*, 1-x manuscripts; doi:10.3390/w60x000x

OPEN ACCESS

water

ISSN 2073-4441

www.mdpi.com/journal/water

Review

Evolution of Marine Organisms under Climate Change at Different Levels of Biological Organisation

Ben P. Harvey [1,*], Balsam Al-Janabi [2], Stefanie Broszeit [3,4], Rebekah Cioffi [5,6], Amit Kumar [7], Maria Aranguren-Gassis [8], Allison Bailey [9], Leon Green [10], Carina M. Gsottbauer [11], Emilie F. Hall [12], Maria Lechler [13], Francesco P. Mancuso [3], Camila O. Pereira [14], Elena Ricevuto [7], Julie B. Schram [15], Laura S. Stapp [16], Simon Stenberg [17] and Lindzai T. Santa Rosa [18]

1 Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, SY23 3DA, UK

2 GEOMAR, Helmholtz Centre for Ocean Research, Duesternbrookerweg 20, Kiel 24105, Germany; E-Mail: baljanabi@geomar.de

3 *Scienze Ambientali, Università di Bologna,Via S. Alberto 163, I-48100 Ravenna, Italy;* E-Mails: stefbroszeit@gmail.com (S.B.); francesco.mancuso4@unibo.it (F.P.M.)

4 Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, PL1 3DH, UK

5 School of Biological Sciences, Plymouth University, Drake Circus, Plymouth, PL4 8AA, UK; E-Mail: rebekah.cioffi@plymouth.ac.uk

[6] Marine Biology and Ecology Research Centre, School of Marine Science and Engineering, Plymouth University, Plymouth, PL4 8AA, UK
[7] Stazione Zoologica "Anton Dohrn", Punta San Pietro, Ischia (NA) 80077, Italy; E-Mails: amit.kumar@szn.it (A.K.); elena.ricevuto@szn.it (E.R.)
[8] Ecology and Animal Biology Department, University of Vigo, Campus Lagoas Marcosende, Vigo 36210, Spain; E-Mail: aranguren@uvigo.es
[9] Norwegian Polar Institute, Fram Centre, Tromsø, NO-9296, Norway; E-Mail: allison.bailey@npolar.no
[10] Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, 405 30, Sweden; E-Mail: leon.green@hotmail.com
[11] School of Biological Sciences, Queen's University Belfast, Belfast, BT9 7BL, UK; E-Mail: cgsottbauer01@qub.ac.uk
[12] Marine Biology and Ecology Research Centre, School of Marine Science and Engineering, Plymouth University, Plymouth, PL4 8AA, UK; E-Mail: emilie.hall@plymouth.ac.uk
[13] Dipartimento di Scienze, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, Potenza 85100, Italy; E-Mail: maria.lechler@googlemail.com
[14] Instituto Oceanográfico da Universidade de São Paulo, Praça do Oceanográfico, 191, Sala 139, Cidade Universitária, São Paulo 05508-120, Brazil; E-Mail: copereira@usp.br
[15] Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA; E-Mail: jbschram@uab.edu
[16] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Integrative Ecophysiology, Postfach 120161, D-27570 Bremerhaven, Germany; E-Mail: laura.stapp@awi.de
[17] Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (NMBU), PO Box 5003, Ås 1432, Norway; E-Mail: simon.stenberg@nmbu.no
[18] School of Biological Sciences, University of Essex, Essex, CO4 3SQ, UK; E-Mail: ltorre@essex.ac.uk

***** Author to whom correspondence should be addressed; E-Mail: beh14@aber.ac.uk; Tel.: +44-(0)-1970-623-111 (ext. 4187).

External Editor: Sam Dupont

Received: 4 March 2014; in revised form: 9 October 2014 / Accepted: 12 November 2014 / Published:

Abstract: Research to date has suggested that both individual marine species and ecological processes are expected to exhibit diverse responses to the environmental effects of climate change. Evolutionary responses can occur on rapid (ecological) timescales, and yet studies typically do not consider the role that adaptive evolution will play in modulating biological responses to climate change. Investigations into such responses have typically been focused at particular biological levels (e.g., cellular, population, community), often lacking interactions among levels. Since all levels of biological organisation are sensitive to global climate change, there is a need to elucidate how different processes and hierarchical interactions will influence

species fitness. Therefore, predicting the responses of communities and populations to global change will require multidisciplinary efforts across multiple levels of hierarchy, from the genetic and cellular to communities and ecosystems. Eventually, this may allow us to establish the role that acclimatisation and adaptation will play in determining marine community structures in future scenarios.

Keywords: ocean acidification; climate change; acclimation; evolutionary potential; adaptation; biological organisation; biologically-relevant scales

1. Introduction

Evolutionary processes play a fundamental role in the organisational structure of biological systems and the diversity of life [1]. It is possible for evolution to occur on a rapid ecological timescale, that may allow organisms to avoid extinction following environmental change [2]. One environment which is arguably changing faster than others is the marine environment [3], where increasing levels of atmospheric CO_2 are causing the seawater temperature and carbonate chemistry of surface waters to change at geologically unprecedented rates [4]. Future warming and altered ocean chemistry (broadly termed climate change throughout the present review) are recognised as pervasive and detrimental anthropogenic influences on marine life [5–9]. Climate change is expected to impose strong selection pressure on fitness-related traits, impacting on populations and ecosystems [10–14], and yet most future projections of community dynamics and population persistence in marine organisms do not consider the role of evolution and adaptive capacity [15–17].

The potential for genetic adaptation in response to climate change has been acknowledged [17,18], and adaptive evolution may represent a critical mechanism which could alleviate some of the negative consequences expected with future climate change [19]. However, the relatively limited number of studies means that evidence is still somewhat scarce [20]. A number of recent reviews outline the role of adaptive evolution in the face of climate change, including the need for determining species' capacity for evolutionary adaptation and physiological acclimatisation, the distinctions between evolutionary and phenotypically plastic responses, and summaries of the different experimental approaches (e.g., molecular tools, quantitative genetics, standing genetic variation, and experimental evolution). They also outline possible directions for future research (for reviews, see [16,17,19–23], and references therein). A glossary for some of the terms commonly used in this review is given in Box 1.

Studies investigating biological responses to climate change will often be carried out with a particular focus, whether that be physiology, evolutionary biology or community ecology. Such focus naturally means that other interacting facets of eco-evolution are often neglected [24]. Clearly, there are many important inter-disciplinary studies that do bridge this gap (e.g., [25–27]), however, there are still often disparities in the extent (if at all) that adaptive evolution is considered by different disciplines when determining a species' response under a changing environment. Inter-disciplinary work that links eco-evolution through biological hierarchies is not a new concept having been raised by numerous influential comparative physiologists in the 1950s, such as C. Ladd Prosser [28]. We believe this idea bears reiterating, and consider modern science to possess the necessary advancements in technology and communication required to begin incorporating this concept into future research.

There are a number of factors that mediate evolutionary processes, but their effects are highly dependent on the level of biological organisation that is considered (e.g., intra-individual, whole-organism, population, community and ecosystem, see Figure 1). The underlying mechanisms of how these levels of hierarchy will interact to influence fitness in the face of climate change are poorly understood, but are important in determining whether individual populations and communities will persist at levels comparable to the present day [16].

This review will focus on factors that can modulate adaptive evolution at different levels of biological organisation, by considering the response of marine organisms at these different levels in terms of the consequences for fitness traits (*i.e.*, lifetime reproductive success). We discuss: (1) what molecular and cellular mechanisms exist that can influence fitness and drive adaptive evolution; (2) how changes in life history and behavioural characteristics of organisms can influence lifetime reproductive success; (3) how demographic processes (gene frequencies, population size and turnover) and genetic architecture (heritability, imprinting, genetic correlations and diversity) of the population will influence adaptive evolution; and (4) how changes in species interactions and community composition influence the magnitude and direction of adaptive evolution of populations.

1. **Box 1.** Glossary for terms used in this article.

Acclimation: Reversible process of an organism to adjust to experimental conditions. When the process is induced by natural environmental changes, it is called **acclimatisation**.

Bottleneck effect: Reduction in population size due to environmental events, leading to a strong reduction of the variation in the gene pool.

Effective population size: Size of a hypothetical ideal population with random mating that corresponds to population genetic processes within the focal wild population.

Epigenetics: Heritable changes in gene regulation processes that are not caused by changes in the DNA sequence.

Evolution: Genetic changes in a population over generations. It is said to be microevolution when these changes occur over relatively short timescales, rather than on geological scales (macroevolution).

Evolutionary rescue: Genetic adaptation of populations that allows them to recover from demographic effects and avoid extinction.

Experimental evolution: Controlled experiment that exposes populations to new environmental conditions for multiple generations to observe for genetic adaptation.

Fitness: The potential for individuals of a given genotype to survive and pass their genes to future generations by influencing either their own reproductive success or that of related individuals.

Genetic adaptation: A process of transgenerational selection of genes to maximise or maintain the relative fitness of a population in a given environment.

Phenotypic buffering: Type of phenotypic plasticity, in which no difference in the response of a trait to a given environment might be observed because plasticity in a physiological process allows an organism to maintain fitness.

Phenotypic plasticity: Phenotypic adjustment to the environment without any genetic change.

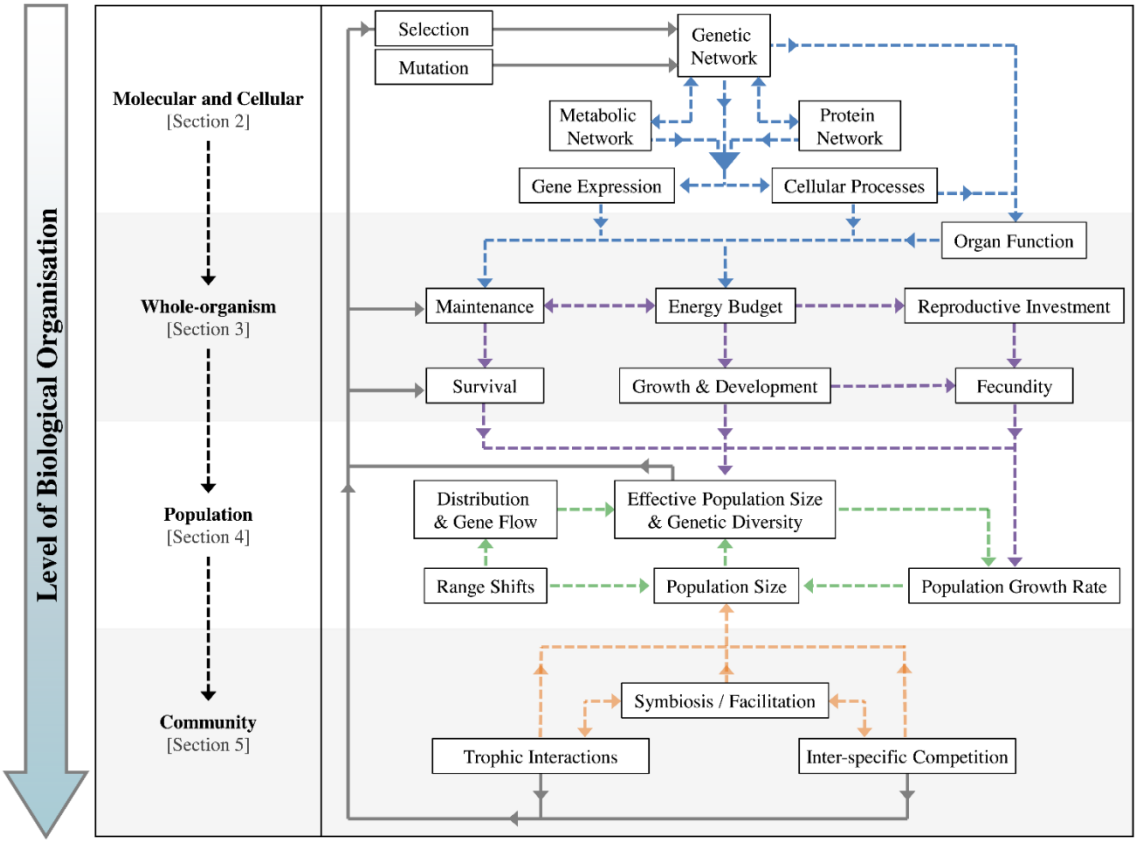
Quantitative genetics: Method to partition the observed phenotypic variance among relatives (of known genetic relatedness) into their environmental and genetic components.

Selection: Non-random reproduction or survival of individuals of a particular phenotype.

2. Role of Molecular and Cellular Processes in Evolutionary Responses

Molecular and cellular level studies can provide several approaches for improving our understanding of the potential for adaptation in response to climate change. These can include characterising an organism's capacity to acclimatise to changing environmental conditions, as well as establishing a more mechanistic understanding of the response of organisms to abiotic factors at different levels of intra-individual biological organisation, such as the nature of sub-lethal cellular stress [29]. Eventually this might enable us to investigate whether genetic adaptation can occur at a sufficient rate to maintain the physiological functioning required for survival and reproduction, and gain important insights into energy allocation and physiological responses due to climate change, as well as other biotic and abiotic stressors [22]. However, the distribution of a species is shaped by both a species' physiological limits and biotic interactions with co-existing species, and therefore, cellular and molecular studies alone may only provide part of the picture.

2. **Figure 1.** Conceptual diagram of the factors modulating evolution at different levels of biological organisation (molecular, cellular, whole-organism, population and community), that will determine the response of marine organisms to future climate change. The arrow on the left represents the increased biological complexity (going from top to bottom). Single-headed arrows indicate the direction of the effect with the level of biological organisation indicated by different colours. Effects originating from the molecular and cellular (dashed blue), whole-organism (dashed purple), population (dashed green) and community (dashed orange). Double-headed arrows indicate that there is feedback between two factors, as well as the effect, and the solid grey arrows indicate a feedback loop. Note that the depiction of factors is conceptual and not comprehensive.



2.1. Biochemical Reactions and Gene Expression

Within the organism, protein activity is often thought to underlie variations in fitness (for discussion, see [30]). Fitness at the biochemical level could be simply considered as the ability of proteins to function (within their respective intra- and extra-cellular setting) in order to integrate the diverse functions of cells and organelles [31]. Proteins are responsible for crucial functions in all biological processes [31], and evolutionary changes can occur through changes in the proteins themselves (e.g., post-translational modifications), the encoding gene(s) of those proteins, or the transcription of those encoding genes [31].

Fitness-related traits can be influenced through genetic variation in these proteins, such as the collinearity of gene mutations, whereby the point mutations in the DNA sequence will correspondingly change the sequence of amino acids in a protein [32]. These biochemical consequences can influence protein function and in turn, tolerances to environmental conditions [33]. For example, a minor mutation (only two amino-acids out of 334) in a dehydrogenase enzyme in the temperate mussel *Mytilus galloprovincialis* (Lamarck,

1819) resulted in higher thermal tolerance towards warm conditions [34]. Alternatively, enzymes possessing alternative alleles, such as for lactate dehydrogenase-B in cold- and warm-adapted populations of the killifish (*Fundulus heteroclitus* L., 1776) [35], may be able to confer adaptation potential for thermal tolerance through variable allele frequencies.

In order to produce adaptive phenotypes, changes may be required in multiple combinations of alleles [36]. Allelic changes are embedded within genetic networks and hence, will not occur independently to other changes, since any allelic changes at a particular locus will influence only one aspect of a genetic network [37]. These genetic networks essentially consist of the genes which encode the transcription factors as the input for each coding gene, and the *cis*-regulatory modules that control the appropriate phases of expression of these genes [38]. Gene regulatory networks control the expression of genes in any given developmental process [39], including fitness-related traits, and therefore, any changes in the networks could play an important role in adaptive evolution and climate change responses [37].

Environmental effects may cause changes either in specific genes within the network, influencing their gene expression, or affect the gene regulatory network as a whole [40]. Genetic networks will primarily be influenced by current environmental conditions and maternal effects (the latter described in Section 2.3), and these changes will, in turn, alter the protein and metabolic networks that influence gene regulation (*via* a feedback loop reaction) [37]. Changes in genetic networks may influence plastic responses and facilitate adaptive evolution by providing a rapid response to the changing environmental conditions. However, if the genetic regulatory network is influenced by other factors that do not follow the changing environmental conditions, such as photoperiod [41] or even biotic interactions [42] (discussed further in Section 5), then adaptive evolution might require a restructuring of the genetic network in order to conform to the novel environmental conditions [37].

Currently it remains unclear whether the few examples that demonstrate observable adaptive evolution of traits in response to climate change (e.g., body size [43], migration timing [44], thermal responses [45]) are dictated by various independent genes (within their respective genetic networks), or by fewer key regulatory genes within their genetic or metabolic networks. This is important to consider since any changes in the 'upstream' network genes could have extensive and numerous effects on traits [37], and yet the network itself may also provide some redundancy and buffering against perturbations, whereby changes to regulatory genes do not influence the genes they regulate [46]. Eventually, it may be possible to identify common genetic (e.g., collinearity in the gene order between genomes [47]) and physiological mechanisms underlying species responses [17]. However, studies demonstrating a clear link between the genetic variation and phenotypic variation for the majority of traits are scarce (but for example, see [48]). Therefore, any studies of genetic variation should focus on traits with more straightforward or measurable relationships to fitness [17,21].

Establishing the evolutionary significance of cellular-level plasticity (*i.e.*, the changes in the expression levels of stress-related genes, e.g., [49]) requires demonstration of a heritable component of expression variation, or allelic variation in the coding genes themselves [17,50]. Accurately estimating selection responses requires the genetic component of this variation (in regulatory responses) to be related to the fitness of the organism [51,52] in order to ascertain the fitness-related consequences for the individual and the population. This highlights the need to investigate transcriptome profile responses in terms of survival, fecundity, or other ecologically important traits that determine lifetime reproductive success (but see [30] for a discussion on the limitations in the link between the transcriptome and the phenotype), and importantly, ascertain whether sufficient genetic variation exists in that trait [53].

2.2. Cellular Processes and Organ Function

Cellular and organ functioning during stressful conditions will primarily be dictated by changes at the genomic and biochemical level (Section 2.1). The principal factor determining the underlying cellular stress response (a universally conserved mechanism to protect macromolecules within cells from damage [54]) depends on the extent of stress-induced disturbances (reviewed by [55]). During moderate stress, resources may be shifted from anabolic (e.g., protein biosynthesis) towards vital processes for cellular homeostasis (e.g., ion regulation; [55]) to maintain cellular integrity and ensure short-term survival. However, on longer time scales such shifts may not be feasible and might lead to a reduction in organism performance (e.g., reduced growth rates or fecundity) since the organismal energy budget can be considered as the sum of all cellular energy budgets [55].

Such trade-offs in physiological functions could have important fitness consequences, but may not be apparent when only observing the whole organism level. For instance, a study on the effects of ocean acidification on the reef-building coral, *Acropora millepora* (Ehrenberg, 1834), reported major changes in gene expression and cell physiology long before phenotypic effects were observed, in this case, a decrease in calcification rates [54]. Thus, cellular functioning might play a central role in linking environmental conditions to an organism's fitness [56], and the plasticity and adaptive evolution of cellular processes may be an important influence on species resilience towards changing environmental conditions.

Adaptive evolution in cellular function may be possible through gene duplication [57–59], whereby paralogous genes (*i.e.*, gene copies) that perform a particular function either increase their expression (increased gene dosage) or diverge their functions through mutation [60]. This divergence can be achieved by one of the copies acquiring a new function, or through a partial loss-of-function mutation of both copies that complement each other [61], while retaining the full set of functions (termed sub-functionalisation [60]). This sub-functionalisation is a relatively common mechanism for functionally related proteins [61], such as components of cell signalling pathways, and may facilitate evolution of advantageous traits: e.g., a changed pH optima of proteins [62], a beneficial trait for maintaining acid-base homeostasis in response to ocean acidification.

2.3. Epigenetics and Trans-Generational Plasticity

The environment experienced by an organism can shape the phenotype of their offspring, and is termed trans-generational plasticity (e.g., [63]). Trans-generational plasticity can be due to maternal or paternal effects, genomic imprinting, gene expression or other epigenetic processes. These epigenetic effects (whether a gene is being expressed or not) can be transmitted through the germ line [64], which can allow for transmission through meiosis to the succeeding generation, constituting a heritable, epigenetic change [65]. For example, five weeks exposure to elevated pCO_2 during the reproductive conditioning of Sydney rock oysters (*Saccostrea glomerata*, Gould 1850) reduced the development time and increased the body size of their larvae through trans-generational plasticity [66].

Mechanisms exist that should allow these epigenetic changes to result in localised changes in the DNA sequence, such as changes in the activity of chromatin-modifying enzymes [65]. Providing they exert the same functional effect, any epigenetic effects can potentially become a genetic change, and exert a selectable phenotypic response [65]. During climate change, the environmental conditions that induce these epigenetic effects (like temperature) will persist (albeit progressively increasing) and therefore with each

successive generation, the epigenetic response could actually result in continued DNA change in selected regions of the genome [65].

The gene regulatory network, responsible for many fitness-related traits (Section 2.1), is initiated by maternal transcripts and proteins, which cascade into subsequent gene regulatory interactions [67]. Early genes that function during development (such as for larval morphology) can be influenced by the fitness traits of the maternal parent (e.g., by changes in egg size or provisioning [67]), and therefore it may be possible that parental exposure to climate change can cause DNA (or heritable, epigenetic) changes that promote adaptive evolution in key regulatory genes, or the genetic network as a whole.

3. Role of Whole-Organism Physiological and Behavioural Responses

Marine organisms possess a range of reproductive and developmental strategies that have important implications for their fitness [68]. Different reproductive modes, life histories, and demographic processes can influence these strategies [69–71]. In this section we focus on how climate change, specifically ocean acidification and warming, can influence the physiology and behaviour of the individuals, affecting their survival and fitness. It is important to consider the factors that influence selection at this level of biological organisation in order to link individual phenotypes, which are in turn driven by transcriptional and cellular processes, to population-level effects.

3.1. Maintenance and Energetic Trade-Offs

The capacity to maintain metabolic processes under environmental stress may support (or promote) the retention of particular life history traits (such as reproductive output) that may ultimately determine a species' biogeography [72,73]. A recent study using an *in situ* transplant experiment with polychaetes, found that species capable of maintaining their metabolic rates (under stress) were able to migrate into or even colonise areas characterised by chronically elevated levels of pCO_2 [26]. This high-CO_2 tolerance was achieved in the polychaetes *via* acclimatisation for *Amphiglena mediterranea* (Leydig, 1851) and by adaptation for *Platynereis dumerilii* (Audouin & Milne-Edwards, 1834) [26]. However, such resilience often comes at a cost [74]. The individuals of *P. dumerilii* were smaller in body size compared to nearby populations in lower pCO_2 conditions, attributed to increases in maintenance costs due to a higher mean metabolic rate under chronic exposure to elevated pCO_2. Since the size (in several polychaete species) can determine the maximum numbers of eggs that a female produces, this resilience could result in reduced reproductive output [26]. Although the study did not empirically test this, any reallocation of energy away from reproduction would clearly have important implications for lifetime reproductive success.

Fitness-related traits can be genetically correlated to each other and, depending on strength and direction of selection, influence the potential for adaptive evolution (for more detail, see [17]). Briefly, a positive correlation could include a co-tolerance to multiple stressors (e.g., developing sea urchin larvae obtaining tolerance to low pH and therefore also temperature [75]), or a selection for a particular trait providing tolerance for another trait (e.g., growth and disease resilience in Sydney rock oyster (*S. glomerata*) providing tolerance to high pCO_2, [66]). If the intra-individual physiological mechanisms (Section 2) and an organism's response during climate change are nonlinearly related, then there is a need to understand what physiological trade-offs are occurring that are influencing their fitness related traits. Fitness trade-offs will certainly influence potentially selected traits, if other energetically maintained traits are selected over survival or reproductive output.

3.2. Life-History Stages

Research into physiological responses to climate change has demonstrated that fitness traits, such as reproduction and development, are likely to be disproportionally affected [76–78]. Since natural selection acts upon lifetime reproductive success, climate change can reduce fitness through impacts on early life-history stages, such as an increase in developmental duration or number of defects [79–81]. However, many marine species have complex life histories, and despite early life history stages being considered to be particularly vulnerable to climate change [82], there is increasing evidence that selection pressures act on each life stage differently (e.g., [83]). Phenotypic carry-over effects can also occur between life history stages (as well as trans-generationally, Section 2.3) that could exacerbate or alleviate the impacts on fitness-related traits. For example, exposure to stressful conditions during the larval stage can reduce the juvenile fitness if those conditions continue (e.g., [84]). This may be particularly important given that different stages of ontogeny may utilise different habitats (e.g., [85]) or exhibit different behaviour. Hence, impacts considered on individual life-stages may not accurately estimate the fitness response of a given species [86].

3.3. Behavioural Responses

Organismal behaviour is mediated by multiple external and internal sensory inputs that may be changed directly and indirectly by climate change [87]. The plastic behavioural responses observed in organisms are largely a direct physiological response to a changing environment, since the nervous system is under biochemical and physiological control [88]. Hence, changes in the underlying physiological condition (see Section 2) could influence behavioural performance by constraining an ecologically-relevant behaviour, such as swimming activity [89]. A study in coral reef fish found that small temperature increases (<3 °C) contributed to changes in animal personality (activity, boldness, aggression), thought to be linked to individual responses in energy metabolism [90].

Changing environments can also modulate behaviour by interfering with sensory inputs and neural functioning. For example, elevated levels of pCO_2 are hypothesised to remodel the sensory pathway of the GABA-A system of marine organisms, including the larval clownfish (*Amphiprion percula* Lacepède, 1802), damselfish (*Neopomacentrus azysron* Bleeker, 1877), and gastropod *Gibberulus gibbosus* (Röding, 1798) [91,92], causing sensory and behavioural impairment, including learning ability [93]. This phenomenon is thought to be associated with ion regulatory mechanisms during high CO_2 exposure (accumulation of intracellular HCO_3^- and Cl^-), which interfere with neurotransmitter functions (for more details, see [92]). Impaired learning regarding the identity of predators during high pCO_2, or diminished detection of the olfactory cues for settlement (for instance) influence fitness by negatively affecting the survivorship of the individual [93,94]. Sensory pathways occur in differing complexities with receptors and messenger systems of different adaptive potential [95]. Hence, knowing the mechanistic pathway of a behavioural response is important for determining the evolutionary potential of an organism or indeed a trait. Linking these pathways with their genes is important for finding out if organisms can adapt, in order to cope behaviourally with environmental stressors [96]. Behavioural traits may be more evolutionary labile than other traits [97], and may contribute to or hinder adaptation [19,98].

4. Role of Population-Level Responses

Focusing on population-level organisation is crucial for connecting the fitness responses of lower levels (individual/population) to changes in higher levels (species/community). The analysis of microevolution in

populations requires an understanding of how environmental changes influence evolutionary processes such as gene flow, mutation, genetic drift and natural selection [99]. Historically, the concept and investigation of population level adaptation in the marine environment was largely dismissed; it was assumed that marine connectivity would maintain high levels of gene flow between populations via adult and larval dispersal [100], and so impede local adaptation. However, new evidence compiled by Sanford and Kelly [101] shows that microevolution is not restricted to organisms with low dispersal abilities. Through a literature survey Sanford and Kelly [101] found that 66% of marine invertebrates with planktonic life stages for dispersal, *i.e.*, meroplankton, present highly adaptive differentiation at the population level (e.g. *Haliotis rufescens*, Table 1). Depending on the taxa investigated, the planktonic dispersal stages of the identified (66%) invertebrates experienced brief (up to a few days as with some corals, sea anemones or ascidians) to prolonged (several weeks to longer, some crustaceans and gastropods [100]) planktonic larval durations.

4.1. Demographic Processes

Populations can respond to environmental pressures more rapidly through range shifts and phenotypic plasticity rather than through evolutionary adaptation [102]. Evolutionary responses are likely to vary depending on the cost of adaptation, timescale, life-history and dispersal ability in addition to other factors [19]. Different evolutionary responses have been previously investigated and require a variety of techniques (for a survey of selected reference studies see Table 1). Understanding genetic variation, as well as specific population dynamics, is crucial to explore the potential for evolutionary rescue [103]. For example, populations in isolated environments, such as the Baltic Sea, may also undergo isolation and develop genetic endemism as a result of local extinctions or adaptation by evolutionary rescue [104]. Therefore, population size and genetic variation in the context of the intensity and duration of environmental selection pressures must be considered [105] to identify what part of the population (*i.e.*, the effective population size [106]) contributes to the next generation.

3. **Table 1.** Published studies investigating population level evolutionary responses to climate change (including ocean acidification) in marine species.

Taxonomic Affiliation	Response Variable(s)	Driver	Method(s)	Evolutionary Response	Ref.
Spermatophyta: *Zostera marina*	Growth rate Survival	T	F	Genotypic complementarity	[107]
Coccolithophyceae: *Emiliania huxleyi*	Growth rate Production rate: (PIC)	OA	LS	Selection of genotypes Direct positive adaptation	[108]
Gephyrocapsa oceanica	Growth rate Carbon fixation	OA	LS	Selection of genotypes (Adaptation)	[109]
Diatomophyceae: *Thalassiosira pseudonana*	Phytosynthetic efficiency	OA	LS	No adaptation	[110]
Anthozoa: *Acropora millepora*	Thermal and physiological tolerance	T	F	Natural selection	[111]
Pocillopora damicornis	Coral bleaching (thermal tolerance)	T ES	CG	Local adaptation or acclimation	[112]
Bivalvia: *Mytilus trossulus*	Growth rate Survival	T	TE	Possible thermal adaptation	[113]
Gastropoda: *Haliotis rufescens*	Genetic polymorphism	T	SNP	Local adaptation Genetic differentiation	[114]
Polychaeta: *Platynereis dumerilii*	Body size	OA	TE	Genetic adaptation	[26]
Amphiglena mediterranea	Body size	OA	TE	Physiological plasticity	[26]
Amphipoda: *Orchestia gammarellus*	Growth Thermal tolerance	T	LS	Selection	[115]
Cirripedia: *Semibalanus balanoides*	Genetic polymorphism	T D	TE	Balancing selection Local adaptation	[116]
Copepoda: *Tigriopus californicus*	Survival (LT_{50}) Thermal plasticity	T	LS	Low adaptation potential	[117]
Decapoda: *Uca pugnax*	Developmental rate	T	CG	Selection on variation Local adaptation	[118]
Echinoidea: *Heliocidaris erythrogramma armigera*	Hatching success	T	QG	Genotype-by- environment interaction	[119]
Strongylocentrotus purpuratus	Gene expression: thermal resistance	T	CG	Selection of thermally sensitive genes	[120]
Strongylocentrotus purpuratus	Larval body size	OA	CG	Heritability correlates with high-pCO_2	[121]
Centrostephanus rodgersii	Cleavage and gastrulation stage	T OA	QG	Heritable genetic variation for sires	[75]
Centrostephanus rodgersii	Embryonic development	T OA	CG	Varying expansion of population	[122]
Teleostei: *Gadus morhua*	Body shape	T	CG	Counter-gradient variation	[123]
Fundulus heteroclitus	Thermal tolerance	T	LS	Selection Regulation of heat shock proteins	[124]

Notes: Selective driver: abbreviated as T - temperature; OA - ocean acidification; ES - environmental stability; D - desiccation. Method: F - field experiment; LS - laboratory selection experiment; CG - common garden experiment; TE - transplant experiment; SNP - outlier SNP analysis; QG - quantitative genetics.

Populations may have an increased chance of persistence if they react to changing climatic conditions with higher phenotypic plasticity. Should this plasticity occur in a fitness-related trait, then this may present a heritable variation for selection to act upon (e.g., [23,125]). This mechanism would thereby allow for a faster non-mutational selection [126]. Populations that are maladapted to climate change will likely

experience an initial decline and thus, a reduced effective population size [127]. Phenotypic buffering, a type of phenotypic plasticity, represents an important mechanism for maintaining population performance under stressful conditions until adaptive evolution can "catch up" and sufficiently improve population fitness [23,128]. For example, genetically diverse populations of the seagrass *Zostera marina* (L., 1758) showed quicker recovery following sub-lethal temperature stress when compared to less diverse populations [107]. This buffering effect was expressed due to the complementarity of different genotypes (e.g., facilitation) that maintained ecosystem functioning, and may promote adaptive evolution [107].

4.2. Environmental Variability

The potential for adaptation under naturally low or fluctuating pH can be studied in regions of upwelling along the continental coast of (Western) North America [129]. A transcriptomic analysis of sea urchin larvae (*Strongylocentrotus purpuratus* Stimpson, 1857) collected from a naturally variable low pH upwelling site revealed that larvae under present day conditions initiated a robust transcriptional response, but only a muted response to near future conditions [130]. These exposures to transient extreme conditions may be sufficient to provide populations with a selection for tolerance (e.g., [131]). However, the question then becomes whether selection for one stressor will provide increased tolerance to another. Quantitative genetics is a technique that may help answer this type of question because it allows partitioning of the observed phenotypic variance of a population among relatives (with known genetic relatedness) into their environmental and genetic components [132], in a synchronic approach (*sensu* [23]). Numerous studies have demonstrated evolutionary adaptive capacity using quantitative genetics (as reviewed in [71]).

In the absence of mutations, adaptive evolution relies on the genetic variation in physiological tolerances [133], this is because in turn, the variation of physiological tolerances influences the likelihood of extinction [121,134]. These tolerance traits in natural populations are termed standing genetic variation, and arguably the most important influence maintaining this adaptive variation is spatially varying selection [135]. For most species, the temperature gradient across their distribution (e.g., 30 °C difference between the pole and equator [121]) will greatly exceed the expected future temperature change (3.7–4.8 °C, [136]). In contrast, pH gradients are often relatively homogenous when compared to predicted change (0.3–0.5 pH units by 2100 [136]; but see [129,137,138]). Therefore, populations may possess greater adaptive variation for temperature tolerance, but have less adaptive variation for pH tolerance [117].

It is crucial to distinguish between microevolutionary (genetic) and phenotypic (plastic) responses at the population level. Many past studies have lacked this focus, but identifying the drivers responsible for changes in fitness traits should be given more attention in future studies (e.g. [19,139]). Non-genetic evidence can also be lacking, missing potential patterns, such as in situations of counter gradient variation whereby genetic and environmental influence can oppose each other [140]. This was the case for the genetic divergence of body shape between two populations of juvenile Atlantic cod (*Gadus morhua* L., 1758), in which phenotypic differences were mitigated by environmental influences [123]. Even the positive, negative or neutral correlation between two fitness traits may accelerate, slow down, or not impact adaptive evolution [17]. As such, local environmental variability must be considered when determining population responses.

4.3. Modes of Population-Level Response

Examination of time series data reveals evolutionary responses to climate change, such as direct allochronic studies which include a mixture of populations that are on their way to adaptation or extinction (reviewed in depth by [102]). These studies can show that the selection of genotypes is an immediate

mechanism of population-level adaptation. Multi-generational analysis of selection of the coccolithophore (*Emiliania huxleyi* (Lohmann) Hay & Mohler, 1967) has provided evidence for evolutionary adaptation responses detected by selection of genotypes and direct positive adaptation to increased pCO_2 by mutation [108]. However, it is important to emphasise that the rate of adaptation for single-celled organisms, due to their fast generation times, will likely differ along with the mechanisms utilised when compared to multi-cellular organisms. Future studies should be optimised by an interdisciplinary approach, including abiotic changes driven by climate change, biological networks, and the relationship between the phenotypic and genetic analysis, for a better understanding of future climate change impacts on the evolution of populations.

5. Community Composition and Interactions

While studies of evolution on single species and populations are already underway (either *in situ* or in the laboratory), the potential of communities and ecosystems to evolve as a unit in response to changing environments has not yet received as much attention. This is partially due to the complex nature of communities. Another important reason is that for several decades, ecological and evolutionary time scales were thought to diverge widely and this has led to very different thought models of evolution and ecology [141]. In particular, it was thought that evolution takes place in time frames that cannot influence ecology, while the effect of ecology on evolution has been studied in some prominent examples. For instance, in the Atlantic cod (*Gadus morhua* L., 1758) fishing pressure led to earlier age at maturation [142]. However, the dynamic effect of evolution on ecology is an emerging field of study since it was recognised that evolution of ecologically relevant traits can influence contemporary communities [143,144].

If community composition is altered, the coevolution between interacting species will be driven and/or modified by their interactions within the community [145,146]. This diffuse coevolution means that the selection of a specific trait in one species may depend on the presence of another species [147], making species identity and uniqueness a plastic response in community-level responses [148]. Therefore, the effects of future climate change on communities will likely be complex [149], and influence the outcomes of competition, facilitation (e.g. [150]) and trophic interactions (e.g., predator-prey [151,152], and plant-herbivore [153,154]).

5.1. Changes to Community Dynamics

The fast population turnover of single-celled phytoplankton represents a great opportunity to study experimental evolution and to quantify evolutionary and plastic responses of populations to future climate change [155]. Phytoplankton communities represent a pivotal role in marine ecosystem functioning [155], forming the base of the marine food web and crucial for global biogeochemical cycles [156]. Under current conditions of dissolved inorganic carbon, many phytoplankton species are not fully saturated for growth and photosynthesis, and therefore, will benefit from the addition of CO_2 (e.g., [157–159]). However, any selection for fast growth, despite providing competitive ability through size (but see [160]), may come at the cost of reduced resilience to pCO_2 [161]. This was shown by a study ([161]) that used genetically distinct isolates of phytoplankton species (sixteen strains of the diatom *Skeletonema marinoi*, Sarno & Zingone 2005 and eight strains of dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen, 1985) and found that slow-growing cultures generally responded positively to elevated pCO_2, while fast-growing cultures either showed neutral or negative responses. Hence, the effects of climate change need to be considered holistically in terms of both ecological performance as well as physiological tolerance.

The enormous diversity of phytoplankton and the variety of environmental stressors makes it unthinkable to experimentally test all the possible trait responses in every phytoplankton group. The difficulty lies in establishing whether this evolutionary potential can be realised, and whether results from laboratory experiments can be related to natural populations (see [155]). Therefore, understanding the mechanistic effects of future climate change on key functional groups (e.g., [162]) will require a deeper understanding, across biological hierarchies, of the direct effects on their physiology (molecular and cellular), basic biology (whole-organism), as well as estimates of gene flow, population size, and recombination rates (population) [155].

In order to extrapolate from the organism and individual species' responses to the community level, we also need to understand the response of the ecological interactions within the community. For example, any increased biomass associated with higher atmospheric CO_2 may be indirectly mediated by the presence of grazers (indirect trophic interactions, e.g., [163]), or regulated by heterotrophs of the same community (e.g., [164]). Similarly, phytoplankton responses associated with climate change can lead to bottom-up control (e.g., [165]), or, due to sufficient food availability to marine organisms may provide physiological homeostasis (e.g., *Mytilus edulis* L.; 1758 [166]). As such, if the effects of climate change differ between similar co-existing species (e.g., [167]), it may indirectly influence selection by causing ecological release; reducing the need for competitive traits.

In addition to the direct effects, future climate change may have indirect effects on other communities. Where CO_2 is a resource for organisms, it can play an important role leading to changes in community competition (e.g., [168]). For example, opportunistic turf- and mat-forming algae have been demonstrated to inhibit other taxa (e.g., [169]) and outcompete kelp recruitment (e.g., [170]), inducing phase shifts. Species in diverse communities tend to have lower effective population sizes compared to when they are in isolation due to the competitive interactions [171]. This typically increases the role of genetic drift compared to selection, and might reduce the rates of adaptive evolution [172]. Climate change might reduce those inter-specific interactions (e.g., bottom-up control releasing resource limitation [165]) and thereby enhance the potential for adaptation, through reductions in genetic drift. Alternatively, climate change may increase competition (e.g., [173]) and amplify changes in mean population size, increasing extinction risks, as well as decreasing adaptation rates (Figure 1). This may be further exacerbated through co-extinctions, due to

increased habitat and biodiversity loss, whereupon one species is dependent on another that is already extinct [174].

The presence of co-occurring species might enable adaptation by initiating coevolutionary interactions (e.g., [175]), however, it has also been suggested that increasing biodiversity may begin to inhibit that subsequent adaptation (e.g. [171]). This is due to an increased number of species in an assemblage, increasing the chance that a current species will possess traits that would predispose the species towards favourable selection under future environmental conditions, and could restrict the opportunity of other co-occurring species to adapt. Species-specific adaptation mechanisms could ultimately feedback to influence ecosystem functioning [143]. For example, three bacterial species that were raised together had higher productivity compared to the same species that adapted in isolation [176]. This was due to the inter-specific competition that caused them to select for specialisations in their resource use (niche partitioning [177]), leading to a complementary adaptation [176]. Hence understanding whether the evolutionary potential can be realised will require investigations that utilise realistically diverse assemblages (e.g., [169,173]). It does however, also raise the challenge of understanding whether future ecosystems will become sustained ecosystems (with fewer species that are selected for their favourable traits), or more evolved ecosystems as a whole. This is crucial given the extensive research regarding biodiversity and ecosystem multi-functionality in present day communities (for more details, see [178]).

5.2. Habitat Fragmentation and Biological Invasions

Anthropogenic climate change is expected to reorganise patterns of species diversity [179,180]. One possible approach for investigating the selection response using naturally assembled communities is through the use of natural analogues for future climate change, such as CO_2 vents [26], or coastal upwelling sites [114,181]. These areas provide long-term chronic exposure to novel environmental conditions, and allow experimental work to capture an organism's response in fitness-related traits [26, 182], such as reproductive success. Moreover, organism responses will include carry-over effects (between life-history stages and trans-generationally), as well as being influenced by other ecological interactions, such as competition and trophic interactions. Yet, (a caveat) for those species that are not direct-developing, these sites may be confounded by larvae received from outside of the site, with different environmental conditions, likely reducing selection pressure.

For long-lived sessile foundation species, such as reef-building corals, evidence suggests that acclimatisation and adaptation will be essential for population persistence in the face of climate change [25], given that any range shifts are likely to be slow [183]. A recent transplant experiment utilising the table top coral (*Acropora hyacinthus* Dana, 1846) found that acclimatisation and adaptive responses (mirrored in the patterns of gene expression) allowed this faster-growing coral to inhabit areas of the reef that far exceeded their expected temperature tolerances [25]. This tolerance to elevated temperature might be associated with either the coral host (e.g., [48,184]), or their associated *Symbiodinium* (e.g., [185]). In contrast, experimental work investigating the coral reefs at the shallow volcanic CO_2 seeps (in Papua New Guinea) found an overall reduction in diversity and recruitment in the coral communities pre-acclimated to high pCO_2, thought to be associated with shifts in competitive interactions [173]. This highlights that the adaptive evolution of coral reef communities is possible and driven by abiotic factors (Court Jester hypothesis, [186]), however, community-level interactions (such as the increased competition in high pCO_2) may equal or exceed these fitness-related responses (*i.e.*, survival), and lead to adaptive evolution being driven by biotic

factors (Red Queen hypothesis, [186]) Clearly, the relative roles of biotic and abiotic factors will be stressor-specific reaffirming the need to investigate the adaptive evolution responses with multiple stressors in realistic communities.

Biological invasions are important drivers of change in marine communities, particularly coastal communities (e.g., [187,188]). Increases in temperature may facilitate species' range shifts, thereby aiding invasion [188]. One particular example of this is the 'tropicalisation' of the Mediterranean Sea, where, invasions and establishments have been made possible due to increasing annual mean temperatures all year around [189]. The integration of novel species may influence evolutionary processes by altering existing interactions (e.g., [190]) or population growth rates (see [191]). Alternatively, both the native and non-native species may be able to achieve coevolution if their co-existence can maximise their habitat use [192]. Although native species might be able to overcome the invasion of some non-natives, some may become less adapted to the new conditions and be out competed by invasive organisms, which exhibit greater adaptability or the ability to demonstrate strong fitness effects [188].

6. Future Directions

It is inevitable that increasing ocean acidification will be accompanied by changes in other abiotic factors, and therefore interactions with other stressors (*i.e.*, temperature, nutrients, hypoxia or salinity) are extremely likely [193]. For both single and multiple stressors, there is a crucial need to incorporate the potential for adaptation to future climate change, to reliably determine the sensitivity and mechanisms for adaptation of marine organisms.

Adaptation capacity will be driven through a number of mechanisms with different taxonomic and functional groups utilising a variety of processes. Species with large population sizes and fast population turnover rates, such as phytoplankton, are likely to demonstrate the potential to achieve the adaptation rates required for future climate change (e.g., [108]), making them a model species for laboratory experimental evolution. However, these experiments will likely be carried out in the absence of more complex trophic and ecological interactions. In order to clarify the effects of anthropogenic climate change on community- and ecosystem-levels, future research should be directed towards identifying key species, and establishing their interactions with coexisting species, particularly if those ecological interactions vary with season or ontogeny [194,195]. The choice of species could be associated with the needs for either ecosystem's services or functioning, such as the disproportionate role that coccolithophores play in the carbon-cycle, or societal needs, such as for food security, or possibly in an ecological context, being habitat forming or a keystone species.

Given the differential sensitivities and responses of different life-stages, future research needs to identify which life-stages are most affected by climate change and the key interactions (among species and to different climatic scenarios) within ecosystems [196]. The negative results from short-term studies on early life-stages often make it difficult to extrapolate to longer-term impacts [197–199], especially when multiple stressors interact, since the sensitivity of early life-stages may not be representative when responses are considered across all ontogeny and life-stages. The exposure of previous generations to environmental conditions will influence the response of subsequent generations (*i.e.*, carry-over effects). As such, the use of chronic long term multigenerational experiments should contribute to our understanding of both developmental and trans-generational plasticity [108,155,198]. An additional important consideration is the current local-scale variability of environmental conditions. If the adverse conditions that we expect by the

end of the century are already being experienced by marine organisms, and are within the range of the current environmental variability (e.g., CO_2-enriched upwelling, Kiel Fjord, western Baltic Sea [138]), then these transient extreme conditions may result in a pre-selection for tolerance (e.g., [131]). This pre-selection might be achieved through the divergent selection of specific genes in candidate loci (e.g., [114]), and contribute to the maintenance of positive life-history traits.

Phenotypic plasticity may provide the potential for species to achieve sufficient tolerance in the short-term, such that they may actually be able to achieve adaptation to environmental change. To attain a mechanistic understanding of this process will require an interdisciplinary approach, including investigations at different levels of biological hierarchy. This is because the capacity of a species' phenotypic plasticity might be set at the cellular level, for example through changes in oxygen demand via mitochondrial activity. However, it is important to consider that these responses might be first observed through changes in abundance (or distribution), using more phenomenological approaches at the population level. Alternatively, the persistence of a species could be attributed to its dispersal ability and the availability of suitable habitat and hence potential spatial scale of gene flow. As such, research needs to be carried out at biologically-relevant scales. Therefore, a crucial first step in understanding responses at the population level will require linking the intra-individual physiology (e.g., transcriptional and cellular responses) to the fitness-related traits of the whole-organism, in order to more reliably estimate the effects of climate change on contemporary population demographics into the future.

7. Conclusions

Biotic factors such as competition and trophic interactions shape marine communities at local spatial scales and over relatively short timescales. Other extrinsic factors, such as oceanic and atmospheric environmental conditions will influence patterns of biodiversity over longer timescales, and at regional or global scales [186]. Since climate change is occurring rapidly, it is likely that biotic interactions may play a more important role, compared to abiotic factors, when it comes to evolution (*i.e.*, the Red Queen hypothesis [200]). As such, establishing the association between local environmental conditions and the genomic-physiological features of key species, that are known to be influential in communities (including their interactions with co-existing species), should elucidate how community processes will be affected, and whether evolutionary potential can be realised. However, investigating broader spatial scales will require determining the link between the genomic-physiological responses of contemporary populations and population dynamics. This could establish a deeper understanding between the physiological stress responses of marine organisms to both biotic and abiotic factors, and critical (yet often unknown) demographic processes such as effective population size.

Both adaptation and acclimatisation may enable organisms to persist in future oceans, and understanding how factors at different levels of biological hierarchy will influence these important evolutionary responses to climate change is crucial. Future research needs to investigate biological responses both spatially and temporally, by utilising spatially representative replication across different scientific disciplines and research institutes, in an effort to integrate responses and adaptive mechanisms at regional or global scales. This will help to achieve direct comparisons and a more integrative picture of the responses at the community and ecosystem levels. Only then can we establish whether the future organisational structure of marine ecosystems will resemble the communities of today, and what role acclimatisation and adaptation will play in the persistence of marine organisms.

Acknowledgments

Thanks to Sam Dupont, Piero Calosi, Niall McKeown, Pippa Moore, Paul Shaw, John Spicer and the two anonymous reviewers for their suggestions and feedback that greatly improved this manuscript. The collaboration on this manuscript was made possible due to the CeMEB Advanced Course on Marine Evolution Under Climate Change, funded by the Swedish Royal Academy of Science and organised by Sam Dupont, Piero Calosi, Pierre De Wit, Narimane Dorey, Géraldine Fauville, and Greg "George" Puncher. Funding for Ben Harvey was provided by an Institute of Biological, Environmental and Rural Sciences Ph.D. Studentship and Training Budget. Financial support for Balsam Al-Janabi and Laura S. Stapp was provided by the project BIOACID phase II of the German Federal Ministry of Education and Research (BMBF; FKZ 03F0655A/B), Laura S. Stapp also received a travel grant of the Helmholtz Graduate School for Polar and Marine research (POLMAR). Stefanie Broszeit funded by an assegno di ricerca di Università di Bologna. Amit Kumar and Elena Ricevuto were funded by a PhD Studentship from the Stazione Zoologica Anton Dohrn di Napoli. Funding for Rebekah Cioffi was provided by a Plymouth University Ph.D Studentship. Alison Bailey was provided funding by the Norwegian Research Council and Norwegian Polar Institute. Leon Green participated through funding from the Swedish Royal Academy of Science. Carina M. Gsottbauer was funded by the Integrated Aquatic Resources Management between Ireland, Northern Ireland and Scotland (IBIS) (www.loughs-agency.org/ibis), project 2859 supported by the European Union's Cross-Border Territorial Co-operation Programme for Northern Ireland, the Border Region of Ireland and Western Scotland (INTERREG IVA) programme managed by the Special EU Programmes Body (www.seupb.eu). Emilie Hall had funding provided by the Plymouth University Marine Institute and School of Marine Science & Engineering University Research Studentship. Maria Lechler was funded by MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca). Francesco P. Mancuso has funding from TETRIS - Observing, modelling and testing synergies and trade-offs for the adaptive management of multiple impacts in coastal systems" (PRIN 2011, Italian Ministry of Education, University and Research). Camila O. Pereira is thankful to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the PhD Scholarship. Research Grant No. 2012/14032-9. Julie B. Schram was funded by the Biology Department, University of Alabama at Birmingham, and travel funding from the Endowed Professorship in Polar and Marine Biology provided to James B. McClintock. Simon Stenberg was supported by the Research Council of Norway, Grant No. 222364/F20. Funding for Lindzai T. Santa Rosa was provided by the Brazilian Agency for Higher Education (Capes).

Author Contributions

Original concept, drafting and editing manuscript: Ben Harvey. Group leaders: molecular and cellular responses – Amit Kumar; whole-organism - Rebekah Cioffi; population-level responses – Balsam Al-Janabi; community composition and interactions – Stefanie Broszeit. Figure 1 – Ben Harvey. All other authors contributed with concept development, writing and commented on the manuscript at all stages.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Schmitz, O.J. Global climate change and the evolutionary ecology of ecosystem functioning. *Annu. N. Y. Acad. Sci.* **2013**, *1297*, 61–72.
2. Bell, G.; Gonzalez, A. Evolutionary rescue can prevent extinction following environmental change. *Ecol. Lett.* **2009**, *12*, 942–948.
3. Burrows, M.T.; Schoeman, D.S.; Buckley, L.B.; Moore, P.; Poloczanska, E.S.; Brander, K.M.; Brown, C.; Bruno, J.F.; Duarte, C.M.; Halpern, B.S.; *et al*. The pace of shifting climate in marine and terrestrial ecosystems. *Science* **2011**, *334*, 652–655.
4. Doney, S.C.; Schimel, D.S. Carbon and climate system coupling on timescales from the precambrian to the anthropocene. *Annu. Rev. Environ. Resour.* **2007**, *32*, 31–66.
5. Halpern, B.S.; Walbridge, S.; Selkoe, K.A.; Kappel, C.V.; Micheli, F.; D'Agrosa, C.; Bruno, J.F.; Casey, K.S.; Ebert, C.; Fox, H.E.; *et al*. A global map of human impact on marine ecosystems. *Science* **2008**, *319*, 948–952.
6. Dupont, S.; Dorey, N.; Thorndyke, M. What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? *Estuar. Coast. Shelf Sci.* **2010**, *89*, 182–185.
7. Kroeker, K.; Kordas, R.; Crim, R.; Singh, G. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.* **2010**, *13*, 1419–1434.
8. Harvey, B.P.; Gwynn-Jones, D.; Moore, P.J. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecol. Evol.* **2013**, *3*, 1016–1030.
9. Kroeker, K.J.; Kordas, R.L.; Crim, R.; Hendriks, I.E.; Ramajo, L.; Singh, G.S.; Duarte, C.M.; Gattuso, J.-P. Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Glob. Change Biol.* **2013**, *19*, 1884–1896.
10. Pörtner, H.-O. Physiological basis of temperature-dependent biogeography: Trade-offs in muscle design and performance in polar ectotherms. *J. Exp. Biol.* **2002**, *205*, 2217–2230.
11. Somero, G.N. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Front. Zool.* **2005**, *2*, 1.
12. Widdicombe, S.; Spicer, J. Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *J. Exp. Mar. Biol. Ecol.* **2008**, *366*, 187–197.
13. Somero, G.N. The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **2010**, *213*, 912–920.
14. Van der Putten, W.H.; Macel, M.; Visser, M.E. Predicting species distribution and abundance responses to climate change: Why it is essential to include biotic interactions across trophic levels. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2010**, *365*, 2025–2034.
15. Stockwell, C.A.; Hendry, A.P.; Kinnison, M.T. Contemporary evolution meets conservation biology. *Trends Ecol. Evol.* **2003**, *18*, 94–101.
16. Munday, P.L.; Warner, R.R.; Monro, K.; Pandolfi, J.M.; Marshall, D.J. Predicting evolutionary responses to climate change in the sea. *Ecol. Lett.* **2013**, *16*, 1488–1500.
17. Sunday, J.M.; Calosi, P.; Dupont, S.; Munday, P.L.; Stillman, J.H.; Reusch, T.B.H. Evolution in an acidifying ocean. *Trends Ecol. Evol.* **2014**, *29*, 117–125.
18. Dupont, S.; Portner, H. Marine science: Get ready for ocean acidification. *Nature* **2013**, *498*, 429–429.
19. Gienapp, P.; Teplitsky, C.; Alho, J.S.; Mills, J.A.; Merilä, J. Climate change and evolution: Disentangling environmental and genetic responses. *Mol. Ecol.* **2008**, *17*, 167–178.

20. Merilä, J.; Hendry, A.P. Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evol. Appl.* **2014**, *7*, 1–14.
21. Chevin, L.-M.; Lande, R.; Mace, G.M. Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biol.* **2010**, *8*, doi:10.1371/journal.pbio.1000357.
22. Hoffmann, A.A.; Sgrò, C.M. Climate change and evolutionary adaptation. *Nature* **2011**, *470*, 479–485.
23. Reusch, T.B.H. Climate change in the oceans: Evolutionary *versus* phenotypically plastic responses of marine animals and plants. *Evol. Appl.* **2013**, *7*, 104–122.
24. Blondel, J. From biogeography to life history theory: A multithematic approach illustrated by the biogeography of vertebrates. *J. Biogeogr.* **1987**, *14*, 405–422.
25. Palumbi, S.R.; Barshis, D.J.; Traylor-Knowles, N.; Bay, R.A. Mechanisms of reef coral resistance to future climate change. *Science* **2014**, *344*, 895–898.
26. Calosi, P.; Rastrick, S.P.S.; Lombardi, C.; de Guzman, H.J.; Davidson, L.; Jahnke, M.; Giangrande, A.; Hardege, J.D.; Schulze, A.; Spicer, J.I.; *et al*. Adaptation and acclimatization to ocean acidification in marine ectotherms: An *in situ* transplant experiment with polychaetes at a shallow CO_2 vent system. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2013**, *368*, doi: 10.1098/rstb.2012.0444.
27. Queirós, A.M.; Fernandes, J.A.; Faulwetter, S.; Nunes, J.; Rastrick, S.P.S.; Mieszkowska, N.; Artioli, Y.; Yool, A.; Calosi, P.; Arvanitidis, C.; *et al*. Scaling up experimental ocean acidification and warming research: From individuals to the ecosystem. *Glob. Change Biol.* **2014**, doi:10.1111/gcb.12675.
28. Prosser, C.L. Physiological variation in animals. *Biol. Rev.* **1955**, *30*, 229–261.
29. Somero, G.N. The physiology of global change: Linking patterns to mechanisms. *Annu. Rev. Mar. Sci.* **2012**, *4*, 39–61.
30. Feder, M.E.; Walser, J.C. The biological limitations of transcriptomics in elucidating stress and stress responses. *J. Evol. Biol.* **2005**, *18*, 901–910.
31. Berg, J.M.; Tymoczko, J.L.; Stryer, L. *Biochemistry*; WH Freeman: New York, NY, USA, 2002; p. 1026.
32. Griffiths, A.J.F.; Miller, J.H.; Suzuki, D.T.; Lewontin, R.C.; Gelbart, W.M. Gene-protein relations. In *An Introduction to Genetic Analysis*, 7th ed.; Griffiths, A.J.F., Ed.; WH Freeman: New York, NY, USA, 2000.
33. Kassahn, K.S.; Crozier, R.H.; Pörtner, H.O.; Caley, M.J. Animal performance and stress: Responses and tolerance limits at different levels of biological organisation. *Biol. Rev. Camb. Philos. Soc.* **2009**, *84*, 277–292.
34. Fields, P.A.; Rudomin, E.L.; Somero, G.N. Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): Sequence-function linkages and correlations with biogeographic distribution. *J. Exp. Biol.* **2006**, *209*, 656–667.
35. Powers, D.A.; Schulte, P.M. Evolutionary adaptations of gene structure and expression in natural populations in relation to a changing environment: A multidisciplinary approach to address the million-year saga of a small fish. *J. Exp. Zool.* **1998**, *282*, 71–94.
36. Norry, F.M.; Larsen, P.F.; Liu, Y.; Loeschcke, V. Combined expression patterns of QTL-linked candidate genes best predict thermotolerance in *Drosophila melanogaster*. *J. Insect Physiol.* **2009**, *55*, 1050–1057.

37. Franks, S.J.; Hoffmann, A.A. Genetics of climate change adaptation. *Annu. Rev. Genet.* **2012**, *46*, 185–208.
38. Davidson, E.H. *The Regulatory Genome: Gene Regulatory Networks in Development and Evolution*; Academic Press: San Diego, CA, USA, 2006.
39. Davidson, E.; Levin, M. Gene regulatory networks. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4935.
40. Williams, S.E.; Shoo, L.P.; Isaac, J.L.; Hoffmann, A.A.; Langham, G. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* 2008, *6*, e325.
41. Bradshaw, W.E.; Holzapfel, C.M. Light, time, and the physiology of biotic response to rapid climate change in animals. *Annu. Rev. Physiol.* **2010**, *72*, 147–166.
42. Atkinson, N.J.; Urwin, P.E. The interaction of plant biotic and abiotic stresses: From genes to the field. *J. Exp. Bot.* **2012**, *63*, 3523–3543.
43. Gardner, J.L.; Peters, A.; Kearney, M.R.; Joseph, L.; Heinsohn, R. Declining body size: A third universal response to warming? *Trends Ecol. Evol.* **2011**, *26*, 285–291.
44. Kovach, R.P.; Gharrett, A.J.; Tallmon, D.A. Genetic change for earlier migration timing in a pink salmon population. *Proc. R. Soc. Biol. Sci. Ser. B* **2012**, *279*, 3870–3878.
45. Karell, P.; Ahola, K.; Karstinen, T.; Valkama, J.; Brommer, J.E. Climate change drives microevolution in a wild bird. *Nat. Commun.* **2011**, *2*, 208.
46. Runcie, D.E.; Garfield, D.A.; Babbitt, C.C.; Wygoda, J.A.; Mukherjee, S.; Wray, G.A. Genetics of gene expression responses to temperature stress in a sea urchin gene network. *Mol. Ecol.* **2012**, *21*, 4547–4562.
47. Keller, B.; Feuillet, C. Colinearity and gene density in grass genomes. *Trends Plant Sci.* **2000**, *5*, 246–251.
48. Barshis, D.J.; Ladner, J.T.; Oliver, T.A.; Seneca, F.O.; Traylor-Knowles, N.; Palumbi, S.R. Genomic basis for coral resilience to climate change. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 1387–1392.
49. Todgham, A.E.; Hofmann, G.E. Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO_2-driven seawater acidification. *J. Exp. Biol.* **2009**, *212*, 2579–2594.
50. Wray, G.A. The evolutionary significance of *cis*-regulatory mutations. *Nat. Rev. Genet.* **2007**, *8*, 206–216.
51. Barrett, R.D.; Hoekstra, H.E. Molecular spandrels: Tests of adaptation at the genetic level. *Nat. Rev. Genet.* **2011**, *12*, 767–780.
52. Davidson, W.S. Adaptation genomics: next generation sequencing reveals a shared haplotype for rapid early development in geographically and genetically distant populations of rainbow trout. *Mol. Ecol.* **2012**, *21*, 219–222.
53. Edwards, S.V. Next-generation QTL mapping: Crowdsourcing SNPs, without pedigrees. *Mol. Ecol.* **2013**, *22*, 3885–3887.
54. Kaniewska, P.; Campbell, P.R.; Kline, D.I.; Rodriguez-Lanetty, M.; Miller, D.J.; Dove, S.; Hoegh-Guldberg, O. Major cellular and physiological impacts of ocean acidification on a reef building coral. *PLoS ONE* **2012**, *7*, e34659.
55. Sokolova, I.M.; Frederich, M.; Bagwe, R.; Lannig, G.; Sukhotin, A.A. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar. Environ. Res.* **2012**, *79*, 1–15.

56. Seebacher, F.; Franklin, C.E. Determining environmental causes of biological effects: The need for a mechanistic physiological dimension in conservation biology. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2012**, *367*, 1607–1614.
57. Buckley, B.A.; Gracey, A.Y.; Somero, G.N., The cellular response to heat stress in the goby Gillichthys mirabilis: a cDNA microarray and protein-level analysis. *J. Exp. Biol.* **2006**, *209*, 2660-2677.
58. Wapinski, I.; Pfeffer, A.; Friedman, N.; Regev, A., Natural history and evolutionary principles of gene duplication in fungi. *Nature* **2007**, *449*, 54-61.
59. Raffaele, S.; Farrer, R.A.; Cano, L.M.; Studholme, D.J.; MacLean, D.; Thines, M.; Jiang, R.H.Y.; Zody, M.C.; Kunjeti, S.G.; Donofrio, N.M.; Meyers, B.C.; Nusbaum, C.; Kamoun, S., Genome evolution following host jumps in the Irish potato famine pathogen lineage. *Science* **2010**, *330*, 1540-1543.
60. Hartl, D.L. *Essential Genetics: A Genomics Perspective*; Jones and Bartlett Publishers: London, UK, 2014.
61. Pires-daSilva, A.; Sommer, R.J. The evolution of signalling pathways in animal development. *Nat. Rev. Genet.* **2003**, *4*, 39–49.
62. Garcia-Moreno, B. Adaptations of proteins to cellular and subcellular pH. *J. Biol.* **2009**, *8*, 98.
63. Salinas, S.; Munch, S.B. Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecol. Lett.* **2012**, *15*, 159–163.
64. Jaenisch, R.; Bird, A. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nat. Genet.* **2003**, *33*, 245–254.
65. Turner, B.M. Epigenetic responses to environmental change and their evolutionary implications. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 3403–3418.
66. Parker, L.M.; Ross, P.M.; O'Connor, W.A.; Borysko, L.; Raftos, D.A.; Pörtner, H.-O. Adult exposure influences offspring response to ocean acidification in oysters. *Glob. Change Biol.* **2012**, *18*, 82–92.
67. Garfield, D.A.; Runcie, D.E.; Babbitt, C.C.; Haygood, R.; Nielsen, W.J.; Wray, G.A. The Impact of gene expression variation on the robustness and evolvability of a developmental Gene Regulatory Network. *PLoS Biol.* **2013**, *11*, doi:10.1371/journal.pbio.1001696.
68. Suchanek, T.H. The role of disturbance in the evolution of life history strategies in the intertidal mussels *Mytilus edulis* and *Mytilus californianus*. *Oecologia* **1981**, *50*, 143–152.
69. Dawson, M.N. Phylogeography in coastal marine animals: A solution from California? *J. Biogeogr.* **2001**, *28*, 723–736.
70. Sherman, C.D.H.; Hunt, A.; Ayre, D.J. Is life history a barrier to dispersal? Contrasting patterns of genetic differentiation along an oceanographically complex coast. *Biol. J. Linn. Soc.* **2008**, *95*, 106–116.
71. Sunday, J.M.; Crim, R.N.; Harley, C.D.G.; Hart, M.W. Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS ONE* **2011**, *6*, e22881.
72. Brown, J.H.; Gillooly, J.F.; Allen, A.P.; Savage, V.M.; West, G.B. Toward a metabolic theory of ecology. *Ecology* **2004**, *85*, 1771–1789.
73. Kooijman, S.A.L.M. *Dynamic Energy Budget Theory for Metabolic Organisation*; Cambridge University Press: Cambridge, UK, 2010; p. 514.
74. Holcomb M., McCorkle D.C., Cohen A.L. Long-term effects of nutrient and CO_2 enrichment on the temperate coral *astrangia poculata* (Ellis and Solander, 1786). *J. Exp. Mar. Biol. Ecol.* **2010**, *386*, 27-33.

75. Foo, S.A.; Dworjanyn, S.A.; Poore, A.G.B.; Byrne, M. Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: Performance of early embryos. *PLoS ONE* **2012**, *7*, e42497.
76. Langenbuch, M.; Pörtner, H.O. High sensitivity to chronically elevated CO_2 levels in a eurybathic marine sipunculid. *Aquat. Toxicol.* **2004**, *70*, 55–61.
77. Kurihara, H. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Mar. Ecol. Prog. Ser.* **2008**, *373*, 275–284.
78. Pörtner, H.-O. Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view. *Mar. Ecol. Prog. Ser.* **2008**, *373*, 203–217.
79. O'Connor, M.I.; Bruno, J.F.; Gaines, S.D.; Halpern, B.S.; Lester, S.E.; Kinlan, B.P.; Weiss, J.M. Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 1266–1271.
80. Arnold, K.; Findlay, H.; Spicer, J.; Daniels, C.; Boothroyd, D. Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). *Biogeosciences* **2009**, *6*, 1747–1754.
81. McDonald, M.R.; McClintock, J.B.; Amsler, C.D.; Rittschof, D.; Angus, R.A.; Orihuela, B.; Lutostanski, K. Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. *Mar. Ecol. Prog. Ser.* **2009**, *385*, 179–187.
82. Byrne, M. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: Vulnerabilities and potential for persistence in a changing ocean. *Oceanogr. Mar. Biol.* **2011**, *49*, 1–42.
83. Miller, N.A.; Paganini, A.W.; Stillman, J.H. Differential thermal tolerance and energetic trajectories during ontogeny in porcelain crabs, genus *Petrolisthes*. *J. Therm. Biol.* **2013**, *38*, 79–85.
84. Emlet, R.B.; Sadro, S.S. Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). *Integr. Comp. Biol.* **2006**, *46*, 334–346.
85. Werner, E.E.; Gilliam, J.F. The ontogenetic niche and species interactions in size-structured populations. *Annu. Rev. Ecol. Syst.* **1984**, *15*, 393–425.
86. Dupont, S.; Dorey, N.; Stumpp, M.; Melzner, F.; Thorndyke, M.C. Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Mar. Biol.* **2012**, *160*, 1835–1843.
87. Ferrari, M.C.O.; Manassa, R.P.; Dixson, D.L.; Munday, P.L.; McCormick, M.I.; Meekan, M.G.; Sih, A.; Chivers, D.P. Effects of ocean acidification on learning in coral reef fishes. *PLoS ONE* **2012**, *7*, e31478.
88. Candolin, U.; Wong, B.B.M. *Behavioural Responses to a Changing World: Mechanisms and Consequences*; Oxford University Press: Oxford, UK, 2012; pp. 208–210.
89. Dissanayake, A.; Ishimatsu, A. Synergistic effects of elevated CO_2 and temperature on the metabolic scope and activity in a shallow-water coastal decapod (*Metapenaeus joyneri*; Crustacea: Penaeidae). *ICES J. Mar. Sci.* **2011**, *68*, 1147–1154.
90. Biro, P.A.; Beckmann, C.; Stamps, J.A. Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proc. R. Soc. Biol. Sci. B* **2010**, *277*, 71–77.

91. Watson, S.-A.; Lefevre, S.; McCormick, M.I.; Domenici, P.; Nilsson, G.E.; Munday, P.L. Marine mollusc predator-escape behaviour altered by near-future carbon dioxide levels. *Proc. R. Soc. Biol. Sci. B* **2014**, *281*, 20132377.
92. Nilsson, G.E.; Dixson, D.L.; Domenici, P.; McCormick, M.I.; Sørensen, C.; Watson, S.-A.; Munday, P.L. Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nat. Clim. Change* **2012**, *2*, 201–204.
93. Chivers, D.P.; McCormick, M.I.; Nilsson, G.E.; Munday, P.L.; Watson, S.-A.; Meekan, M.G.; Mitchell, M.D.; Corkill, K.C.; Ferrari, M.C.O. Impaired learning of predators and lower prey survival under elevated CO_2: A consequence of neurotransmitter interference. *Glob. Change Biol.* **2014**, *20*, 515–522.
94. Munday, P.L.; Dixson, D.L.; Donelson, J.M.; Jones, G.P.; Pratchett, M.S.; Devitsina, G.V.; Døving, K.B. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 1848.
95. Goh, C.S.; Bogan, A.A.; Joachimiak, M.; Walther, D.; Cohen, F.E. Co-evolution of proteins with their interaction partners. *J. Mol. Biol.* **2000**, *299*, 283–293.
96. Greenwood, A.K.; Wark, A.R.; Yoshida, K.; Peichel, C.L. Genetic and neural modularity underlie the evolution of schooling behavior in threespine sticklebacks. *Curr. Biol.* **2013**, *23*, 1884–1888.
97. Blomberg, S.P.; Garland, T.; Ives, A.R. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **2003**, *57*, 717–745.
98. Bradshaw, A.D.; McNeilly, T. Evolutionary response to global climatic change. *Ann. Bot. Lond.* **1991**, *67*, 5–14.
99. Bohonak, A.J. Dispersal, gene flow, and population structure. *Q. Rev. Biol.* **1999**, *74*, 21–45.
100. Hellberg, M.E.; Burton, R.S.; Neigel, J.E.; Palumbi, S.R. Genetic assessment of connectivity among marine populations. *Bull. Mar. Sci.* **2002**, *70*, 273–290.
101. Sanford, E.; Kelly, M.W. Local adaptation in marine invertebrates. *Ann. Rev. Mar. Sci.* **2011**, *3*, 509–535.
102. Merilä, J. Evolution in response to climate change: In pursuit of the missing evidence. *BioEssays* **2012**, *34*, 811–818.
103. Gonzalez, A.; Ronce, O.; Ferriere, R.; Hochberg, M.E. Evolutionary rescue: An emerging focus at the intersection between ecology and evolution. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2013**, *368*, doi:10.1098/rstb.2012.0404.
104. Johannesson, K.; Smolarz, K.; Grahn, M.; André, C. The future of Baltic Sea populations: Local extinction or evolutionary rescue? *Ambio* **2011**, *40*, 179–190.
105. Bell, G. Evolutionary rescue and the limits of adaptation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2013**, *368*, doi:10.1098/rstb.2012.0080.
106. Cuveliers, E.L.; Volckaert, F.A.M.; Rijnsdorp, A.D.; Larmuseau, M.H.D.; Maes, G.E. Temporal genetic stability and high effective population size despite fisheries-induced life-history trait evolution in the North Sea sole. *Mol. Ecol.* **2011**, *20*, 3555–3568.
107. Reusch, T.B.H.; Ehlers, A.; Hämmerli, A.; Worm, B. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 2826–2831.
108. Lohbeck, K.T.; Riebesell, U.; Reusch, T.B.H. Adaptive evolution of a key phytoplankton species to ocean acidification. *Nat. Geosci.* **2012**, *5*, 346–351.

109. Jin, P.; Gao, K.; Beardall, J. Evolutionary responses of a coccolithophorid *Gephyrocapsa oceanica* to ocean acidification. *Evolution* **2013**, *67*, 1869–1878.
110. Crawfurd, K.J.; Raven, J.A.; Wheeler, G.L.; Baxter, E.J.; Joint, I. The response of *Thalassiosira pseudonana* to long-term exposure to increased CO_2 and decreased pH. *PLoS ONE* **2011**, *6*, e26695.
111. Smith-Keune, C.; Oppen, M. Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef. *Coral Reefs* **2006**, *25*, 493–502.
112. D'Croz, L.; Maté, J.L. Experimental responses to elevated water temperature in genotypes of the reef coral *Pocillopora damicornis* from upwelling and non-upwelling environments in Panama. *Coral Reefs* **2004**, *23*, 473–483.
113. Yanick, J.F.; Heath, J.W.; Heath, D.D. Survival and growth of local and transplanted blue mussels (*Mytilus trossulus*, Lamark). *Aquac. Res.* **2003**, *34*, 869–875.
114. De Wit, P.; Palumbi, S.R. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. *Mol. Ecol.* **2013**, *22*, 2884–2897.
115. Gaston, K.J.; Spicer, J.I. Do upper thermal tolerances differ in geographically separated populations of the beachflea *Orchestia gammarellus* (Crustacea: Amphipoda)? *J. Exp. Mar. Biol. Ecol.* **1998**, *229*, 265–276.
116. Schmidt, C.; Heinz, P.; Kucera, M.; Uthicke, S. Temperature-induced stress leads to bleaching in larger benthic foraminifera hosting endosymbiotic diatoms. *Limnol. Oceanogr.* **2011**, *56*, 1587–1602.
117. Kelly, M.W.; Sanford, E.; Grosberg, R.K. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc. R. Soc. Biol. Sci. B* **2012**, *279*, 349–356.
118. Sanford, E.; Holzman, S.B.; Haney, R.A.; Rand, D.M.; Bertness, M.D. Larval tolerance, gene flow, and the northern geographic range limit of fiddler crabs. *Ecology* **2006**, *87*, 2882–2894.
119. Lymbery, R.A.; Evans, J.P. Genetic variation underlies temperature tolerance of embryos in the sea urchin *Heliocidaris erythrogramma armigera*. *J. Evol. Biol.* **2013**, *26*, 2271–2282.
120. Osovitz, C.J.; Hofmann, G.E. Thermal history-dependent expression of the *hsp70* gene in purple sea urchins: Biogeographic patterns and the effect of temperature acclimation. *J. Exp. Mar. Biol. Ecol.* **2005**, *327*, 134–143.
121. Kelly, M.W.; Padilla-Gamiño, J.L.; Hofmann, G.E. Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Glob. Change Biol.* **2013**, *19*, 2536–2546.
122. Pecorino, D.; Barker, M.F.; Dworjanyn, S.A.; Byrne, M.; Lamare, M.D. Impacts of near future sea surface pH and temperature conditions on fertilisation and embryonic development in *Centrostephanus rodgersii* from northern New Zealand and northern New South Wales, Australia. *Mar. Biol.* **2014**, *161*, 101–110.
123. Marcil, J.; Swain, D.P.; Hutchings, J.A. Countergradient variation in body shape between two populations of Atlantic cod (*Gadus morhua*). *Proc. R. Soc. Biol. Sci. B* **2006**, *273*, 217–223.
124. Fangue, N.A.; Hofmeister, M.; Schulte, P.M. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. *J. Exp. Biol.* **2006**, *209*, 2859–2872.
125. Schaum, E.; Rost, B.; Millar, A.J.; Collins, S. Variation in plastic responses of a globally distributed picoplankton species to ocean acidification. *Nature Clim. Change* **2012**, *3*, 298–302.

126. Pistevos, J.C.A.; Calosi, P.; Widdicombe, S.; Bishop, J.D.D. Will variation among genetic individuals influence species responses to global climate change? *Oikos* **2011**, *120*, 675–689.
127. Willi, Y.; Van Buskirk, J.; Hoffmann, A.A. Limits to the adaptive potential of small populations. *Annu. Rev. Ecol. Evol. Syst.* **2006**, *37*, 433–458.
128. Lande, R. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evol. Biol.* **2009**, *22*, 1435–1446.
129. Feely, R.A.; Sabine, C.L.; Hernandez-Ayon, J.M.; Ianson, D.; Hales, B. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science* **2008**, *320*, 1490–1492.
130. Evans, T.G.; Chan, F.; Menge, B.A.; Hofmann, G.E. Transcriptomic responses to ocean acidification in larval sea urchins from a naturally variable pH environment. *Mol. Ecol.* **2013**, *22*, 1609–1625.
131. Saderne, V.; Wahl, M. Differential responses of calcifying and non-calcifying epibionts of a brown macroalga to present-day and future upwelling pCO_2. *PLoS ONE* **2013**, *8*, e70455.
132. Leal, S.M. Genetics and Analysis of Quantitative Traits. *Am. J. Hum. Genet.* **2001**, *68*, 548–549.
133. Hughes, A.R.; Inouye, B.D.; Johnson, M.T.J.; Underwood, N.; Vellend, M. Ecological consequences of genetic diversity. *Ecol. Lett.* **2008**, *11*, 609–623.
134. Pespeni, M.H.; Sanford, E.; Gaylord, B.; Hill, T.M.; Hosfelt, J.D.; Jaris, H.K.; LaVigne, M.; Lenz, E.A.; Russell, A.D.; Young, M.K.; *et al*. Evolutionary change during experimental ocean acidification. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6937–6942.
135. Hedrick, P.W. Genetic Polymorphism in Heterogeneous Environments: The Age of Genomics. *Annu. Rev. Ecol., Evol., Syst.* **2006**, *37*, 67–93.
136. Intergovernmental Panel on Climate Change. *Climate Change 2013—The Physical Science Basis*; Cambridge University Press: Cambridge, UK, 2014.
137. Thomsen, J.; Casties, I.; Pansch, C.; Körtzinger, A.; Melzner, F. Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: Laboratory and field experiments. *Glob. Change Biol.* **2012**, *19*, 1017–1027.
138. Thomsen, J.; Gutowska, M.A.; Saphörster, J.; Heinemann, A.; Trübenbach, K.; Fietzke, J.; Hiebenthal, C.; Eisenhauer, A.; Körtzinger, A.; Wahl, M.; *et al*. Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences* **2010**, *7*, 3879–3891.
139. Dam, H.G. Evolutionary adaptation of marine zooplankton to global change. *Annu. Rev. Mar. Sci.* **2013**, *5*, 349–370.
140. Conover, D.O.; Present, T.M.C. Countergradient variation in growth rate: Compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia* **1990**, *83*, 316–324.
141. Hairston, N.G.; Ellner, S.P.; Geber, M.A.; Yoshida, T.; Fox, J.A. Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* **2005**, *8*, 1114–1127.
142. Olsen, E.M.; Heino, M.; Lilly, G.R.; Morgan, M.J.; Brattey, J.; Ernande, B.; Dieckmann, U. Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature* **2004**, *428*, 932–935.
143. Schoener, T.W. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* **2011**, *331*, 426–429.
144. Palkovacs, E.P.; Marshall, M.C.; Lamphere, B.A.; Lynch, B.R.; Weese, D.J.; Fraser, D.F.; Reznick, D.N.; Pringle, C.M.; Kinnison, M.T. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. Trans. R. Soc. B* **2009**, *364*, 1617–1628.

145. Janzen, D.H. When is it coevolution. *Evolution* **1980**, *34*, 611–612.
146. Turcotte, M.M.; Corrin, M.S.; Johnson, M.T. Adaptive evolution in ecological communities. *PLoS Biol.* **2012**, *10*, doi:10.1371/journal.pbio.1001332.
147. Strauss, S.Y.; Sahli, H.; Conner, J.K. Toward a more trait-centered approach to diffuse (co)evolution. *New Phytol.* **2005**, *165*, 81–89.
148. Lai, S.-M.; Liu, W.-C.; Jordán, F. On the centrality and uniqueness of species from the network perspective. *Biol. Lett.* **2012**, *8*, 570–573.
149. Hale, R.; Calosi, P.; McNeill, L.; Mieszkowska, N.; Widdicombe, S. Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos* **2011**, *120*, 661–674.
150. Diaz-Pulido, G.; Gouezo, M.; Tilbrook, B.; Dove, S.; Anthony, K. High CO_2 enhances the competitive strength of seaweeds over corals. *Ecol. Lett.* **2011**, *14*, 156–162.
151. Ferrari, M.C.O.; McCormick, M.I.; Munday, P.L.; Meekan, M.G.; Dixson, D.L.; Lonnstedt, Ö.; Chivers, D.P. Putting prey and predator into the CO_2 equation—Qualitative and quantitative effects of ocean acidification on predator–prey interactions. *Ecol. Lett.* **2011**, *14*, 1143–1148.
152. Sanford, E.; Gaylord, B.; Hettinger, A.; Lenz, E.A.; Meyer, K.; Hill, T.M. Ocean acidification increases the vulnerability of native oysters to predation by invasive snails. *Proc. R. Soc. Biol. Sci. B* **2014**, *281*, 20132681.
153. O'Connor, M.I. Warming strengthens an herbivore-plant interaction. *Ecology* **2009**, *90*, 388–398.
154. Poore, A.G.B.; Graba-Landry, A.; Favret, M.; Sheppard Brennand, H.; Byrne, M.; Dworjanyn, S.A. Direct and indirect effects of ocean acidification and warming on a marine plant–herbivore interaction. *Oecologia* **2013**, *173*, 1113–1124.
155. Collins, S.; Rost, B.; Rynearson, T.A. Evolutionary potential of marine phytoplankton under ocean acidification. *Evol. Appl.* **2014**, *7*, 140–155.
156. Falkowski, P.G.; Fenchel, T.; Delong, E.F. The microbial engines that drive Earth's biogeochemical cycles. *Science* **2008**, *320*, 1034–1039.
157. Iglesias-Rodriguez, M.; Halloran, P.; Rickaby, R.; Hall, I.; Colmenero-Hidalgo, E.; Gittins, J.; Green, D.; Tyrrell, T.; Gibbs, S.; von Dassow, P.; *et al*. Phytoplankton calcification in a high-CO_2 world. *Science* **2008**, *320*, 336–340.
158. Harley, C.D.G.; Anderson, K.M.; Demes, K.W.; Jorve, J.P.; Kordas, R.L.; Coyle, T.A.; Graham, M.H. Effects of climate change on global seaweed communities. *J. Phycol.* **2012**, *48*, 1064–1078.
159. Hurd, C.L.; Hepburn, C.D.; Currie, K.I.; Raven, J.A.; Hunter, K.A. Testing the effects of ocean acidification on algal metabolism: Considerations for experimental designs. *J. Phycol.* **2009**, *45*, 1236–1251.
160. Tatters, A.O.; Schnetzer, A.; Fu, F.; Lie, A.Y.A.; Caron, D.A.; Hutchins, D.A. Short- *versus* long-term responses to changing CO_2 in a coastal dinoflagellate bloom: Implications for interspecific competitive interactions and community structure. *Evolution* **2013**, *67*, 1879–1891.
161. Kremp, A.; Godhe, A.; Egardt, J.; Dupont, S.; Suikkanen, S.; Casabianca, S.; Penna, A. Intraspecific variability in the response of bloom-forming marine microalgae to changed climate conditions. *Ecol. Evol.* **2012**, *2*, 1195–1207.
162. Boyd, P.W.; Rynearson, T.A.; Armstrong, E.A.; Fu, F.; Hayashi, K.; Hu, Z.; Hutchins, D.A.; Kudela, R.M.; Litchman, E.; Mulholland, M.R.; *et al*. Marine phytoplankton temperature *versus* growth

responses from polar to tropical waters—Outcome of a scientific community-wide study. *PLoS ONE* **2013**, *8*, e63091.
163. Alsterberg, C.; Eklöf, J.S.; Gamfeldt, L.; Havenhand, J.N.; Sundbäck, K. Consumers mediate the effects of experimental ocean acidification and warming on primary producers. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 8603–8608.
164. Witt, V.; Wild, C.; Anthony, K.R.N.; Diaz-Pulido, G.; Uthicke, S. Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef. *Environ. Microbiol.* **2011**, *13*, 2976–2989.
165. Beaugrand, G.; Brander, K.M.; Alistair Lindley, J.; Souissi, S.; Reid, P.C. Plankton effect on cod recruitment in the North Sea. *Nature* **2003**, *426*, 661–664.
166. Melzner, F.; Stange, P.; Trübenbach, K.; Thomsen, J.; Casties, I.; Panknin, U.; Gorb, S.N.; Gutowska, M.A. Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE* **2011**, *6*, e24223.
167. Calosi, P.; Rastrick, S.P.S.; Graziano, M.; Thomas, S.C.; Baggini, C.; Carter, H.A.; Hall-Spencer, J.M.; Milazzo, M.; Spicer, J.I. Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid-base and ion-regulatory abilities. *Mar. Pollut. Bull.* **2013**, *73*, 470–484.
168. Connell, S.D.; Kroeker, K.J.; Fabricius, K.E.; Kline, D.I.; Russell, B.D. The other ocean acidification problem: CO_2 as a resource among competitors for ecosystem dominance. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2013**, *368*, doi:10.1098/rstb.2012.0442.
169. Kroeker, K.J.; Micheli, F.; Gambi, M.C. Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nat. Clim. Change* **2012**, *3*, 156–159.
170. Connell, S.; Russell, B. The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proc. R. Soc. Biol. Sci. B* **2010**, *277*, 1409–1415.
171. de Mazancourt, C.; Johnson, E.; Barraclough, T.G. Biodiversity inhibits species' evolutionary responses to changing environments. *Ecol. Lett.* **2008**, *11*, 380–388.
172. Johansson, J. Evolutionary responses to climate changes: how does competition affect adaptation? *Evolution* **2008**, *62*, 421–435.
173. Fabricius, K.E.; Langdon, C.; Uthicke, S.; Humphrey, C.; Noonan, S.; De'ath, G.; Okazaki, R.; Muehllehner, N.; Glas, M.S.; Lough, J.M. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nat. Clim. Change* **2011**, *1*, 165–169.
174. Dunn, R.R.; Harris, N.C.; Colwell, R.K.; Koh, L.P.; Sodhi, N.S. The sixth mass coextinction: Are most endangered species parasites and mutualists? *Proc. R. Soc. Biol. Sci. B* **2009**, *276*, 3037–3045.
175. Stenseth, N.C.; Smith, J.M. Coevolution in ecosystems: Red queen evolution or stasis? *Evolution* **1984**, *38*, 870–880.
176. Lawrence, D.; Fiegna, F.; Behrends, V.; Bundy, J.G.; Phillimore, A.B.; Bell, T.; Barraclough, T.G. Species interactions alter evolutionary responses to a novel environment. *PLoS Biol.* **2012**, *10*, doi:10.1371/journal.pbio.1001330.
177. Chesson, P. A need for niches? *Trends Ecol. Evol.* **1991**, *6*, 26–28.
178. Byrnes, J.E.; Gamfeldt, L.; Isbell, F.; Lefcheck, J.S.; Griffin, J.N.; Hector, A.; Cardinale, B.J.; Hooper, D.U.; Dee, L.E.; Emmett Duffy, J. Investigating the relationship between biodiversity and ecosystem multifunctionality: Challenges and solutions. *Meth. Ecol. Evol.* **2014**, *5*, 111–124.

179. Pereira, H.M.; Leadley, P.W.; Proença, V.; Alkemade, R.; Scharlemann, J.P.W.; Fernandez-Manjarrés, J.F.; Araújo, M.B.; Balvanera, P.; Biggs, R.; Cheung, W.W.L.; *et al*. Scenarios for global biodiversity in the 21st century. *Science* **2010**, *330*, 1496–1501.
180. Burrows, M.T.; Schoeman, D.S.; Richardson, A.J.; Molinos, J.G.; Hoffmann, A.; Buckley, L.B.; Moore, P.J.; Brown, C.J.; Bruno, J.F.; Duarte, C.M.; *et al*. Geographical limits to species-range shifts are suggested by climate velocity. *Nature* **2014**, *507*, 492–495.
181. Pespeni, M.H.; Chan, F.; Menge, B.A.; Palumbi, S.R. Signs of adaptation to local pH conditions across an environmental mosaic in the California current ecosystem. *Integr. Comp. Biol.* **2013**, *53*, 857–870.
182. Ricevuto, E., Kroeker, K.J., Ferrigno, F., Micheli, F., Gambi, M.C. Spatio-temporal variability of polychaete colonization at CO_2 vents indicates high tolerance to ocean acidification. *Mar. Biol.* **2014**, *161*, 2909-2919.
183. Hughes, T.P.; Baird, A.H.; Bellwood, D.R.; Card, M.; Connolly, S.R.; Folke, C.; Grosberg, R.; Hoegh-Guldberg, O.; Jackson, J.B.C.; Kleypas, J.; *et al*. Climate change, human impacts, and the resilience of coral reefs. *Science* **2003**, *301*, 929–933.
184. Bellantuono, A.J.; Hoegh-Guldberg, O.; Rodriguez-Lanetty, M. Resistance to thermal stress in corals without changes in symbiont composition. *Proc. R. Soc. Biol. Sci. B* **2012**, *279*, 1100–1107.
185. Barshis, D.; Ladner, J.; Oliver, T.; Palumbi, S.R. Lineage specific transcriptional profiles of *Symbiodinium* spp. unaltered by heat stress in a coral host. *Mol. Biol. Evol.* **2014**, *31*, 1343–1352.
186. Benton, M.J. The Red Queen and the Court Jester: Species diversity and the role of biotic and abiotic factors through time. *Science* **2009**, *323*, 728–732.
187. Drake, J.M.; Lodge, D.M. Hull fouling is a risk factor for intercontinental species exchange in aquatic ecosystems. *Aq. Inv.* **2007**, *2*, 121–131.
188. Walther, G.-R.; Roques, A.; Hulme, P.E.; Sykes, M.T.; Pysek, P.; Kühn, I.; Zobel, M.; Bacher, S.; Botta-Dukát, Z.; Bugmann, H.; *et al*. Alien species in a warmer world: Risks and opportunities. *Trends Ecol. Evol.* **2009**, *24*, 686–693.
189. Bianchi, C.N. Biodiversity issues for the forthcoming tropical Mediterranean Sea. *Hydrobiologia* **2007**, *580*, 7–21.
190. Irwin, A.J.; Finkel, Z.V.; Schofield, O.M.E.; Falkowski, P.G. Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. *J. Plankton Res.* **2006**, *28*, 459–471.
191. Levine, J.M.; Vilà, M.; Antonio, C.M.D.; Dukes, J.S.; Grigulis, K.; Lavorel, S. Mechanisms underlying the impacts of exotic plant invasions. *Proc. R. Soc. Biol. Sci. B* **2003**, *270*, 775–781.
192. Schluter, D. Experimental evidence that competition promotes divergence in adaptive radiation. *Science* **1994**, *266*, 798–801.
193. Halpern, B.S.; Selkoe, K.A.; Micheli, F.; Kappel, C.V. Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. *Conserv. Biol.* **2007**, *21*, 1301–1315.
194. Barton, A.D.; Pershing, A.J.; Litchman, E.; Record, N.R.; Edwards, K.F.; Finkel, Z.V.; Kiørboe, T.; Ward, B.A. The biogeography of marine plankton traits. *Ecol. Lett.* **2013**, *16*, 522–534.
195. Reusch, T.B.H.; Boyd, P.W. Experimental evolution meets marine phytoplankton. *Evolution* **2013**, *67*, 1849–1859.
196. Russell, B.D.; Wernberg, T.; Mieszkowska, N.; Widdicombe, S.; Hall-Spencer, J.; Connell, S. Predicting ecosystem shifts requires new approaches that integrate the effects of climate change across entire systems. *Biol. Lett.* **2012**, *8*, 164–166.

197. Hendriks, I.E.; Duarte, C.M. Ocean acidification: Separating evidence from judgment—A reply to Dupont *et al. Estuar. Coast. Shelf Sci.* **2010**, *89*, 186–190.
198. Gaylord, B.; Hill, T.M.; Sanford, E.; Lenz, E.A.; Jacobs, L.A.; Sato, K.N.; Russell, A.D.; Hettinger, A. Functional impacts of ocean acidification in an ecologically critical foundation species. *J. Exp. Biol.* **2011**, *214*, 2586–2594.
199. Vihtakari, M.; Hendriks, I.; Holding, J.; Renaud, P.; Duarte, C.; Havenhand, J. Effects of ocean acidification and warming on sperm activity and early life stages of the Mediterranean mussel (*Mytilus galloprovincialis*). *Water* **2013**, *5*, 1890–1915.
200. Van Valen, L. A new evolutionary law. *Evol. Theor.* **1973**, *1*, 1–30.