From behaviour to bathymetric ranges: examining the responses of marine invertebrates to hydrostatic pressure

By

Justine Ammendolia

A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the

requirements for the degree of

Master of Science

Department of Ocean Sciences

Memorial University of Newfoundland

July 2017

St. John's Newfoundland

Abstract

Although hydrostatic pressure is one of the most prominent abiotic drivers of faunal bathymetric ranges, it is one of the least understood. As climate change drives warmer temperatures, it is hypothesized that benthic communities may undergo vertical shifts from shallow to deeper depths. Expanding our understanding of the impact of pressure on marine organisms is therefore important. Here, I first synthesized and analyzed >130 studies reporting survival of >260 shallow and deep-sea taxa after exposure to non-native pressure. Many deep-sea species survived and bred under low or atmospheric pressure (slightly below sea surface depth), especially those from higher latitudes, and tolerance in adults was influenced by phylum. Next, I used high-pressure chambers to test the response of several subtidal echinoderms to various pressure levels, durations and pH conditions. Responses to acute pressure shifts suggest that deep-sea species are relatively tolerant to depressurization, but shallow-water species are less likely to maintain critical behaviours if moved to pressures beyond their current bathymetric ranges.

Acknowledgements

I would like to thank my adviser Annie Mercier for her invaluable support and encouragement during this career step. She truly has contributed significantly to the foundation and development of my scientific career and I appreciate her unconditional patience as a mentor. Special acknowledgements go to Jean-François Hamel, for his continuous encouragement and collaboration.

There are many other people I would like to extend my gratitude whom have enhanced the quality of my research and overall graduate experience. This work would not be possible without CDRF and the accommodating management of Stephen Hills and the immensely helpful technical support of Gordon Nash. I thank my committee members, Suzanne Dufour and Bill Driedzic, for their constructive contributions towards my research and to Field Services (Department of Ocean Sciences, Memorial University) for specimen collections. I am thankful for the experience of participating on a deep-sea trawl facilitated by the Department of Fisheries and Oceans Canada and the Canadian Coast Guard. My research was funded by grants awarded to Annie Mercier by NSERC and CFI. Funding from the School of Graduate Studies, the Faculty of Science and the Department of Ocean Science allowed me the opportunity to present at the following conferences: CSEE, IMCC and ASLO. Furthermore I would like to thank the whole Mercier Lab: Emy Montgomery, Camilla Parzanini, Matt Osse, Bruno Gianasi, Jiamin Sun and Leah Robertson.

I sincerely appreciate the professional support and friendship of Emy Montgomery and Camilla Parzanini. Also I would like to thank Jacquelyn Saturno for her encouragement, sacrifices and commitment. Lastly, I want to express my gratitude to

Melissa, Dustin, Veronica, Carlo, Chloe and Lucciano Ammendolia for always being there to celebrate my accomplishments and for not disowning me during downs of this educational expedition; they are my inspiration for pushing the bar at each stage in my career.

Table of Contents

Abstract i

Acknowledgements ii

Table of Contents iv

List of Tables vii

List of Figures viii

Co-Authorship Statement xi

Chapter 1: General Introduction 1

1.1 Pressure in the marine environment 2

1.2 Colonization of high-pressure environments 4

1.3 Methodological considerations in the study of pressure exposure 5

1.4 Interactions of pressure in an acidifying ocean 7

1.5 Research gaps 8

1.6 Focal organisms 10

1.7 Goals of the research and chapter structure 11

1.8 References 13

Chapter 2: Vertical migrations in the ocean and the deep source-sink hypotheses: insights from pressure tolerance investigations 20

2.1 Abstract 21

2.2 Background and Introduction 22

2.3 Methods 32

2.3.1 Data collection and treatment *32*

2.3.2 Statistical analysis *34*

2.4 Results 36

2.4.1 Adults *36*

2.4.1.1 Survival time of deep-sea adults at atmospheric pressure 36

2.4.1.2 Pressure tolerance experiments 40

2.4.2 Larvae *43*

2.4.2.1 Survival time of deep-sea larvae at atmospheric pressure 43

2.4.2.2 Pressure tolerance experiments 46

2.5 Discussion 49

2.5.1 Pressure tolerance of adults *50*

2.5.2 Pressure tolerance of early life stages *56*

2.5.3 General conclusions *59*

2.6 Acknowledgements 60

2.7 References 61

2.8 Tables **75**

2.9 Figures **81**

Chapter 3: Life under pressure: an experimental study of behavioural responses to hydrostatic pressure and other stressors in echinoderms **87**

3.1 Abstract **88**

3.2 Introduction **89**

3.3 Methods **95**
3.3.1 Collection and maintenance *95*
3.3.2 Equipment and experimental conditions *96*
3.3.3 Response to pressure *97*
3.3.4 Combined response to pressure and pH *100*
3.3.5 Response metrics *101*
3.3.5.1 Body metrics and weight variations in all species 101
3.3.5.2 Righting times in S. droebachiensis and L. polaris 101
3.3.5.3 Ingestion index in S. droebachiensis 102
3.3.5.4 Ingestion index in L. polaris 103
3.3.5.5 Final feeding position in L. polaris 104
3.3.5.6 Post-trial and post-recovery in L. polaris 105
3.3.5.7 Health indices in C. frondosa 105
3.3.6 Statistical analysis *107*

3.4 Results **108**
3.4.1 Response to pressure *108*
3.4.1.1 Strongylocentrotus droebachiensis 108
3.4.1.2 Leptasterias polaris 109
3.4.1.3 Cucumaria frondosa 111
3.4.2 Combined response to pressure and pH *114*
3.4.2.1 Strongylocentrotus droebachiensis 114
3.4.2.2 Leptasterias polaris 115
3.4.2.3 Cucumaria frondosa 116

3.5 Discussion **117**
3.5.1 Response to pressure *117*
3.5.2 Combined response to pressure and pH *123*

3.6 Acknowledgements **127**

3.7 References **128**

3.8 Tables **136**

3.9 Figures **143**

Chapter 4: General Conclusions **153**

4.1 Thesis summary **154**

4.2 Future directions **157**

4.3 References **160**

Appendices **163**

Appendix A: Datasets **164**
Appendix B: Supplementary experiment for *S. droebachiensis* **285**
Appendix C: Statistical results **287**

List of Tables

Table 2-1: List of variables collected from the literature with their definitions and the scales/categories that were compared. 75

Table 2-2: Summary of variables used in FAMD analyses and results from each test. 78

Table 3-1 Bathymetric ranges (m) of focal species (rounded to the nearest hundred; e.g. 0.1 = 0 MPa = atmospheric pressure) with corresponding pressure tested. Note that for all three focal species the maximum depth of occurrence is based on only one record that met the search criteria described in the methods. The references used came from studies that conducted multi-species surveys by reputable institutions. 136

Table 3-2: Summary of experiments, including trial conditions and response variables measured. 137

Table 3-3: Summary of results from 24-h, 72-h and 216-h experiments under ambient pH conditions, showing whether they are consistent with tolerance or acclimation to pressure by three different species. Combinations that were not tested are indicated with NT. 138

Table 3-4: Summary of results from 24-h exposure to atmospheric and medium pressure (within natural depth range) under either acidified or ambient pH conditions, showing whether they are consistent with tolerance to pressure alone, acidification alone, and pressure-acidification combination. 139

Table 3-5: Survival rates (%; post-trial and post-recovery) associated with the 24-h, 72-h and 216-h pressure exposure trials under all pH conditions. Variable that were not tested are indicated with NT. 140

Table 3-6: Proportion of sea stars *L. polaris* found in the various feeding positions after exposure to three pressure levels under either ambient or low pH. Pressure did not affect the final feeding positions of *L. polaris* after 24-h ($H=2.47$, $df=2$, $p=0.291$). In two-way comparisons, there was no significant effect of pressure ($F_{1, 44}=1.02$, $p=0.751$) or pH on the final positions ($F_{1, 44}=0.28$, $p=0.598$), and no interaction between the factors ($F_{1, 44}=2.54$, $p=0.118$). 141

Table 3-7: Activity of *L. polaris* within the pressure vessel over a 216-h period at either atmospheric or high pressure (0 and 22 MPa; simultaneous trials). Scores included: feeding (arms wrapped around mussel, stomach everted on the prey), near mussels (no evident foraging or attempted opening of mussels), active (not visible from camera view because individuals were climbing on the sides of the pressure vessel), paralyzed (individual had not changed position for >8 hours). Proportion (%) of individuals displaying a given activity is shown in brackets. 142

List of Figures

Figure 2-1: Survival time in the laboratory at atmospheric pressure for adult individuals (mean ± SE, *n* = 4-82) from chemosynthetic environments with regards to (A) phylum (Ar=Arthropoda; Ch=Chordata; Cn=Cnidaria; Ec=Echinodermata; Mo=Mollusca; Po=Porifera) and (B) geographic location (see Table 2-1 for outline of abbreviations)...81

Figure 2-2: Minimum survival time in the laboratory at atmospheric pressure for adult individuals (mean ± SE, *n* = 3-35) from non-chemosynthetic environments with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Ch=Chordata; Cn=Cnidaria; Ec=Echinodermata; Mo=Mollusca; Po=Porifera), (B) geographic location (see Table 2-1 for outline of abbreviations) and (C) depth range. 82

Figure 2-3: Survival (%) of adult individuals (mean ± SE, *n* =3-59) from chemosynthetic environments tested under experimental pressure conditions with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Mo=Mollusca), and (B) depth range. 83

Figure 2-4: Survival (%) for adult individuals (mean ± SE, *n* =3-105) from non-chemosynthetic environments tested under experimental pressure conditions with regards to (A) phylum (Ar=Arthropoda; Cn=Cnidaria; Ec=Echinodermata; Fo= Foraminifera; Mo=Mollusca; Po=Porifera), (B) geographic location (Table 1 for outline of abbreviations) and (C) depth range. 84

Figure 2-5: Survival (%) of larvae (mean ± SE, *n* = 4-15) from chemosynthetic environments with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Mo=Mollusca), (B) geographic location (see Table 2-1 for outline of abbreviations), and (C) depth range. 85

Figure 2-6: Survival (%) of larvae (mean ± SE, *n* = 7-111) from non-chemosynthetic environments with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Cn=Cnidaria; Ec=Echinodermata; Mo=Mollusca), (B) geographic location (see Table 2-1 for outline of abbreviations), and (C) depth range. 86

Figure 3-1 (previous page): Response of *S. droebachiensis* to pressure. (A) Feeding index (mean ± SE, *n* = 12-24) during 24-h exposure to pressures within and beyond its natural bathymetric distribution. (B) Time to right to 90° (mean ± SE, *n* = 12-24) immediately after the 24-h trial. (C) Time to right to 180° (mean ± SE, n = 12-24) immediately after the 24-h trial. (D) Comparison of time to right to 90° (mean ± SE, *n* = 6) pre-trial, post-trial or post-recovery following 72-h exposure. (E) Comparison of time to right to 180° (mean ± SE, *n* = 6) pre-trial, post-trial or post-recovery following 72-h exposure. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b, c) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 (Appendix C) for full statistical results. ...144

Figure 3-2: Post-trial condition of individuals immediately after pressure exposure for 72 and 216 h. (A) *S. droebachiensis*; individual on the left was exposed to 0 MPa and had

healthy spines (hs); individual on the right was exposed to 25 MPa and had weak spines (ws); both individuals were tested in different pressure vessels. (B) *L. polaris*; after exposure to 0 MPa for 72 h, individuals were climbing and had healthy arms (ha) indicative of good health. (C) *L. polaris*; after exposure to 25 MPa for 72 h individuals all had convoluted arms (ca) and everted stomachs (es). (D) *L. polaris* after exposure to 22 MPa for 216 h individuals all had convoluted arms (ca) and everted stomachs (es) from stress. (E) *C. frondosa*; after exposure to 25 MPa this individual was found dead and eviscerated upon removal from the IPOCAMP; gonads (go) and intestines (in) are visible. Scale bars represent 4 cm. 145

Figure 3-3: Feeding indices and righting of *L. polaris* post-trial and post-recovery following a 24-h exposure to pressures within and beyond its natural bathymetric distribution. (A) Feeding indices (mean ± SE, n = 12-24). (B) Time (mean ± SE, n = 12-24) required to right itself to 180°. (C) Time (mean ± SE, n = 6) required to right itself to 180° pre-trial, post-trial or post-recovery following 72-h exposure to pressure. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results. 146

Figure 3-4: Time to feed, cloacal movement rate, time to escape response and time to anchor in *C. frondosa* measured either pre-trial, post-trial or post-recovery following 24-h exposure to pressures within and beyond its natural distribution (mean ± SE, n = 6). (A) Time to initiate feeding. (B) Cloacal movements. (C) Time to initiate escape from predator. (D) Time to anchor firmly to substrate. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results. 147

Figure 3-5: Time to feed, cloacal movement rate, time to escape response and time to anchor in *C. frondosa* measured either pre-trial, post-trial or post-recovery following 72-h exposure to pressures within and beyond its natural distribution (mean ± SE, n = 6). (A) Time to initiate feeding. (B) Cloacal movements. (C) Time to initiate escape from predator. (D) Time to anchor firmly to substrate. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results. 148

Figure 3-6: Feeding and righting of *S. droebachiensis* following 24-h exposure to ambient or low-pH conditions under pressures within and beyond its natural bathymetric distribution. (A) Feeding indices (mean ± SE, n = 12-24). (B) Time (mean ± SE, n = 12-24) required to right itself to 180°. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results. 149

Figure 3-7: Feeding and righting in *L. polaris* following 24-h exposure to ambient and acidified conditions under pressures within its natural bathymetric distribution. (A) Feeding indices (mean ± SE, *n* = 12-24). Times to right: (B) post-trial to 90° (C) post-trial to 180°, (D) post-recovery to 180°. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results. 150

Figure 3-8: Cloacal movement and time to anchor (mean ± SE, *n* = 6) in *C. frondosa* measured pre-trial, post-trial and post-recovery following 24-h exposure to ambient and acidified conditions under pressures within its natural distribution. Cloacal movements (A) pre-trial, (B) post-trial, and (C) post-recovery. Time to anchor (D) pre-trial, (E) post-trial and (F) post-recovery. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results. 151

Figure 3-9: Time to initiate feeding and predator escape response (mean ± SE, *n* = 6) in *C. frondosa* following 24-h exposure to ambient and acidified conditions under pressures within its' natural distribution. (A) Time to feed. (B) Time to escape. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results. 152

Co-Authorship Statement

The research described herein, including data collection and analysis, and all written work, was performed by Justine Ammendolia under the supervision and guidance of Annie Mercier, with technical and intellectual input from Jean-Francois Hamel. The authorship of journal contributions arising from the thesis chapters will therefore be:

Chapter 2 and Chapter 3: J. Ammendolia, J-F. Hamel, A. Mercier**.**

CHAPTER 1

General Introduction

1.1 Pressure in the marine environment

Hydrostatic pressure is one of the most prominent abiotic parameters of the marine environment. Unlike other oceanographic factors that present complex spatio-temporal patterns, a linear relationship exists between pressure and depth, which is continuous throughout the ocean from the surface waters to the abyssal plains (Pradillon and Gaill 2007; Young and Tyler 1993). Hydrostatic pressure primarily affects biological organisms by imposing forces of compression that result in 0.1 MPa for every 10 m into the water column (Macdonald 1997; Pradillon et al. 2004). The combination of temperature and pressure gradients is considered the primary factor responsible for limiting the bathymetric distribution of marine species (Pradillion 2011). As pressure tolerance varies interspecifically, the upper and lower limits of species' distributions may range from dozens to thousands of meters (Tyler and Young 1998). An antagonistic relationship exists between the two variables, whereby low temperatures and high pressure elicit negative physiological effects on biological processes (Brown and Thatje 2014; Morris et al. 2015). Animals occupying different depth ranges can be summarily separated into the following classifications: shallow-water species found above 200 m, eurybathic species spreading from shallow waters to below 200 m, and deep-sea species restricted to depths below 200 m (Rodríguez et al. 2007; Thurber et al. 2014; Webb et al. 2010).

Although it is tempting to infer pressure tolerances of species based on their natural bathymetric distribution, it is difficult to do so given the diversity of ontogenetic development modes in marine animals (Gage and Tyler 1999). Notably, various phyla of marine invertebrates (e.g. Echinodermata, Arthropoda, Mollusca) have complex

pelagobenthic life cycles, giving rise to life stages that can occupy a wide range of depths (Gage and Tyler 1999). For instance, species from the phylum Echinodermata can cross a wide spectrum of pressures as free swimming early life stages and be found inside a more narrow range of pressure as sessile or sedentary benthic adults (Byrne et al. 2009). It has been postulated through laboratory studies that pressure tolerance is both species-specific and age-dependent (Mestre et al. 2009; Oliphant et al. 2011; Tyler and Young 1998).

Because pressure and temperature interact to affect the survival of marine animals, they are responsible for imposing a physiological bottleneck on the vertical distribution of animals (see review by Brown and Thatje 2014). The unique relationship between these two key environmental factors has been well examined on a number of different phyla (e.g. Aquino-Souza et al. 2008; Brown and Thatje 2011; Macdonald and Teal 1975; Mestre et al. 2009; Oliphant et al. 2011; Thatje et al. 2010; Tyler and Dixon 2000). However, few studies have focused on testing the effects of hydrostatic pressure, a necessity to further our understanding how animals are affected in physiological, genomic and behavioral processes.

The most common reaction elicited in species exposed to high hydrostatic pressure is a reduction of the volume of internal compartments (Balny et al. 2002; Pradillion 2011). Pressure is capable of modifying the equilibrium and rate constants of enzymatic reactions depending on volume changes, which can critically affect the activity of enzymes and efficiency of ligand binding (Balny et al. 2002; Somero 1992). High pressure also affects the properties of the lipid bilayers by modifying the reaction rates of membrane-associated processes like active transport, membrane fluidity and synaptic transmission (Macdonald 1984). Enzymatic efficiency is also modulated through amino-

acid sequences that can limit metabolic rates (Siebenaller and Somero 1978). Animals respond to changes in pressure by maintaining homeostasis through involuntary changes on genetic, molecular, and biochemical scales (Molina-García 2002; Pradillion 2011; Pradillon et al. 2004). Some of the best-studied mechanisms are changes in the genomic expression of heat shock proteins (HSP) (Barros et al. 2015; Cottin et al. 2012; Morris et al. 2015). High-pressure exposure of shallow-water animals induces cellular stress and protein degradation, which causes an upregulation of heat-shock proteins (HSP) (Cottin et al. 2008; Feder and Hofmann 1999; Morris et al. 2015). Such molecular markers typically indicate the degree of non-lethal stress associated with thermal thresholds but also demonstrate poor physiological state (Morris et al. 2015; Ravaux et al. 2003; 2009). Physiological effects of high pressure can include but are not limited to low rates of aerobic metabolism (Cottin et al. 2008; Oliphant et al. 2011; Thatje and Robinson 2011) and behaviour quantified by convulsions and spasms (Macdonald and Gilchrist 1978; Thatje and Robinson 2011). Overall, despite these findings, our understanding of how pressure affects biological processes within and across multiple taxa remains incomplete.

1.2 Colonization of high-pressure environments

By furthering our understanding of how marine organisms cope with pressure we can answer one of the most critical questions in deep-sea evolutionary ecology: does the deep sea act as a sink or source of oceanic biodiversity (Gage and Tyler 1999; Miglietta et al. 2011)? Since the 1970s two competing hypotheses have been proposed to explain this phenomenon. The submergence hypothesis commonly postulated in pressure studies states that shallow-water animals migrated downwards into the deep sea in an isothermal ocean that was featured in either past geological events and/or during modern times at

high latitudes (Aquino-Souza 2006; Tyler and Young 1998; Tyler and Dixon 2000; Villalobos et al. 2006). As shallow-water animals were able to adapt to high pressures, the migrating taxa radiated to bathyal and abyssal depths (Jablonski 2005; Kiel et al. 2012; Kussakin 1973). By contrast, the high-latitude emergence hypothesis states the opposite: that animals from deep depths migrated and populated shallow depths at high latitudes in the northern and southern hemispheres (Hessler and Thistle 1975; Wägele 1989; Wilson 1999). Thus, in order for us to comprehend the evolution/adaptation of shallow-water and deep-sea fauna in terms of their sensitivity to pressure, it is important to understand how biological structures and processes differ between these two groups (Somero 1992).

1.3 Methodological considerations in the study of pressure exposure

Our knowledge regarding the effects of hydrostatic pressure on marine invertebrates remains rudimentary due to the logistical complications in performing pressure experiments in the laboratory (Shillito et al. 2001). Overall, there is limited availability of high-pressure aquaria that can maintain flow-through conditions for in vitro experiments (Shillito et al. 2014; 2015). The difficulty in acquiring multiples of such pressure systems often imposes limitations on the ability of scientists to replicate experiments (Brooke and Young 2009).

The study of the biological effects of pressure was initially revolutionized by the development of pressure systems used by Childress (Childress 1971; 1976). These experiments contributed a significant portion of knowledge to the field, but systems were logistically constrained to small volumes of static water that could only maintain live animals over the span of a few hours (Childress 1976; George and Marum 1974; Wilcock

et al. 1978). As a result, studies often tested acute physiological and behavioural responses of animals that were either small or in the early stages of ontogenetic development (Childress 1971; Childress et al. 1984; Mickel and Childress 1982a; Schlieper 1968). The study of larvae also remained a focus since adult body sizes and more developed metabolisms required larger vessels capable of maintain flow-through conditions (Brooke and Young 2009; Shillito et al. 2015). These studies were also constrained by the fact that investigators had no control over the rate of pressurization and depressurization being tested (Brown and Thatje 2011). The innovation of the PICCEL (Pressurized Incubators for the Culture of Cells, Embryos and Larvae) addressed the issue by allowing the control of the application of pressure, providing oxygenated water throughout the experiment and offering the possibility to observe experimental animals at the microscopic level while under pressure (Pradillon et al. 2004). However the small volume design (50-100 ml) remained only suitable for embryos and small organisms. It is important to note that different working groups have independently developed several models of pressure systems that have variations in structure and function (e.g. Jannasch et al. 1996; Koyama et al. 2005a; Mestre et al. 2009; Miyake et al. 2007; Quetin and Childress 1980).

The experimental study of hydrostatic pressure was greatly enhanced with the development of a large-volume pressure system called IPOCAMP (Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds) (Shillito et al. 2001). This system enables scientists to hold larger quantities of water (~19 L) at pressure under a continuous flow-through mode, as described in Shillito et al. (2014). Through a built-in temperature control, the system enables the study of thermo- and barotolerance of fairly

large live animals (Ravaux et al. 2009). It has initially been used to hold and test a number of species from deep-sea chemosynthetic habitats (Shillito et al. 2001). Significant to the field, the IPOCAMP allows scientists to maintain animals under pressurized conditions for longer periods, from hours to weeks (Shillito et al. 2014). Another key aspect of the system is its visualization capacity, which allows users to observe and/or record what is occurring inside the pressurized chamber (Shillito et al. 2006). This is critical, as changes in hydrostatic pressure may induce modifications in morphology and behaviour (Begg et al. 1982).

1.4 Interactions of pressure in an acidifying ocean

The anthropogenic generation of large quantities of carbon dioxide (CO_2) is driving the acidification of oceanic waters (Byrne et al. 2009; Gooding et al. 2009). The burning of fossil fuels results in the increase of partial pressure of atmospheric CO_2 (ρCO_2), which is absorbed by the global ocean. Once it dissolves in water, CO_2 undergoes a series of reactions. Carbonic acid is formed and then dissociates to produce hydrogen ions, bicarbonate ions and carbonate, all of which are maintained in equilibrium. As there is an increase in the concentration of hydrogen ions in seawater the pH subsequently decreases resulting in ocean acidification (OA) (Caldeira and Wickett 2003). Oceanic absorption of atmospheric carbon is predicted to increase from the present levels of 300-380 ppm to 450-1000 ppm in 2100 (Pachauri et al. 2014).

Despite the relevance of pH as an abiotic factor in the modern marine environment, little to no research has been done on the biological effects of ocean acidification at various pressures (depths). In one of the rare investigations, Barry et al. (2004) injected CO_2 into deep-sea environments *in situ* at 3600 m depth to determine the

effects on infaunal communities. In proximity to the injection sites, organisms such as flagellates, amoebae, and nematodes experienced high mortalities due to high CO_2 concentrations. However, it remains unclear whether deep-sea organisms are more sensitive to fluctuations in pH by way of their inherent piezophily (adaptation to high pressure) and whether pressure and increased pH can be considered additive stressors on marine life. This question is pertinent since the effects of ocean acidification are expected to occur at all depths of the marine environment and might limit the ability for shallow-water animals to colonize deeper colder regions as they migrate under the threat of ocean warming (Guinotte et al. 2006; Ramirez-Llodra et al. 2011). To date no laboratory studies have been conducted that examine combined responses to increased pressure and seawater acidity.

1.5 Research gaps

Although hydrostatic pressure is a key parameter of marine environments, our knowledge of its role on the behaviour and ecology of adult organisms remains rudimentary (Brown and Thatje 2014; Pradillion 2011; Pradillon and Gaill 2007). The majority of previous studies that have tested the effects of pressure have been restricted by temporal and logistical challenges, preventing the maintenance of animals for more than a few hours (e.g. Aquino-Souza et al. 2008; Mestre et al. 2009; Oliphant et al. 2011; Villalobos et al. 2006). Even though these experiments provide useful information to help further our understanding of acute tolerances, they do not elucidate responses over longer periods of one to several days. Moreover, most of the few studies on pressure to date have examined the effects of short-term exposure to pressure conditions on the development and survival of embryos and larvae, without consideration for the adult

stages (Tyler and Young 1998; Villalobos et al. 2006). Although the relationship between temperature and pressure has been relatively well studied (e.g. Aquino-Souza et al. 2008; Brown and Thatje 2011; Oliphant et al. 2011; Thatje et al. 2010; Tyler and Dixon 2000), the interactions of pressure and factors like pH have been virtually unexplored.

There is an inevitable setback with the experimental design of pressure studies, namely the pressure differential between holding and experimental conditions. As it is not logistically feasible to maintain animals at their native pressure, most animals from pressure experiments are maintained and acclimated to ambient surface pressure prior to experimental studies. Although Shillito et al., (2006) tested shrimp from hydrothermal vents within minutes post collection, quickly bringing the animals back to the equivalent of 1000-m depth after resurfacing, this in itself would cause a high degree of stress to the animals. Other approaches have included gradually pressurizing animals to experimental depths in a series of small intervals in order to reduce stress. For instance, New et al., (2014) used an acclimation pressure interval of 1 MPa every 5 min on animals that had been living at atmospheric pressure. Overall, the sensitivities of animals at the whole-organism level to the depressurization/repressurization process are not fully understood (Dixon et al. 2002).

In addition to the inherent shortcomings that exist in the experimental methodology of testing responses to pressure, to date no review has been done to specifically compare pressure tolerances (survival rate/percent and survival time) of shallow-water and deep-sea animals. The putative role of important factors like life stage, phylum, collection depth and habitat type have not yet been synthetized or analyzed.

1.6 Focal organisms

The experimental segment of the present thesis revolves around members of the phylum Echinodermata. Echinoderms are well suited to experimental studies because of their complex life histories and broad distribution across both the shallow-water and deep-sea oceanic environments. Animals are relatively accessible for collections and can be maintained in laboratory conditions for long periods of time. While several species of echinoderms have been the subject of previous studies on pressure tolerance, few investigations have studied the responses of adults. The following shallow-water adult echinoderms were selected based on their broad distribution in temperate, cold and polar subtidal environments as well as their overall importance to the coastal ecosystems of eastern Canada: green sea urchin (*Strongylocentrotus droebachiensis*), polar sea star (*Leptasterias polaris*) and orange-footed sea cucumber (*Cucumaria frondosa*). These three species are respectively omnivorous, carnivorous and herbivorous (phytoplankton feeder) and exhibit two larval feeding strategies (planktotrophy for the first two, lecithotrophy for the third). All the species selected have distinct behaviours that could be tested in pressurized lab conditions, and they play important ecological roles. As a ubiquitous keystone species, *S. drobachiensis* exerts intensive grazing pressure on kelp beds, resulting in cascading effects on the population dynamics of other species (Mann and Breen 1972). Similarly, *L. polaris* was chosen because it exemplifies highly successful benthic predators. Its diet primarily consists of molluscs, including scallops, whelks and mussels. High abundance of this species occurs in proximity to subtidal mussel beds where there is strong inter- and intra- specific competition (Gaymer et al. 2001). Lastly, subtidal populations of the slow-growing *C. frondosa* comprise a

substantial proportion of the biomass on hard-bottom marine ecosystems of eastern Canada (Hamel and Mercier 1998) and have considerable economic value (Mercier and Hamel 2008a; So et al. 2010; So et al. 2011).

1.7 Goals of the research and chapter structure

This investigation aimed to further our knowledge of how hydrostatic pressure, a poorly studied yet crucial abiotic parameter, affects the biology and overall survival of both shallow-water and deep-sea marine invertebrates. As there are many knowledge gaps in the literature of biological interactions with pressure, both within and across phyla, this study aimed to examine the effects of hydrostatic pressure at both levels. One of the major objectives was to use the IPOCAMP high-pressure systems to simulate a range of high-pressure conditions that would be critical in establishing survival thresholds of several species of Echinodermata ubiquitous to the North Atlantic ecosystem. This thesis is composed of four chapters.

In Chapter 2, data were collated and synthesized the findings from 134 studies to analyze how pressure tolerance (survival percent and time) varied between larvae and adults from shallow-water and deep-sea environments. The pressure tolerance of species was examined by testing the relationship between tolerance and individuals': (i) depth stratum of collection, (ii) geographic location of origin, and (iii) phylum. Findings were used to help better understand the bathymetric movement of animals. Specifically the following theories were tested: submergence (shallow to deep) hypothesis, high-latitude emergence (deep to shallow) hypothesis and an alternate hypothesis (dubbed the parsimony hypothesis), which would explain colonization as a bi-directional movement of species between the shallow-water and deep-sea environments.

In Chapter 3, experiments were conducted with two IPOCAMP systems to test three focal echinoderm species under three pressure regimes that represent depths representative of their natural bathymetric distributions and one extreme value beyond their natural range of occurrence. The three echinoderm species selected represent the main extant classes, Echinoidea, Asteroidea, and Holothuroidea. The duration of the trials were adjusted to test both acute (24 h) and slightly longer (72 h to 9 d) exposures to pressure, as well as the effects of combined stressors (acidity and pressure) over short-term trials (24 h).

In Chapter 4, the general conclusions from this investigation were summarized and the ecological significance of hydrostatic pressure tolerance among marine invertebrates in a changing ocean was discussed. This study expanded on the potential implications of expected vertical migrations for marine communities at different depths. Lastly, future directions for research in the study of hydrostatic pressure in the marine environment were discussed.

1.8 References

Aquino-Souza R, Hawkins S, Tyler P (2008) Early development and larval survival of *Psammechinus miliaris* under deep-sea temperature and pressure conditions. Journal of the Marine Biological Association of the UK 88(3): 453-461

Aquino-Souza R (2006) Pressure and temperature effects on planktonic stages of benthic invertebrates with regard to their potential for invasion of the deep sea. Doctoral Thesis. University of Southhampton.

Balny C, Masson P, Heremans K (2002) High pressure effects on biological macromolecules: from structural changes to alteration of cellular processes. Biochimica Et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology 1595(1): 3-10

Barros I, Divya B, Martins I, Vandeperre F, Santos RS, Bettencourt R (2015) Post-capture immune gene expression studies in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* acclimatized to atmospheric pressure. Fish and Shellfish Immunology 42(1): 159-170

Barry JP, Buck KR, Lovera CF, Kuhnz L, Whaling PJ, Peltzer ET, Walz P, Brewer PG (2004) Effects of direct ocean CO_2 injection on deep-sea meiofauna. Journal of Oceanography 60(4): 759-766

Begg DA, Rebhun LI, Hyatt H (1982) Structural organization of actin in the sea urchin egg cortex: microvillar elongation in the absence of actin filament bundle formation. Journal of Cell Biology 93(1): 24-32

Brooke SD and Young CM (2009) Where do the embryos of *Riftia pachyptila* develop? Pressure tolerances, temperature tolerances, and buoyancy during prolonged embryonic dispersal. Deep Sea Research Part II: Topical Studies in Oceanography 56(19): 1599-1606

Brown A and Thatje S (2014) Explaining bathymetric diversity patterns in marine benthic invertebrates and demersal fishes: physiological contributions to adaptation of life at depth. Biological Reviews 89(2): 406-426

Brown A and Thatje S (2011) Respiratory response of the deep-sea amphipod *Stephonyx biscayensis* indicates bathymetric range limitation by temperature and hydrostatic pressure. PLoS One 6(12): e28562

Byrne M, Ho M, Selvakumaraswamy P, Nguyen HD, Dworjanyn SA, Davis AR (2009) Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. Proceedings of the Royal Society of London B: Biological Sciences 276(1663): 1883-1888

Caldeira K and Wickett ME (2003) Oceanography: anthropogenic carbon and ocean pH. Nature 425(6956): 365-365

Childress JJ (1971) Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. Biological Bulletin 141(1): 109-121

Childress J (1976) Effects of pressure, temperature and oxygen on the oxygen consumption rate of the midwater copepod *Gaussia princeps*. Marine Biology 39(1): 19-24

Childress J, Arp A, Fisher Jr C (1984) Metabolic and blood characteristics of the hydrothermal vent tube-worm *Riftia pachyptila*. Marine Biology 83(2): 109-124

Company J and Sardà F (1998) Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. Deep Sea Research Part I: Oceanographic Research Papers 45(11): 1861-1880

Cottin D, Brown A, Oliphant A, Mestre NC, Ravaux J, Shillito B, Thatje S (2012) Sustained hydrostatic pressure tolerance of the shallow water shrimp *Palaemonetes varians* at different temperatures: insights into the colonisation of the deep sea. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 162(4): 357-363

Cottin D, Ravaux J, Leger N, Halary S, Toullec JY, Sarradin PM, Gaill F, Shillito B (2008) Thermal biology of the deep-sea vent annelid *Paralvinella grasslei*: *in vivo* studies. Journal of Experimental Biology 211(14): 2196-2204

Dixon DR, Dixon LR, Shillito B, Gwynn JP (2002) Background and induced levels of DNA damage in pacific deep-sea vent polychaetes: the case for avoidance. Cahiers De Biologie Marine 43(3/4): 333-336

Feder ME and Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology 61(1): 243-282

Gage JD and Tyler PA. (1999) Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press.

Gaymer CF, Himmelman JH, Johnson LE (2001) Use of prey resources by the seastars *Leptasterias polaris* and *Asterias vulgaris*: a comparison between field observations and laboratory experiments. Journal of Experimental Marine Biology and Ecology 262(1): 13-30

George RY and Marum JP (1974) The effects of hydrostatic pressure on living aquatic organisms III. behavior and tolerance of euplanktonic organisms to increased

hydrostatic pressure. Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie 59(2): 175-186

Gooding RA, Harley CD, Tang E (2009) Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. Proceedings of the National Academy of Sciences USA 106(23): 9316-9321

Guinotte JM, Orr J, Cairns S, Freiwald A, Morgan L, George R (2006) Will human-induced changes in seawater chemistry alter the distribution of deep-sea scleractinian corals? Frontiers in Ecology and the Environment 4(3): 141-146

Hamel J-F and Mercier A (1998) Diet and feeding behaviour of the sea cucumber *Cucumaria frondosa* in the St. Lawrence Estuary, eastern Canada. Canadian Journal of Zoology 76(6): 1194-1198

Hessler R and Thistle D (1975) On the place of origin of deep-sea lsopods. Marine Biology 32(2): 155-165

Jablonski D (2005) Mass extinctions and macroevolution. Paleobiology 31(5): 192-210

Jannasch HW, Wirsen CO, Doherty KW (1996) A pressurized chemostat for the study of marine barophilic and oligotrophic bacteria. Applied Environmental Microbiology 62(5): 1593-1596

Kiel S, Wiese F, Titus AL (2012) Shallow-water methane-seep faunas in the Cenomanian western interior seaway: no evidence for onshore-offshore adaptations to deep-sea vents. Geology 40(9): 839-842

Koyama S, Kobayashi H, Inoue A, Miwa T, Aizawa M (2005) Effects of the piezo-tolerance of cultured deep-sea eel cells on survival rates, cell proliferation, and cytoskeletal structures. Extremophiles 9(6): 449-460

Kussakin O (1973) Peculiarities of the geographical and vertical distribution of marine isopods and the problem of deep-sea fauna origin. Marine Biology 23(1): 19-34

Macdonald A (1997) Hydrostatic pressure as an environmental factor in life processes. Comparative Biochemistry and Physiology Part A: Physiology 116(4): 291-297

Macdonald A and Gilchrist I (1978) Further studies on the pressure tolerance of deep-sea crustacea, with observations using a new high-pressure trap. Marine Biology 45(1): 9-21

Macdonald AG and Teal J (1975) Tolerance of oceanic and shallow water crustacea to high hydrostatic pressure. 22(3): 131-144

Macdonald AG (1984) The effects of pressure on the molecular structure and physiological functions of cell membranes. Philosophical Transactions of the Royal Society of London B: Biological Sciences 304(1118): 47-68

Mann K and Breen P (1972) The relation between lobster abundance, sea urchins, and kelp beds. Journal of the Fisheries Board of Canada 29(5): 603-605

Mercier A and Hamel J-F (2008) Depth-related shift in life history strategies of a brooding and broadcasting deep-sea asteroid. Marine Biology 156(2): 205-223

Mestre NC, Thatje S, Tyler PA (2009) The ocean is not deep enough: pressure tolerances during early ontogeny of the blue mussel *Mytilus edulis*. Proceedings of the Royal Society of London B: Biological Sciences 276(1657): 717-726

Mickel TJ and Childress J (1982a) Effects of pressure and pressure acclimation on activity and oxygen consumption in the bathypelagic mysid *gnathophausia ingens*. Deep Sea Research Part A Oceanographic Research Papers 29(11): 1293-1301

Mickel TJ and Childress JJ (1982b) Effects of pressure and temperature on the EKG and heart rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). The Biological Bulletin 162(1): 70-82

Miglietta MP, Faucci A, Santini F (2011) Speciation in the sea: Overview of the symposium and discussion of future directions. Integrative and Comparative Biology 51(3): 449-455

Miyake H, Kitada M, Tsuchida S, Okuyama Y, Nakamura K (2007) Ecological aspects of hydrothermal vent animals in captivity at atmospheric pressure. Marine Ecology 28(1): 86-92

Molina-García AD (2002) The effect of hydrostatic pressure on biological systems. Biotechnology and Genetic Engineering Reviews 19(1): 3-54

Morris J, Thatje S, Ravaux J, Shillito B, Fernando D, Hauton C (2015) Acute combined pressure and temperature exposures on a shallow-water crustacean: novel insights into the stress response and high pressure neurological syndrome. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 181: 9-17

New P, Brown A, Oliphant A, Burchell P, Smith A, Thatje S (2014) The effects of temperature and pressure acclimation on the temperature and pressure tolerance of the shallow-water shrimp *Palaemonetes varians*. Marine Biology 161(3): 697-709

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. Journal of Experimental Biology 214(7): 1109-1117

Pachauri RK, Allen M, Barros V, Broome J, Cramer W, Christ R, Church J, Clarke L, Dahe Q, Dasgupta P (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.

Pradillion F (2011) High hydrostatic pressure environments. In: Bell, E. (ed.), Life at extremes: environments, organisms, and strategies for survival. CABI, UK: 271-295

Pradillon F and Gaill F (2007) Pressure and life: some biological strategies. Reviews in Environmental Science and Bio/Technology 6(1-3): 181-195

Pradillon F, Shillito B, Chervin J, Hamel G, Gaill F (2004) Pressure vessels for *in vivo* studies of deep-sea fauna. High Pressure Research 24(2): 237-246

Quetin LB and Childress JJ (1980) Observations on the swimming activity of two bathypelagic mysid species maintained at high hydrostatic pressures. Deep Sea Research Part A. Oceanographic Research Papers 27(5): 383-391

Ramirez-Llodra E, Tyler PA, Baker MC, Bergstad OA, Clark MR, Escobar E, Levin LA, Menot L, Rowden AA, Smith CR (2011) Man and the last great wilderness: human impact on the deep sea. PLoS One 6(7): e22588

Ravaux J, Cottin D, Chertemps T, Hamel G, Shillito B (2009) Hydrothermal shrimps display low expression of heat-inducible hsp70 gene in nature. Marine Ecology Progress Series 396: 153-156

Ravaux J, Gaill F, Le Bris N, Sarradin PM, Jollivet D, Shillito B (2003) Heat-shock response and temperature resistance in the deep-sea vent shrimp *Rimicaris exoculata*. Journal of Experimental Biology 206(14): 2345-2354

Rodríguez E, López-González PJ, Gili JM (2007) Biogeography of Antarctic sea anemones (Anthozoa, Actiniaria): what do they tell us about the origin of the Antarctic benthic fauna? Deep Sea Research Part II: Topical Studies in Oceanography 54(16): 1876-1904

Schlieper C (1968) High pressure effects on marine invertebrates and fishes. Marine Biology 2(1): 5-12

Shillito B, Gaill F, Ravaux J (2014) The IPOCAMP pressure incubator for deep-sea fauna. Journal of Marine Science and Technology Taiwan 22: 97-102

Shillito B, Ravaux J, Sarrazin J, Zbinden M, Sarradin P, Barthelemy D (2015) Long-term maintenance and public exhibition of deep-sea Hydrothermal fauna: the AbyssBox project. Deep Sea Research Part II: Topical Studies in Oceanography 121: 137-145

Shillito B, Jollivet D, Sarradin P, Rodier P, Lallier F, Desbruyères D, Gaill F (2001) Temperature resistance of *Hesiolyra bergi*, a polychaetous annelid living on deep-sea vent smoker walls. Marine Ecology Progress Series 216: 141-149

Shillito B, Le Bris N, Hourdez S, Ravaux J, Cottin D, Caprais JC, Jollivet D, Gaill F (2006) Temperature resistance studies on the deep-sea vent shrimp *Mirocaris fortunata*. Journal of Experimental Biology 209(5): 945-955

Siebenaller J and Somero GN (1978) Pressure-adaptive differences in lactate dehydrogenases of congeneric fishes living at different depths. Science 201(4352): 255-257

So JJ, Hamel J-F, Mercier A (2010) Habitat utilisation, growth and predation of *cucumaria frondosa*: Implications for an emerging sea cucumber fishery. Fisheries Management and Ecology 17(6): 473-484

So JJ, Uthicke S, Hamel J-F, Mercier A (2011) Genetic population structure in a commercial marine invertebrate with long-lived lecithotrophic larvae: *Cucumaria frondosa* (echinodermata: Holothuroidea). Marine Biology 158(4): 859-870

Somero G (1992) Biochemical ecology of deep-sea animals. Experientia 48(6): 537-543

Thatje S and Robinson N (2011) Specific dynamic action affects the hydrostatic pressure tolerance of the shallow-water spider crab *Maja brachydactyla*. Naturwissenschaften 98(4): 299-313

Thatje S, Casburn L, Calcagno JA (2010) Behavioural and respiratory response of the shallow-water hermit crab *Pagurus cuanensis* to hydrostatic pressure and temperature. Journal of Experimental Marine Biology and Ecology 390(1): 22-30

Thurber A, Sweetman A, Narayanaswamy B, Jones D, Ingels J, Hansman R (2014) Ecosystem function and services provided by the deep sea. Biogeosciences 11(14): 3941-3963

Tyler P and Dixon D (2000) Temperature/pressure tolerance of the first larval stage of *Mirocaris fortunata* from Lucky Strike hydrothermal vent field. Journal of the Marine Biological Association of the UK 80(4): 739-740

Tyler P and Young C (1998) Temperature and pressure tolerances in dispersal stages of the genus *Echinus* (Echinodermata: Echinoidea): prerequisites for deep-sea invasion and speciation. Deep Sea Research Part II: Topical Studies in Oceanography 45(1): 253-277

Villalobos FB, Tyler PA, Young CM (2006) Temperature and pressure tolerance of embryos and larvae of the Atlantic seastars *Asterias rubens* and *Marthasterias*

glacialis (Echinodermata: Asteroidea): potential for deep-sea invasion. Marine Ecology Progress Series 314: 109-117

Wägele JW. (1989) Evolution und phylogenetisches system der isopoda: stand der forschung und neue erkenntnisse. E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller),

Webb TJ, Vanden Berghe E, O'Dor R (2010) Biodiversity's big wet secret: the global distribution of marine biological records reveals chronic under-exploration of the deep pelagic ocean. PLoS One 5(8): e10223

Wilcock S, Wann K, Macdonald A (1978) The motor activity of *Crangon crangon* subjected to high hydrostatic pressure. Marine Biology 45(1): 1-7

Wilson GD (1999) Some of the deep-sea fauna is ancient. Crustaceana 72(8): 1019-1030

Young CM and Tyler PA (1993) Embryos of the deep-sea echinoid *Echinus affinis* require high pressure for development. Limnology and Oceanography 38(1): 178-181

CHAPTER 2

Vertical migrations in the ocean and the deep source-sink hypotheses: insights from pressure tolerance investigations

2.1 Abstract

It has been postulated that, throughout geological times, faunal migrations between the shallow-water and deep-sea environments have resulted in the broad colonisation of oceanic depths we see today. Attempts have been made to explain the current bathymetric ranges of animals by the submergence (shallow to deep) hypothesis and the high-latitude emergence (deep to shallow) hypothesis, but there has so far been no clear consensus. Here, we explore empirical support for both hypotheses, in addition to a newly proposed parsimony hypothesis, which would explain colonization as a bi-directional movement of species between the shallow-water and deep-sea environments. We collated and analyzed data from 134 studies reporting the pressure tolerance of adults and embryos/larvae of 261 species obtained from different regions and depths. Subsets of the main database were used to test whether the ability to tolerate a change in pressure is influenced by (i) depth stratum of origin, (ii) geographic location of origin, and (iii) phylum. This review revealed stronger empirical support for the general tolerance of deep-water taxa to atmospheric pressure than for tolerance of shallow-water taxa to increases in pressure (both following sudden shifts). Overall, species from bathyal depths survived longer under atmospheric pressure than those from abyssal depths. Deep-sea species also survived better than shallow-water species to pressurization trials. If tolerance to non-native pressures is taken as a predictor of potential for vertical migration, empirical and experimental data currently lend more support to the parsimony and/or the emergence hypotheses. Species collected at depth from tropical locations were less tolerant to a pressure change than those from northern latitudes, emphasizing the confounding impact of thermotolerance. Lastly, phylum was a more significant driver of pressure tolerance

for adults than for larvae, although this might be a result of the generally shorter study duration for the latter. Taken together, these findings provide a valuable overview of the current state of knowledge and offer a framework for further investigation of vertical movements of marine species across depths, which will be particularly useful in predicting ecosystem shifts in the face of climate change.

2.2 Background and Introduction

The most fundamental division of the world ocean involves the distinction between shallow-water and deep-sea environments (Webb et al. 2010). The transition is typically considered to occur at ~200 m, the average depth of the continental shelf break (Ramirez-Llodra et al. 2011; Thistle 2003). While oceanic depths have always exerted great fascination, they were initially believed to be barren. Edward Forbes was the first to propose that little to no life existed below 600 m, and is thus considered to be the father of the azoic theory (Forbes 1844). Even following more exploration in the 1960s, the deep sea remained depicted as a so-called harsh environment exhibiting greater stability and lesser biodiversity than its shallow-water counterpart (Sanders 1968). However, this oversimplified view is now being challenged by a growing number of publications based on species from both chemosynthetic and non-chemosynthetic environments (e.g. Shillito et al. 2015; Miyake et al. 2007; Kádár et al. 2006a; 2008a; Mercier and Hamel 2008b; 2009; Mercier et al. 2011a; 2013). Paradigms regarding the low biodiversity of the deep sea were revised when for the first time hundreds of benthic macro-faunal species were recovered from trawls (Gage and Tyler 1999; Hessler and Sanders 1967) and exceptional ecosystems such as hydrothermal vents were discovered (Corliss and Ballard 1977). Over the last few decades, hot spots of productivity have been highlighted in the deep sea,

including vents, seamounts, and coral and sponge gardens, which rival some of the most diversified environments on the planet. In fact, recent extrapolations suggest that deep-sea habitats could host over 10 million species (Ramirez-Llodra et al. 2011), i.e. more than the total number of species currently known to populate Earth (Mora et al. 2011).

The realization that the deep sea likely fosters high levels of biodiversity, and the fact that depths >200 m cover more than 60% of the globe, gave rise to theories on whether species of the deep sea evolved within this environment or following the migration of species from the shallows (Gage and Tyler 1999; Locket 1977). The majority of studies focusing on the concept of deep-sea and shallow-water colonization developed over the 1970s (Hessler et al. 1979; Hessler and Thistle 1975; Kussakin 1973; Menzies et al. 1973), although some theories were postulated a few decades earlier (Dahl 1954; Wolff 1960). While deep-sea research has intensified over the past 30 years, the role of the deep ocean as a sink or source of biodiversity remains obscure (Gage and Tyler 1999; Miglietta et al. 2011). Answering this question is complicated by ongoing disputes over the importance that certain abyssal and bathyal depths played as sinks or sources at different geological times (Bik et al. 2010; Rex et al. 2005).

The submergence (shallow to deep) hypothesis postulates that higher-level taxa from shallow waters migrated downwards during various geological periods to radiate biodiversity at bathyal and abyssal depths (Jablonski 2005; Kussakin 1973). This migration is suggested to have occurred multiple times throughout geological history, especially during the late Mesozoic and early Cenozoic periods, when the water column was isothermal across low latitudes (Jablonski et al. 1983; Menzies et al. 1973; Wilson 1999). Extinct deep-sea fauna identified in fossil records from these periods have been

linked to current shallow-water animals whose ancestors would have colonized these areas (Cottin et al. 2012; Kussakin 1973). The small temperature difference between these shallow and deep depths greatly reduced the physiological barrier that prevented animals from expanding their typical bathymetric range (Gage and Tyler 1999; Raupach et al. 2009). In fact it is argued that the colonization of the deep sea by shallow-water animals is continuously ongoing in permanently isothermal areas, like Antarctica and regions of deep-water formation (Oliphant et al. 2011; Tyler and Young 1998; Wolff 1960). It is postulated that during this downwards migration shallow-water species evolved to adapt to high-pressure conditions, thus undergoing speciation (Clarke et al. 1992). Some animals are thought to have adapted so well that they migrated to more specialized habitats like hydrothermal vents and cold seeps (Kiel and Little 2006). Support for this downward migration cites the close phylogenetic and taxonomic relationships that exist between shallow and deep-sea species (Distel et al. 2000; Tokuda et al. 2006). It is speculated that prior to the invasions from the shallows, the deep sea had substantially less fauna than presently (Kussakin 1973). One of the predictions from this hypothesis is that shallow-water animals would need to demonstrate physiological and developmental plasticity in order to successfully invade the deep sea and thrive under extreme conditions, which include: high hydrostatic pressure, low temperatures, complete darkness and minimal nutrients from primary production (Hessler and Wilson 1983; Pradillon et al. 2004). If adaptation to these conditions proves too difficult for invading species, the surviving species will specialize, thus resulting in taxonomic isolation from shallow relatives (Hessler and Wilson 1983).

The opposing hypothesis states that modern deep-sea invertebrates evolved from ancestors that occupied the same bathymetric environment (Hessler and Thistle 1975). Known as the high-latitude emergence hypothesis, it is theorized that, at high latitudes in the northern and southern hemispheres, animals from deep depths migrated and populated shallow depths (Hessler et al. 1979; Hessler and Thistle 1975; Wägele 1989; Wilson 1999). The fact that the level of biodiversity currently present in the deep sea could hardly be a product of small colonisations from shallow waters is proposed to support the emergence hypothesis (Birstein 1963; Hessler and Sanders 1967; Hessler and Thistle 1975). In addition, many deep-sea families have the vast majority of their species diversity restricted to deep bathymetric ranges, with no primitive or less evolved shallow-water representatives, questioning the possibility that these lineages migrated from shallower waters (Hessler and Wilson 1983; Raupach et al. 2009). It has been proposed that some deep-sea isopod lineages have evolved *in situ* within the deep sea based on the occurrence of morphologically primitive shallow-water taxa that lack eyes and are related to considerably more evolved deep-sea relatives. Overall, the evolutionary history of bathyal and abyssal species and their connection to shallow-water relatives is difficult to ascertain because retrieving live deep-sea animals for phylogenetic analysis is a challenge and many taxa lack clear fossil records (Raupach et al. 2009).

An alternative to the two previously described theories might be described as a parsimony hypothesis, based on previously proposed bi-directional movement of species between the shallow-water and deep-sea environments (Carney 2005). One of the few case studies in support of this hypothesis involves Antarctic microfossils of Foraminifera from shallow and deep-sea populations that have been discovered to derive from both

depths, showing an active exchange of fauna (Hayward 2001; Lipps and Hickman 1982). Similarly, a combination of early-derived and late-branching lineages found in deep-sea populations of nematodes evokes dynamic exchanges between fauna from different depths (Bik et al. 2010). Overall, the parsimony hypothesis has not formally been discussed in the literature in spite of gathering evidence, which might warrant looking into an intermediate source depth coinciding with peak biodiversity.

Of equal relevance is the temporal scale over which these various types of migrations might occur (e.g. individual lifetime, multiple generations, evolutionary time scales). However, because of experimental limitations, this aspect remains difficult to predict or infer from findings. The initial work preoccupied with the shallow-deep or deep-shallow migration theories mainly approached this discussion from an ecological and fossil record perspective, focusing on evidence that described the natural (past or present) bathymetric distributions of various phyla (Hessler et al. 1979; Hessler and Wilson 1983; Kussakin 1973; Menzies et al. 1973; Thistle and Hessler 1976). Gradually, laboratory-based studies were conducted, which examined the tolerance of extant animal taxa to hydrostatic pressures within and beyond their natural distributions (see review by Brown and Thatje 2014). Responses were measured from various angles, including: physiology (Childress 1976; Childress and Thuesen 1993; Ravaux et al. 2009; 2013) behaviour (New et al. 2014; Shillito et al. 2006; Thatje et al. 2010; Wilcock et al. 1978) development (Tyler and Dixon 2000; Villalobos et al. 2006; Young and Tyler 1993) and genomics (Barros et al. 2015; Dixon et al. 2002; Morris et al. 2015; New et al. 2014).

The bathymetric distribution of species in the ocean is primarily controlled by the presence of temperature and hydrostatic pressure gradients (Cottin et al. 2012; Menzies et

al. 1973). The interactions between both factors (i.e. high hydrostatic pressure and low temperature) have antagonistic effects on the survival of most shallow-water organisms (Mestre et al. 2009). Thus, depending on physiological thresholds to these factors, the habitat range of organisms is defined by an upper and lower vertical limit (Kiel et al. 2012; Mestre et al. 2009; Wilson et al. 2007). Depth ranges that bridge the shallow-water and deep-sea ranges are considered eurybathic (Rodríguez et al. 2007). The selective pressures exerted by abiotic factors on survival supports the hypothesis that there is a biodiversity bottleneck that exists with increasing depths, notably extreme depths greater than 2000-3000 m (Carney 2005). While the tolerance of marine organisms to changes in temperature is a widely studied field, the impact of hydrostatic pressure is comparatively understudied, thus creating a bias in our understanding of bathymetric adaptations (Macdonald 1997).

Unlike temperature, hydrostatic pressure exhibits a linear gradient from the surface to the bottom of the ocean (Pradillon and Gaill 2007; Tyler and Young 1998). The compression forces of pressure result in an increase of 1 MPa (~10 bar or ~10 atm) for every 100 m in the water column (Macdonald 1997; Pradillon et al. 2004). Exposure to high hydrostatic pressure affects physiological and biochemical processes that are reflected at the level of the whole organism, causing the loss of motor function and even mortality (Macdonald and Teal 1975; Morris et al. 2015). The neural and muscular dysfunctions associated with exposure to pressure in invertebrate metazoans are symptoms typical of high-pressure neurological syndrome (HPNS) observed in vertebrates (Jain 1994; Morris et al. 2015). Even if the effects of HPNS are sub-lethal or cause temporary paralysis, it is inferred that this would jeopardize basic ecological

behaviours like foraging and escaping from predators, which are essential to survival (Munro et al. 2015; Oliphant et al. 2011). However, the full range of chemo-physiological effects from hydrostatic pressure is incompletely understood across different phyla of marine organisms (Pradillon and Gaill 2007). It is worth noting that fewer studies have focused on the pressure tolerance of deep-sea species from non-chemosynthetic environments as opposed to those from hydrothermal vents and cold seeps (Dixon et al. 2002; Gaill et al. 1997; Lee 2003; Marsh et al. 2001; Martinez et al. 2001; Pradillon et al. 2001; Pruski and Dixon 2003; Ravaux et al. 2009; 2013; Shillito et al. 2001; 2004; 2006; Smith et al. 2013; Tyler and Dixon 2000). While chemosynthetic environments are characterized by extreme temperatures (Shillito et al. 2001), high sulphide concentrations (Ravaux et al. 2003) and fluctuating ranges of abiotic conditions (Ravaux et al. 2009), high pressure is typical of both types of environments (Ravaux et al. 2009).

Studies have shown that the effects of pressure vary not only across taxa but also intraspecifically throughout ontogeny (Aquino-Souza 2006; Tyler and Dixon 2000; Villalobos et al. 2006; Yoshiki et al. 2006; 2008; 2011). Both Mollusca and Echinodermata larvae are capable of surviving and developing under pressures that typically exceed the natural distribution of their adult stages (e.g. Aquino-Souza et al. 2008; Brown and Thatje 2014; Mestre et al. 2009; Smith and Thatje 2012; Tyler and Young 1998; Tyler and Dixon 2000; Villalobos et al. 2006; Young et al. 1997). Although such studies suggest that the high tolerances of larvae provide sufficient evidence for the deep-sea colonization hypothesis, few studies have investigated the effects on adults (Young et al. 1993) or even on settlement processes and juveniles. In fact, high-pressure

tolerance is greatest at the early embryonic stages during cleavage but subsequently decreases with further development (Pradillon and Gaill 2007; Tyler and Dixon 2000). For instance, juveniles of various echinoderm species have been found to settle at pressures exceeding the bathymetric distribution of adults but they expressed delayed development, which resulted in high levels of mortality (Gage and Tyler 1981a; 1981b; Sumida et al. 2000).

Despite the gaps in knowledge and some of the shortcomings of the shallow to deep-sea migration hypothesis, few empirical studies have expanded on the alternative possibility that deep-sea taxa could migrate upwards (emergence hypothesis) or in both directions (parsimony hypothesis) from either shallow to deep environments or even to either environment from a pelagic region (500-1000 m). This is largely due to the fact that deep-sea organisms are expensive to collect and logistically difficult to maintain alive in laboratory conditions (Shillito et al. 2001; 2015). Despite a shortage of supporting evidence, it has been claimed that animals collected from depths below 1500 m are incapable of surviving at atmospheric pressure for long-term periods (Dixon et al. 2004; Pruski and Dixon 2003).

Structurally, marine invertebrates lack internal gas spaces that will cause damage from gas expansion upon depressurization. Therefore, adult animals that are retrieved can be used for laboratory investigations if there is no cell or tissue damage (Dixon et al. 2004). A number of studies have shown that animals collected between 200 and 2500 m can thrive for several years, reproduce and grow at atmospheric pressure, under true-to-native temperature regimes (Hamel et al. 2010; Mercier and Hamel 2008b; Sun et al. 2010). Other species collected from depths of ~2000 m have also been observed to

survive at atmospheric pressure in laboratory conditions (Smith et al. 2013). Even the hydrothermal shrimp, *Mirocaris fortunata* collected from specialized habitats (vent communities) have been maintained at atmospheric pressure for over a year (Shillito et al. 2015). Maintenance of deep-sea animals at atmospheric pressure is not limited to adults, as many studies have been able to rear deep-sea larvae at atmospheric pressure as well. For example, Epifanio et al., (1999) reared megalopa larvae of *Bythograea thermydron* collected from 2600 m to undergo metamorphosis. It has also been found that some embryos of *A. pompejana* from 2500 m depths were capable of developing at 0.1 MPa (Pradillon et al. 2005). Although there have been some reported abnormalities with the development of deep-sea species at atmospheric pressure, a variety of deep-sea cnidarians and echinoderms have been developed from spontaneous egg release until successful settlement and early juvenile growth (Mercier et al. 2011a; 2014), even to the next reproductive generation (Mercier et al. 2014). The ability for some animals to adapt and survive could presumably give rise to new populations (Marsh et al. 2001; Pradillon et al. 2005; Young and Tyler 1993). Thus, tolerances for lower pressures suggest that larvae of deep-sea taxa are capable of penetrating upper bathymetric ranges where adults can thrive as well.

Overall, there is accumulating evidence that deep-sea animals are physiologically equipped to expand their range upwards, which can be seen to balance similar evidence provided in support of shallow-water species tolerating high pressures for short periods. The objective of the present review is therefore to examine and critically assess the full scope of evidence in favour of both hypotheses to tease out major findings and identify a way forward. As 80% of the marine biosphere lies below depths of 500 m, it is critical to

understand adaptations to hyperbaric conditions in order to determine evolutionary patterns (Jaenicke 1983; Jannasch and Taylor 1984; Somero 1992). Climate change is warming ocean surface temperatures and predicted to drive the vertical migrations of benthic organisms (Morris et al. 2015). The movements of marine species across depths could potentially alter existing ecosystems, emphasizing the importance of furthering our understanding of the colonization theories in the face of predicted climatic shifts. While hydrostatic pressure is another strong driver of bathymetric distributions, its effects on marine benthic invertebrates remain poorly understood.

The aim of our study is to present an unbiased perspective of the deep-sea and shallow-water migration hypotheses based on empirical evidence of pressure tolerance. Specifically, we will (1) review published data on the responses of both shallow and deep-sea animals, at various life stages, to pressures within and beyond their bathymetric range; (2) include previously unpublished data; (3) identify key trends of relevance to colonization theories; and (4) critically discuss how the evidence may be interpreted (i.e. whether tolerance to certain pressure variations relates to ancestral provenance or future invasion). Three main hypotheses will be examined: whether animals from shallow-water and deep-sea environments have elicit variability in survival responses based on the (i) depth of collection and (ii) geographic location of origin, and lastly, (iii) whether phylum drives the ability to tolerate pressure.

2.3 Methods

2.3.1 Data collection and treatment

Efforts were made to collect the largest possible dataset on the response of shallow-water and deep-sea marine invertebrates exposed to pressures within and beyond their natural bathymetric range. Empirical data from 134 studies were collected for 261 species, which included the following phyla: Annelida, Arthropoda, Brachiopoda, Chaetognatha, Chordata, Cnidaria, Echinodermata, Foraminifera, Heterokontophyta, Mollusca, Porifera, Sipuncula and Vestimentifera (Table A1 in Appendix A). The categorical and numerical variables compiled from the studies are listed and defined in Table 2-1. As definitions of oceanic zones vary in the literature, the present study followed the basic strata outlined in Gage and Tyler (1999): 20-200 m (subtidal or shallow); >200-2000 m (bathyal) and >2000-6000 m (abyssal). These depth ranges also parallel the shelf, slope and abyssal zones defined by Woolley et al. (2016).

Analysis considered each experiment (even if reported in the same study) as an individual data point (record) as long as different pressures, durations and/or life stages were used. Due to differences in developmental modes across the species studied, only the general universal life stages were considered (larva, adult). Species-specific life stage categories (e.g. nauplii) were omitted. In addition to the data collected from published papers, new empirical data were added from long-term laboratory observations for several species (see data in Table A1). Only whole animals collected from the wild were considered in the statistical analysis and cell cultures from animals collected at depths or animals reared in laboratory for multiple generations were omitted (no habitat type was assigned to either case). If studies recorded that animals were captured at both shallow

(<200 m) and deep (≥200 m) depths, and the specimens were tested together without distinguishing the results from the different collection depths, the data were omitted. In cases where the collection depth range was <100 m, the greatest depth was selected since the differential in pressure was under 1 MPa. Animals that were collected down a continuous gradient of overlapping shallow and deep depths extending more than 50 m were not considered (e.g. samples from plankton tows) but if animals were collected from a range that extended into only two habitat types that was less than 15 m, the deeper of the depth was selected for the analysis.

Studies selected for analysis were limited to records that tested pressure tolerance to a single known pressure value. Thus, experiments that set their test pressure at once (absolute method) were all included. Studies that used a step-wise protocol (increment method) were also selected if the process took less than 25% of the total experimental period. Incremental methods that continuously subjected animals to multiple pressures to demonstrate the overall ability for animals to adapt could not be included since it was not possible to determine any clear pressure-response link.

When studies used different combinations of temperature and pressure, the results of the optimal temperature for survival were selected for tabulation (Table A1) and corresponding analysis. The optimal temperature was selected based on explicit identification by the investigators or, if this was not stated, the temperature resulting in the highest survival rate. Experiments that tested pressure tolerance under incremental thermal conditions that were not explicitly defined to be within the thermal tolerance of the species were excluded as this treatment could have imposed thermal stress and reduced the ability for animals to cope with pressure. Survival proportions of individuals

(%) were ranked into the following categories for analyses: 1 (0-9%), 2 (10-19%), 3 (20-29%), 4 (30-39%), 5 (40-49%), 6 (50-59%), 7 (60-69%), 8 (70-79%), 9 (80-89%) and 10 (90-100%).

In instances where studies stated that individuals (adults or larvae) survived for a time within the laboratory under atmospheric pressure (prior, during or post experimentation) the maximum time of survival was recorded and used in analysis. If investigators reported a range (i.e. the minimum and maximum time) these values were presented in the table. Studies were not generally conducted in an ecotoxicological context (i.e. with formal measure of LC50 values); therefore, survival times were used as they were often provided. It is important to note that this measure is only an estimation of how long the species can survive (at non-native pressure). Studies that did not provide exact maintenance duration were omitted. For instances in which early stages of larvae were reported to develop to or surpass the juvenile stages under atmospheric pressure, post-metamorphic data were omitted. For the analyses survival times were separated into the following categories: 1 (<10 d), 2 (10-60 d), 3 (61-730 d) and 4 (>730 d).

Data were separately analyzed for animals that were collected from their natural habitat and those that were born and reared at atmospheric pressure. As the animals reared at atmospheric pressure were the first generation of animals collected from depth, they were ascribed their parental life history. As chemosynthetic habitats generate their own thermal gradients, no climate zone data were determined for these records.

2.3.2 Statistical analysis

Factor analysis of mixed data (FAMD) was used to examine the strength of associations between experimental variables and the ranked response variables, i.e.

survival time or percent survival (Lê et al. 2008). In order to assess the influence of deep-sea chemosynthetic habitats (e.g. hydrothermal vents and cold seeps) on response variables, records involving animals from chemosynthetic habitats were first grouped with others and analyzed separately from animals from non-chemosynthetic environments. The depth variable (deep or shallow) could not be assessed in any analyses that examined individuals from chemosynthetic environments, as these regions were restricted to the deep-sea. As FAMD analyses require a minimum of three data points for any variable, in some cases depth range (i.e. abyssal, bathyal, subtidal, etc.) could not be tested as data was only sourced from the deep-sea and there was a bias for animals collected from bathyal depths as opposed to abyssal depths. As the survival time measured the length of time that individuals from the deep-sea survived at atmospheric pressure, experimental duration and pressure were not relevant to this response variable.

The survival time of adults at atmospheric pressure was assessed with phylum and geographic location for groups from chemosynthetic (n=44, Table A2) and non-chemosynthetic environments (n=105, Table A3). FAMD was also used to detect associations between phylum, geographic location and depth range for groups from non-chemosynthetic environments (n=108, Table A4). The next tests examined the associations for the survival percent of chemosynthetic groups based on: phylum, pressure and experimental duration (n=72, Table A5) and phylum, geographic location and depth range (n=72, Table A6). The next two tests repeated the groups of factors mentioned above with the addition of the depth variable (i.e. deep or shallow origin) for the survival percent of non-chemosynthetic groups (n=170, Table A7; n=170, Table A8). Larvae from chemosynthetic environments were tested for associations with phylum,

pressure and experimental duration (*n*=22, Table A9) and phylum, geographic location and depth range (*n*=22, Table A10). The final two tests repeated the groups of factors mentioned above with the addition of the depth variable (i.e. deep or shallow origin) for the survival percent of non-chemosynthetic groups (*n*=163, Table A11; *n*=163, Table A12). Survival time of larvae could not be tested due to lack of data; variables and results from FAMD analyses are summarized in Table 2-2.

Pearson correlations were conducted to test the relationships between numerical measurements (pressure and experimental duration) and response variables (percent survival) in Table A13-A16. Significance levels were considered at $p<0.05$. All analyses were carried out using Sigma Plot version 11.0 (Systat Software, USA) and RStudio 2017, version 2.11.1.

2.4 Results

2.4.1 Adults

2.4.1.1 Survival time of deep-sea adults at atmospheric pressure

2.4.1.1a General observations

Despite the extensive logistical limitations with regards to collecting deep-sea species and holding them under atmospheric pressure, a considerable number of studies maintained a collectively high diversity of species in the laboratory. In nearly all cases, individuals collected from the deep sea were depressurized quickly (within minutes to hours) before being transferred to holding facilities on a ship and then in the laboratory. While some researchers have clearly specialized in the maintenance and study of a taxon

from a given location, other laboratories have held and tested several taxa under similar conditions.

Generally, species collected from chemosynthetic environments were obtained from consistent locations and similar depths due to their restricted distributions. For instance, vent mussels, *Bathymodiolus azoricus* were only collected from the North-East Atlantic (NE Atl) at Menez Gwen and Rainbow from depths of 840, 850 and 2300 m. Individuals survived anywhere from 10 d to one full year (e.g. Barros et al. 2015; Bettencourt et al. 2008; 2010; Colaço et al. 2011; Dixon et al. 2004; Kadar et al. 2008a; Martins et al. 2014; Pruski and Dixon 2003). Likewise, *Bathymodiolus childressi* from the Gulf of Mexico (GOM) survived under atmospheric pressure conditions for periods ranging from 15 to 365 d (Arellano and Young 2009). Furthermore, 5 species of Arthropoda from chemosynthetic environments were sampled from the North-West Pacific (NW Pac) off the coast of Japan and maintained for over a year under atmospheric pressure (Hamasaki et al. 2010; Miyake et al. 2007). There are comparatively few examples of chemosynthetic Annelida successfully maintained under atmospheric pressure; e.g. hydrothermal vent tubeworms (*Lamellibrachia luymesi*) were collected and held between 1-15 months in laboratory conditions (Dattagupta et al. 2006). It was noted that most individuals survived despite physical damage to the roots sustained during the collection process.

Parallel investigations focused on non-chemosynthetic environments in the North-West Atlantic (NW Atl) have collected and successfully held 22 different deep-sea species of Cnidaria that were maintained for over 2 years under atmospheric pressure (present study; Mercier and Hamel, 2009; Baillon et al., 2013; Sun et al., 2009, 2010,

2011; Hamel et al., 2010; Mercier et al., 2011a, 2017). Other deep-sea phyla from this area that have shown similar ability to survive at low pressure include: 18 species of Echinodermata, 12 species of Arthropoda, 6 species of Mollusca, and 2 species of Porifera (Table A1). Successful holding also included Polychaeta; i.e. *Neopolynoe acanellae* collected from depths of 466-1406 m (Hamel et al. 2015) and *Ophryotrocha* sp. that was maintained for several generations (Verkaik et al. 2016b). Many other individuals were not only observed to survive at non-native pressure but also grew and reproduced. For instance, the scleractinian coral *Flabellum alabastrum* exhibited growth between 1 and 5 mm $year^{-1}$ over two years (Hamel et al. 2010). Details on other species that successfully reproduced under low-pressure conditions are provided below (section 2.4.2.2), including a delicate species of pycnogonid (*Nymphon hirtipes*), which was collected from depths of 1350-1450 m off eastern Canada (Mercier et al. 2015). Reports from many other regions reveal that a diversity of deep-sea taxa from non-chemosynthetic environments were confirmed to survive at atmospheric pressure for days to months, and beyond (Table A1). Notably, several Arthropoda and Echinodermata (13 and 14 species, respectively) were collected from the tropical West Pacific (Trop W Pac) and maintained in the laboratory for 120 d by Wilson et al. (2013). It is possible that the species could have survived longer than 120 d but the experiments were simply concluded. Among other examples, hagfishes (Chordata) collected from depths of 1200 m survived for a year in laboratory conditions (Drazen, pers. comm.), amphipods (Arthropoda) collected from the NE Atl at depths of 1528-1765 m survived for 60 d (Brown and Thatje 2011), and two coral species (Cnidaria) collected from the

Mediterranean (Med) at depths of 214 and 218 m were held at atmospheric pressure for 517 d to study their growth (Orejas et al. 2008).

2.4.1.1b Analysis of survival time in deep-sea adults

In general, FAMD results for taxa collected from chemosynthetic environments showed that Arthropoda survived longer (mean = 310.7 d) than Mollusca (175.1 d; $p<0.001$) under laboratory conditions. Furthermore, individuals from vents/seeps in the NW Pac survived longer (390 d) than those from the NE Atl and GOM (216 d and 134 d, respectively). Figure 2-1 shows the overall average survival times recorded (not just the data selected for the FAMD analyses), based on phyla and geographic locations.

FAMD analyses on taxa from non-chemosynthetic environments revealed that Echinodermata and Arthropoda collected from the Trop W Pac survived for shorter durations (116.6 d and 120 d, respectively) than the average of other non-chemosynthetic species (518.9 d; $p<0.001$). Furthermore, adults from the Med and North-East Pacific (NE Pac) survived for shorter durations (mean = 347 d and 375 d, respectively) under atmospheric pressure conditions in the laboratory than individuals collected from the other geographic locations studied (518.9 d; $p<0.001$). Lastly, Cnidaria from the NW Atl survived longer (730 d) than the average (518.9 d; $p<0.001$). In FAMD analyses where the depth range of adults from non-chemosynthetic regions was considered, no associations between bathyal or abyssal depth ranges and the other factors were evidenced. Average minimum survival times are presented in Figure 2-2, with regards to phyla, geographic locations and depth ranges.

2.4.1.2 Pressure tolerance experiments

2.4.1.2a General observations

Experimental studies that expose live animals to various pressure levels are relatively novel and still plagued by technical constraints. Unlike the holding of deep-sea species at atmospheric pressure, which can offer good conditions apart from the pressure change, the pressurization of live organisms is invariably subjected to potential biases (e.g. small volumes, static conditions). For this reason, and because flow-through pressure vessels are rare, experiments involving pressure are still of short durations. Experiments were conducted in a variety of different pressure vessels (e.g. IPOCAMP and customized hydraulic pump pressure systems) and also in regular aquaria, which were in some cases designed to chemically mimic hydrothermal vent environments.

Many species from chemosynthetic environments were brought to the surface and then tested under high pressures mimicking their depth of collection (e.g. Boutet et al. 2009; Childress et al. 1984; 1991; Cottin et al. 2008; 2010a; Durand et al. 2010; Dixon et al. 2002; Gaill et al. 1997; Mickel and Childress 1982b; Kadar et al. 2008a; Ravaux et al. 2003; 2013; Shillito et al. 2001; 2004; 2006; 2015). Despite the stress experienced from collection and re-pressurization, survival was generally high for >70% of these studies (Table A1) (e.g. Boutet et al. 2009; Childress et al. 1984; 1991; Company et al. 2004; Cottin et al. 2008; 2010a; Dixon et al. 2002; Durand et al. 2010; Kadar et al. 2008a; Mickel and Childress 1982c; Ravaux et al. 2003; 2013; Shillito et al. 2001). Similarly, when individuals were formally tested at lower pressure conditions (atmospheric) survival remained high for either the control experiments that were run in parallel with pressure treatments (Dixon et al. 2002; Martinez et al. 2001; Shillito et al. 2006; Watsuji et al. 2014) or experiments that simply maintained individuals under atmospheric

pressure conditions (Bettencourt et al. 2008; Colaço et al. 2011; Company et al. 2004; Kadar et al. 2008a; Martins et al. 2014; Miyake et al. 2007). For instance, deep-sea Mollusca maintained under atmospheric pressure had 100% survival following experimental durations ranging from 12 h to a year (Bettencourt et al. 2008; Colaço et al. 2011; Company et al. 2004; Martinez et al. 2001; Kádár et al. 2005; 2006; 2008a). Survival in Arthropoda was not as high, but still generally >50% (only one experiment resulted in 0% survival; Table A1). Furthermore, the hydrothermal vent shrimp *Mirocaris fortunata* collected from 850 m was maintained for 9 d at atmospheric pressure and had a 70% survival rate (Shillito et al. 2006).

Other studies examined the survival of shallow-water individuals from non-chemosynthetic environments under experimental pressures, yielding variable results. In the findings of Chapter 3, shallow-water species of Echinodermata (*Strongylocentrotus droebachiensis* and *Cucumaria frondosa*) collected from the NW Atl had a more limited ability to survive exposure longer than shorter exposures (72 h vs. 24 h) to pressure beyond their natural bathymetric range (2-3 times the deepest depth of natural occurrence). Similarly, after 9 d of pressure exposure, there was 100% mortality of the polar sea stars (*Leptasterias polaris*), demonstrating a lack of tolerance to high pressure over extended experimental durations (9 d vs 24/72 h). The same was noted for a species of shallow-water Mollusca, *Mytilus edulis* (Ammendolia et al., in preparation; Chapter 3). Nevertheless, all else being equal, the analysis of experimental results obtained so far can provide some interesting insight (see below).

2.4.1.2b Analysis of percent survival in shallow-water and deep-sea adults exposed to experimental pressure

According to FAMD analyses of results from experiments conducted on taxa collected from chemosynthetic environments, percent survival of deep-sea Arthropoda tested for longer durations (3782 h or 157 d) under experimental pressures survived less (mean = 70.3%) than the average (79.9%; $p<0.001$). Similarly, Arthropoda specifically from the NE Atl collected at bathyal depths survived less (62.6%) than the average (79.9%; $p<0.001$). By contrast, Mollusca from the NE Atl collected from bathyal depths and Annelida from the NE Pac survived better (84.3% and 96.7%, respectively) than the average (79.9%; $p<0.001$). Overall, there was no correlation between percent survival and experimental pressure level ($R=0.11$, $p=0.371$) but there was a significant negative correlation between survival and trial duration ($R=-0.54$, $p<0.001$). Average percent survival with respect to phyla and depth ranges is presented in Figure 2-3.

FAMD analyses of experimental data involving individuals from non-chemosynthetic environments revealed that deep-sea Cnidaria tested for long durations (17009 h or 709 d) at low/atmospheric pressure (0.1 MPa) survived more (mean = 80.5%) than the average (76.1%; $p<0.001$). In other FAMD analyses, shallow-water Arthropoda from the Trop E Pac collected from subtidal depths survived less (32.1%) than the average (76.1%; $p<0.001$). It was also found that deep-sea Cnidaria from the NW Atl collected from bathyal depths survived better (78.3%; $p<0.001$). Overall, there was a significant negative correlation between percent survival and pressure level ($R=-0.17$, $p=0.025$) but no correlation with experimental duration ($R=0.07$, $p=0.378$). Figure 2-4 shows the average percent survival within phyla, geographic locations and depth ranges.

2.4.2 Larvae

2.4.2.1 Survival time of deep-sea larvae at atmospheric pressure

The literature includes many reports of adult deep-sea species having spawned under atmospheric pressure and the subsequent larvae having successfully developed (Table A1). Similar to data on adults, data on larvae from both chemosynthetic and non-chemosynthetic environments exist.

Information on the development of larvae obtained following the spawning of adults collected from chemosynthetic environments is largely inconsistent. Deep-sea adult ascidians (Chordata) collected in the NW Pacific released larvae under atmospheric pressure conditions that developed into juveniles and survived a maximum of 7 months (Havenhand et al. 2006). Similarly, many deep-sea species sampled from the Nikko Seamount vents of the NW Pacific successfully spawned, including the vent crab (*Austinograea yunhana*), tonguefish (*Symphurus sp.*) and vent shrimp (*Opaepele* spp.) (Miyake et al. 2007). Vent shrimp did not continue development post hatching (Miyake et al. 2007). Despite the fact that vent crab larvae also experienced early mortality, it was speculated that they could potential survive to megalope or juvenile stages under non-native pressure because mortality was unrelated to pressure issues, according to investigators (Miyake et al. 2007). Similar issues were reported in the deep-sea bythograeid crabs: larvae that were obtained in the laboratory successfully metamorphosed into megalopal stages, but individuals died without molting into juveniles after 104 d (Hamasaki et al. 2010). It was suggested that chemical cues from the natural environment were required for moulting and that pressure was not a limiting factor (Hamasaki et al. 2010). Wantanabe et al. (2004) offered similar conclusions when

deep-sea adult barnacles (*Neoverruca*) from the NW Pacific spawned larvae under atmospheric pressure, and none successfully metamorphosed/settled before dying after 183 days.

One of the limitations of assessing larval survival in the literature was that investigators did not always monitor development. For instance, adult deep-sea vent shrimp (*Mirocaris fortunata*) from the NE Alt were collected from 1700 m and maintained at either native or atmospheric pressures (Shillito et al. 2015). Some individuals developed gonads to a gravid state but larval development was not examined. Similarly, despite the fact that adult vent mussels (*Bathymodiolus azoricus*) and cold-seep mussels (*Bathymodiolus childressi*), collected from different geographic regions and maintained for one full year, spawned, the larvae were not monitored (Arellano and Young 2009; Colaço et al. 2006). Overall, it is apparent that most studies focused mainly on testing the ability for adults to spawn and did not examine the fate of the offspring (Colaço et al. 2006; Shillito et al. 2015).

It is worth noting that some investigators obtained larvae directly from chemosynthetic environments and encountered similar issues as those mentioned above. Arellano et al. (2014) collected eggs of the gastropod *Bathynerita naticoidea* and veligers of *Bathymodiolus childressi* from the GOM. Although individuals successfully hatched and developed under atmospheric pressure, the maximum length of survival at this pressure was not assessed. Similarly, Epifanio et al. (1999) collected megalopa larvae and early juveniles of hydrothermal vent crab (*Bythograea thermydron*) from 2500 m. Despite the trauma of collection and transfer to atmospheric pressure, a portion of the megalopae metamorphosed and survived until their 3rd stage of development, while

juveniles survived longer. Some of the individuals were maintained under atmospheric pressure for a period of 201 days. Juvenile demonstrated normal mobility and feeding under atmospheric pressure and their mortality was attributed to molting rather than barotolerance.

There have been even more cases of larvae from non-chemosynthetic environments obtained and raised until settlement under laboratory conditions. In a diversity of deep-sea taxa collected from the NW Atlantic, larvae and resulting juveniles survived for long-term periods (years) at atmospheric pressure, including those of the sea anemones *Allantactis parasitica* (Mercier and Hamel 2009) and *Urticina* sp. (Mercier et al. 2011b), the whelk *Buccinum scalariforme* (Montgomery et al., in press), the sea star *Henricia lisa* (Mercier and Hamel 2008b) and several corals including *Flabellum angulare* (Hamel et al. 2010), *Drifa glomerata* (Sun et al. 2010) and *Drifa* sp. (Sun et al. 2009). Notably, deep-sea Annelida (*Ophryotrocha* sp.) collected at 1500 m reproduced at atmospheric pressure after being maintained for a year; juvenile stages had survival rates of 80%, and some survived to adult stages and successfully reproduce themselves, yielding a total of three generations (Mercier et al. 2014; Verkaik et al. 2016b). Another example is offered by *Nymphon hirtipes* (Arthropoda: Pycnogonida) collected at similar deep-sea depths, whose larvae were successfully reared in the laboratory over a period of 390 d until juvenile stages dispersed (Mercier et al. 2015). As mentioned earlier for chemosynthetic species, many studies did not evaluate the development of larvae from non-chemosynthetic environments, mainly because such investigations require appropriate holding facilities and continuous monitoring over months to years. For instance, offspring of deep-sea Cnidaria including *Drifa* sp., *Drifa glomerata, Flabellum*

angulare and *Urticina* sp. had high survival rates (>70%) to the planula stage (Mercier et al. 2011a; Mercier et al. 2011b; Mercier et al. 2011c; Sun et al. 2009; Sun et al. 2010) but the full potential of these individuals and their ability to survive to later stages was not evaluated due to logistical constraints (Mercier et al. 2014; Sun et al. 2010).

2.4.2.2 Pressure tolerance experiments

2.4.2.2a General observations

Overall, there were very few studies and experiments that had collected larvae from chemosynthetic environments and managed to experimentally test their survival (*n*=22 data points; 8 studies). This can be attributed to the logistical difficulties in collecting species from deep-sea vent or seep communities (Cottin et al. 2008; Pradillon et al. 2001; Pradillon et al. 2005; Ravaux et al. 2009). Generally, larvae of deep-sea vent species of Mollusca (that were spawned from adults collected from the GOM) survived well (>58%) when exposed to atmospheric pressure (Arellano and Young 2011; Arellano et al. 2014). The Arthropoda species *Neoverruca* sp. survived the best in experiments that exposed larvae to lower pressures than to native pressure conditions (Watanabe et al. 2014). Survival ranged from 97-100% over the course of 14-17 d, which were the longest set of experiments conducted within this dataset.

Larvae that were spawned from adults originating from non-chemosynthetic environments generally survived well under various experimental conditions. For instance, species of shallow-water Echinodermata collected from the NE Atl and Antarctica had high survival rates (mostly >80%) when exposed to pressures that are characteristic of depths greater than their natural occurrence (Aquino-Souza et al. 2008; Tyler and Young 1998; Tyler and Dixon 2000; Villalobos et al. 2006). Such experiments

lasted 24-48 h, which can be considered long experimental durations since larvae develop relatively fast. By contrast, other experiments focused on acute shock and tested individuals for as little as 7.2 min (~0.12 h) (Ding et al. 2007). The experimental durations were sometimes limited because of the technologies available. For instance, George and Marum (1974) tested larvae of Arthropoda (12 species), Cnidaria (1 species) and Mollusca (1 species) from either the Cari or NW Atl under experimental pressures for 1 h (6.1-65.5 MPa). Generally, most individuals had 100% mortality, although it should be noted that pressurization lacked acclimation periods and occurred rapidly <1 min.

Generally, there were trends of low survival among shallow-water larvae when exposed to increasing pressures that were beyond the scope of their natural bathymetric range. This was apparent in species of Mollusca, for instance, *Crepidula fornicata* and *Nucella lapillus* collected from the NE Atl and NW Atl, which survived less than the average when exposed to higher pressures (Mestre et al. 2009; Pechenik et al. 1984). This trend was also seen in other phyla, including shallow-water Annelida larvae collected from the NE Atl that had low pressure tolerances for high experimental pressures, i.e. 19.3% survival under 30.4 MPa (Vevers et al. 2010).

2.4.2.2b Analysis of percent survival in shallow-water and deep-sea larvae exposed to experimental pressure

FAMD analyses of data on larvae obtained from chemosynthetic adults/environments revealed that Annelida tested for short durations (73.1 h) under high pressures (11.5 MPa) survived less (mean = 56.9%) than the average (73.1%; $p<0.001$). By contrast, Arthropoda tested under low pressures (6.5 MPa) survived more (84.6%)

than the average (73.1%; $p<0.001$). The results of another FAMD analysis showed that larvae of Annelida from the NE Pac originating from abyssal depths survived less (56.9%) than the average (73.1%; $p<0.001$). There was no correlation between percent survival and pressure level ($R=-0.27$, $p=0.226$) or experimental duration ($R=0.01$, $p=0.996$). Average percent survival of larvae obtained from species grouped by phyla, geographic locations and depth ranges is presented in Figure 2-5.

In general, FAMD analyses conducted on data involving larvae from non-chemosynthetic environments showed that deep-sea Cnidaria tested for long durations (14335 h or 597 d) under lower pressures (mean = 5.8 MPa) survived better (73.6%) than the average (69.7%; $p<0.001$). Another FAMD analysis revealed that larvae of Echinodermata from the NE Atl and of Arthropoda from the NW Pac, both from subtidal depths, survived better (94.7% and 63.1%, respectively) than the average (69.7%; $p<0.001$). By contrast, larvae of shallow-water Echinodermata from the Trop W Pac collected from subtidal depths survived less (15.4%) than the average (69.7%; $p<0.001$). Also, larvae of deep-sea Arthropoda from the NW Pac and Caribbean collected from bathyal depths survived less (63.7% and 10%, respectively) than the average (69.7%; $p<0.001$). Lastly, shallow-water larval Annelida from the intertidal zone survived less (63.1%) than the average (69.7%; $p<0.001$). There was a significant negative correlation between percent larval survival and pressure level ($R=-0.64$, $p=<0.00001$) but not experimental duration ($R=0.11$, $p=0.153$). Average percent survival of larvae belonging to various phyla and obtained from species collected in different geographic locations and depth ranges is presented in Fig. 2-6.

2.5 Discussion

How species colonized the ocean and how vertical migrations will continue to operate in the face of climate change are crucial questions for ecologists and evolutionary scientists. By analyzing a large set of observational and experimental results related to the pressure tolerance of a diversity of marine taxa, the present study attempted to draw the first broad (but likely not comprehensive) picture of the evidence available from empirical data. Findings show that adults and progeny of many deep-sea species can withstand quick depressurization and survive fairly well (thriving, feeding, spawning, developing, growing) at atmospheric pressure when environmental conditions are minimally suitable (e.g. Arellano and Young 2009; 2014; Colaço et al. 2006; Epifanio et al. 1999; Mercier and Hamel 2008b; 2009; Mercier et al. 2011a; Miyake et al. 2007). Most of the successful examples are from higher attitudes; however, the confounding effect of thermotolerance (Pradillon et al. 2001; 2005; Shillito et al. 2006) currently prevents any reliable assessment of the pressure tolerance of taxa collected at depth from lower latitudes, since live animals incur a temperature shock during transport to the surface (Menzies and Wilson 1961; Quetin and Childress 1980; Wilson et al. 2013). On the other hand, shallow-water species, with the possible exception of eurybathic taxa, are not known to survive marked increases in pressure beyond a few hours, although this could be a result of our still limited capacity to maintain optimal environmental conditions at experimental pressure. A final limitation involves marked differences between trial conditions when testing pressure tolerance in larvae and adults (i.e. smaller sample sizes and shorter experimental durations for larvae); therefore results have to be explored and discussed separately, and ontogenetic comparisons remain tentative at best.

Overall, when the measure of pressure tolerance is interpreted as a predictor of recent/ongoing vertical migration, the available data are more strongly supportive of the parsimony or emergence hypotheses, with bathyal taxa exhibiting the most plastic tolerance to pressure variations.

2.5.1 Pressure tolerance of adults

The hypothesis that depth stratum of collection would drive pressure tolerance was supported. A number of investigating teams have successfully maintained deep-sea animals at atmospheric pressure for several years in tanks that either mimicked the conditions of chemosynthetic environments (e.g. Colaço et al. 2006; 2011; Miyake et al. 2007; 2012; Shillito et al. 2015) or were supplied with ambient seawater (e.g. Baillon et al. 2014; Hamel et al. 2010; Mercier and Hamel 2009; Mercier et al. 2013; 2014). In these studies, deep-water animals exhibited key behaviours such as feeding, growth, spawning and development, indicating that they adapted successfully to atmospheric pressure. Further evidence of the resilience of deep-water taxa comes from a field study that transplanted deep-sea Antarctic acorn barnacles (*Bathylasma corolliforme*) from 400 to 25 m; individuals survived at transplanted depths for >2 years (Dayton et al. 1982). Survival in this experiment was not affected by pressure but rather by substrate availability, thus supporting the ability for deep-sea species to adapt to shallow water under otherwise suitable conditions. It should be noted that evidence of deep-sea species surviving atypical pressures (i.e. atmospheric pressure) often lasted months to years (Mercier et al. 2011a; Miyake et al. 2007; Weinberg 1990). By contrast, evidence of shallow-water bivalves (*Mytilus galloprovincialis*) surviving pressure treatments was no longer than 69 d for individuals maintained in sea cages at depth (Galgani et al. 2005) or

28 d in pressurized aquaria for shrimps (Cottin et al. 2012). Differences between the experimental pressure exposure durations used for testing shallow-water and deep-sea pressure tolerances were inherent limiting factors within the available literature. Interestingly, deep-sea animals also exhibited high survival levels under pressure experiments (i.e. re-pressurization). The percent survival of adults after experimental exposure to non-native pressure was overall higher for deep-sea (80%) than shallow-water taxa (68%).

Despite the early assumption that deep-sea animals from depths >1500 m could not survive atmospheric pressure (Dixon et al. 2004; Pruski and Dixon 2003) the evidence summarized here includes several examples of deep-sea animals collected >2000 m surviving very well at atmospheric pressure. Nevertheless, the length of post-collection survival of deep-sea adults at atmospheric pressure was generally longer for taxa collected from bathyal (>200-2000 m) than abyssal depths (>2000-6000 m). This trend was evidenced for both chemosynthetic and non-chemosynthetic species. Exceptions include deep-sea corals of the genus *Flabellum* collected in both zones from the NW Atlantic, which survived equally well (present study; Hamel et al. 2010; Mercier et al. 2011a), suggesting that the threshold (if present) may lie deeper in boreal climates. On the other hand, it cannot be excluded that the numerous cases of abyssal species showing shorter survival at atmospheric pressure results from damage incurred during collection rather than to any real physiological barrier. For instance, depressurisation of animals collected from deep-sea vent communities at 2530 m occurred inside ~90 minutes (Pradillon et al. 2004). When animals undergo acute barotrauma, severe damage to nervous tissue on both a transcriptional and cellular level can occur, as suggested by

Morris et al. (2013) potentially leading to serious injuries or death. Supporting this conclusion, Seo et al. (2013) demonstrated that gradual acclimation to new pressures under laboratory conditions increased the chances of survival for the crustacean *Artemia franciscana*. Due to the lack of control over depressurising conditions during field collections, abyssal species could survive even more than what has been documented up to now, therefore, their potential to survive under atmospheric pressure must not be entirely ruled out.

In addition to depth of collection, geographic location of origin also revealed a marked dichotomy between lower and higher latitudes. Individuals collected from tropical locations generally did not survive as long or in equal proportions to those from northern locations. For instance, both shallow-water and deep-sea species collected in the tropical Eastern Pacific survived less than all those from higher latitudes under experimental conditions or at atmospheric pressure, respectively. Furthermore, in pressurization trials, Arthropoda and Echinodermata collected from the shallow Mediterranean Sea (Young et al. 1997) survived less than the average from other locations, including temperate habitats, when exposed to various pressures. As thermoclines in northern areas occur at ~500 m and stratification generally dissipates over several months, between late fall and spring, animals collected during those periods would not experience as severe a temperature shock as those collected in tropical waters where a more permanent stratification occurs in the first 100 m (Tyler and Young 1998). Ravaux et al. (2003) reported that using insulated collection boxes on ROVs markedly increased the survival of deep-sea species collected at abyssal depths.

Recently, Brown and Thatje (2015) showed that fundamental ecological niches (FENs) or bathymetric habitats of marine animals are controlled by abiotic factors (i.e. temperature, pressure, oxygen concentration). The spatial extent of FENs depends on their geographic location; generally, FENs in tropical regions are geographically and bathymetrically constricted because of the drastic changes in the thermoclines (see above), whereas the inverse exists for northern regions (Brown and Thatje 2015; Tyler and Young 1998). Therefore, the success of bathymetric migrations within tropical regions may also (or primarily) be limited by thermotolerance preventing species from surviving vertical movement. This reasoning applies both ways. For instance, low temperatures can cause physiological stress that increases the mitochondrial oxygen demand in the shallow-water spider crab (*Maja squinado*) and high pressure can complicate processes (i.e. ventilation and circulation) that would maintain aerobic homeostasis (Brown and Thatje 2015; Frederich and Portner 2000). Inversely, hydrothermal vent shrimp (*Microcaris fortunata*) originally from depths of 1700 m were maintained for months at atmospheric pressure and were found to be attracted to heating elements within the aquaria; although this could be due to the animals exhibiting behaviour also displayed in their native hydrothermal vents (Matabos et al. 2015). However, long survival durations and attraction to high temperatures may indicate potential for species to exploit more shallow environments.

Overall, movement in tropical areas might be restricted by thermal physiological bottlenecks that impose species-specific thresholds. The present synthesis does not support previous claims that shallow-water individuals from certain tropical regions would be capable of surviving pressures required for deep-sea migration (Tyler and

Young 1998; Tyler et al. 2000a; Villalobos et al. 2006; Young et al. 1997; 1996). Although investigators have built an impressive understanding of how survival and growth are affected by various pressure conditions, few studies have focused on how vertical movement of animals may be affected by biogeography.

Due to limitations in the available literature, we could not analyze the pressure tolerance on a species-specific level, but our assumption that phylum plays a role in pressure tolerance was supported for the various taxa surveyed. The analyses did not explicitly identify a phylum that was either more resilient or more vulnerable to shifts in pressure. This is in part due to the complexity of the dataset and the number of studies conducted by different investigative teams. This synthesis must therefore be approached with caution as taxa were not tested uniformly among studies, and investigators used different protocols in regards to collection methods and experimental pressure acclimation.

This being acknowledged, deep-sea molluscs from both chemosynthetic and non-chemosynthetic environments often stood out in the analyses; they were found to survive longer at atmospheric pressure than other phyla. However, these conclusions are based on multiple experiments conducted on a limited number of species. Notably, numerous studies have been conducted by the Horta Laboratory in the Azores on the life history, development and physiology of hydrothermal vent mussels (*Bathymodiolus azoricus*) collected from the Mid-Atlantic Ridge at ~850 m depth (Barros et al. 2015; Colaço et al. 2006; 2011; Bettencourt et al. 2008; 2010; Company et al. 2004; Company and Sardà 1998; Martins et al. 2014; Kádár et al. 2006; 2008b). Experimental pressure tolerance of this species was shown to be high, since investigators were able to maintain it for >12

months under laboratory conditions at atmospheric pressure. The ability to adapt to low-pressure conditions by deep-sea mussels may reside in their lack of internal gas compartments, and *B. azoricus* presumably undergoes minimal physical damage from changes in pressure. When considering the total number of deep-water species assessed, rather than the number of experiments in which a given species was used, the phylum Echinodermata stands out for its ability to withstand non-native pressure conditions.

The ability of a taxon to survive pressure shifts could potentially relate to body types and body-plan complexity. In terms of percent survival data under experimental pressure treatments, Foraminifera, Porifera and Annelida did better relative to invertebrate Chordata, possibly because of the latter's phylogenetic position. Maintaining physiological homeostasis when faced with acute pressure stresses could potentially be more difficult in certain animal body designs. By contrast, the simple structure of deep-sea Porifera may have allowed 100% survival of demosponge colonies at atmospheric pressure for ~2 years (Robertson et al. submitted). Therefore, it is unlikely that vertical migrations would involve mass movements of all taxa, but rather would be restricted to those few that are more barotolerant under relatively isothermal conditions. In order to determine the most likely candidates for vertical migrations, further investigations will be required to test the barotolerance of multiple taxa on physiological, developmental and genomic levels. To establish more definite conclusions regarding the link between body design complexity and barotolerance, future studies should examine the responses of multiple species from various phyla under standardized conditions.

2.5.2 Pressure tolerance of early life stages

Inherent to deep-sea research, one of the setbacks with the assessment of pressure tolerance in early life stages is that they generally develop at atmospheric pressure from parents collected at depth. Only a few investigators have successfully collected early life stages (eggs or veligers) directly from great depths in the field (Arellano et al. 2014; Epifanio et al. 1999). Therefore testing the importance of depth of origin on their pressure tolerance can be complicated by collection logistics in the majority of the studies conducted. Moreover, embryos and larvae are transient stages, and most trials involving them are of shorter duration (<24 h) than studies of adult individuals discussed above. In this context, survival rates of larvae whose parents were collected from shallow and deep depths was not significantly different in the dataset examined here, indicating that survival of early life stages is not affected by the provenance of their parents. It should be emphasized, however, that apart from relying on brief trials, studies did not always test the tolerance of the corresponding adult stages (e.g. Aquino-Souza et al. 2008; Childress and Thuesen 1993; Ding et al. 2007; Mestre et al. 2009; Munro et al. 2015; Pechenik et al. 1984; Smith et al. 2013; Smith and Thatje 2012; Smith et al. 2015; Tyler and Young 1998; Tyler and Dixon 2000; Vevers et al. 2010; Villalobos et al. 2006). Although the high-latitude emergence (deep to shallow) hypothesis has been supported chiefly on the basis of echinoderm larvae surviving short-term pressure exposure (e.g. Tyler and Young 1998; Tyler and Dixon 2000; Villalobos et al. 2006), the present synthesis found no evidence that shallow-water larvae can survive long-term maintenance under various pressure conditions. Furthermore, while some shallow-water larvae have been shown to survive pressures representative of depths atypical of their natural bathymetric ranges,

successful metamorphosis/settlement has not been documented, and Brown and Thatje (2014) noted that adults of these species were not found at such depths in nature.

One interesting aspect to highlight from the dataset is that, inversely, many investigators were able to successfully maintain cultures of larvae at atmospheric pressure after obtaining them from adults collected in the deep sea. A number of them have even been able to rear such larvae until settlement into juveniles (e.g. Mercier and Hamel 2008b; 2011b; Miyake et al. 2007; 2012; Sun et al. 2009). At least one species underwent multiple generations (Baillon et al. 2014). It was even reported that the adult tonguefish (Chordata) released larvae that had formed eyes and actively fed at 7 days old (Miyake et al. 2007). This is an intriguing finding as deep-sea fish do not typically survive under atmospheric pressure conditions (Pradillon et al. 2005). Furthermore, adult sea stars, sea anemones and octocorals collected from as deep as 2500 m produced larvae that were reared to settlement and even sexual maturity at atmospheric pressure (e.g. Mercier and Hamel 2008b; 2009; Mercier et al. 2011a; Sun et al. 2010).

As mentioned above, most studies do not directly sample larvae from the deep sea; rather adults are collected which may undergo physical damage that can impact their reproductive capacity. Pradillion et al. (2005) stated that most animals "were dead or moribund upon reaching the surface" from 2500 m depths. Even though larvae are often dissected from collected adults, in most studies the extent of the trauma that gametes or brooded larvae experience from the ascent has yet to be determined. Ultimately, collection methods may negatively affect the health/fitness of animals, including rapid depressurization from the native habitat or use of sampling technology that may inflict physical damage (see above).

Similar to the findings reported earlier for adults, geographic location (of parental collection) had an effect on survival of the larvae. Both percent survival and the survival time at atmospheric pressure were higher and longer, respectively, for collections made in colder geographic locations. In contrast, phylum did not influence the pressure tolerance of larvae in the dataset examined, although data remain too limited to make any strong inferences. Another limitation of the available dataset was that survival time of larvae could not be fully analyzed due to low and biased sample sizes (i.e. there was only enough data for Arthropoda).

Overall research on deep-sea larvae is still in its early stages and the holding conditions might also not provide all the necessary abiotic conditions for optimal embryonic and larval development. Many investigative teams have argued that the ability for deep-sea larvae to survive under laboratory conditions can be negatively affected by environmental conditions other than pressure. Such factors may include poor water conditions, i.e. ammonium levels that induced abnormalities in the eggs (Colaço et al. 2006), and the absence of natural chemical cues required for development or settlement of deep-sea larvae (Hamasaki et al. 2010; Watanabe et al. 2004). Another issue that was found with evaluating the ability of larvae (spawned from deep-sea adults) to tolerate atmospheric pressure is that early life stages can succumb to natural threats, such as cannibalism or disease. Cannibalism was reported in the vent crab (*Austinograea yunhana*) whereby no eggs maintained at the Enoshima Aquarium survived (Miyake et al. 2007). Similarly, many deep-sea ascidians larvae developed until juvenile stages and demonstrated adequate feeding; however, they lived only for 7 months because of a ciliate disease (Havenhand et al. 2006). Ultimately these findings suggest that the full

potential of deep-sea larvae to develop under low-pressure conditions has not been explored due to basic husbandry constraints.

2.5.3 General conclusions

Overall, this review of the pressure tolerance literature has helped synthesize a large body of observational and experimental results related to a broad range of phyla. Based on the tolerance of taxa collected from intermediate (bathyal) depths to both atmospheric pressure and pressurization, the parsimony hypothesis suggesting a bi-directional movement of species was the most strongly supported hypothesis. The high-latitude emergence (deep to shallow) hypothesis also received support from the analyses and numerous reports of long-term holding of deep-sea animals (adults and larvae) under atmospheric pressure conditions. Finally, evidence for the submergence (shallow to deep) hypothesis from empirical data on barotolerance is weak, i.e. restricted to fewer and comparatively short studies. In summary, we found that (i) depth stratum of collection had an effect on the survival of adults but not larvae (with the caveats highlighted), (ii) geographic location of origin impacted pressure tolerances of all ontogenetic stages, whereby individuals from tropical areas where less tolerant to pressure changes than those from northern latitudes (presumably due to the synergistic effect of thermotolerance), and finally (iii) phylum had an effect on pressure tolerance of adults in that more derived taxa appeared to be more sensitive to pressure shifts. Additional research will be required to assess which precise morphological, physiological and/or molecular aspects might drive barotolerance in the various taxa. Importantly, the parsimony hypothesis was shown to be worthy of further investigation.

2.6 Acknowledgements

We would like to thank the members of Mercier Lab for their invaluable support. This research was supported by NSERC and CFI grants to A. Mercier. We would also like to thank the following individuals for informative correspondence: J. Drazen, L. Raymond, J. Company and H. Miyake.

2.7 References

Aquino-Souza R, Hawkins S, Tyler P (2008) Early development and larval survival of *Psammechinus miliaris* under deep-sea temperature and pressure conditions. Journal of the Marine Biological Association of the UK 88(3): 453-461

Aquino-Souza R (2006) Pressure and temperature effects on planktonic stages of benthic invertebrates with regard to their potential for invasion of the deep sea. Doctoral thesis. University of Southampton.

Arellano SM and Young CM (2011) Temperature and salinity tolerances of embryos and larvae of the deep-sea mytilid mussel "*Bathymodiolus*" *childressi*. Marine Biology 158(11): 2481-2493

Arellano SM and Young CM (2009) Spawning, development, and the duration of larval life in a deep-sea cold-seep mussel. Biological Bulletin 216(2): 149-162

Arellano SM, Van Gaest AL, Johnson SB, Vrijenhoek RC, Young CM (2014) Larvae from deep-sea methane seeps disperse in surface waters. Proceedings of the Royal Society of London B: Biological Sciences 281(1786): 20133276

Baillon S, Hamel J, Wareham VE, Mercier A (2014) Seasonality in reproduction of the deep-water pennatulacean coral *Anthoptilum grandiflorum*. Marine Biology 161(1): 29-43

Barros I, Divya B, Martins I, Vandeperre F, Santos RS, Bettencourt R (2015) Post-capture immune gene expression studies in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* acclimatized to atmospheric pressure. Fish and Shellfish Immunology 42(1): 159-170

Bettencourt R, Dando P, Rosa D, Riou V, Colaço A, Sarrazin J, Sarradin P, Santos RS (2008) Changes of gill and hemocyte-related bio-indicators during long term maintenance of the vent mussel *Bathymodiolus azoricus* held in aquaria at atmospheric pressure. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 150(1): 1-7

Bettencourt R, Costa V, Laranjo M, Rosa D, Pires L, Colaco A, Sarradin PM, Lopes H, Sarrazin MJ, Santos RS (2010) Out of the deep-sea into a land-based aquarium environment: investigating innate immunity in the hydrothermal vent mussel *Bathymodiolus azoricus*. Cahiers de Biologie Marine 51(4): 341-350

Bik HM, Thomas WK, Lunt DH, Lambshead PJ (2010) Low endemism, continued deep-shallow interchanges, and evidence for cosmopolitan distributions in free-living marine nematodes (Order Enoplida). BMC Evolutionary Biology 10: 389-2148-10-389

Birstein J (1963) Deep-sea isopod crustaceans of the northwestern Pacific ocean. Institute of Oceanology of the USSR, Akademii Nauk, Moscow

Boutet I, Jollivet D, Shillito B, Moraga D, Tanguy A (2009) Molecular identification of differentially regulated genes in the hydrothermal-vent species *Bathymodiolus thermophilus* and *Paralvinella pandorae* in response to temperature. BMC Genomics 10: 222-2164-10-222

Brown A and Thatje S (2015) The effects of changing climate on faunal depth distributions determine winners and losers. Global Change Biology 21(1): 173-180

Brown A and Thatje S (2014) Explaining bathymetric diversity patterns in marine benthic invertebrates and demersal fishes: physiological contributions to adaptation of life at depth. Biological Reviews 89(2): 406-426

Brown A and Thatje S (2011) Respiratory response of the deep-sea amphipod *Stephonyx biscayensis* indicates bathymetric range limitation by temperature and hydrostatic pressure. PLoS One 6(12): e28562

Carney RS (2005) Zonation of deep biota on continental margins. Oceanography and Marine Biology: An Annual Review 43: 211–227

Childress J (1976) Effects of pressure, temperature and oxygen on the oxygen consumption rate of the midwater copepod *Gaussia princeps*. Marine Biology 39(1): 19-24

Childress J and Thuesen E (1993) Effects of hydrostatic pressure on metabolic rates of six species of deep‐sea gelatinous zooplankton. Limnology and Oceanography 38(3): 665-670

Childress J, Arp A, Fisher Jr C (1984) Metabolic and blood characteristics of the hydrothermal vent tube-worm *Riftia pachyptila*. Marine Biology 83(2): 109-124

Childress J, Fisher C, Favuzzi J, Kochevar R, Sanders N, Alayse A (1991) Sulfide-driven autotrophic balance in the bacterial symbiont-containing hydrothermal vent tubeworm, *Riftia pachyptila jones*. Biological Bulletin 180(1): 135-153

Clarke A, Crame JA, Stromberg J, Barker P (1992) The southern ocean benthic fauna and climate change: a historical perspective [and discussion]. Philosophical Transactions of the Royal Society B: Biological Sciences 338(1285): 299-309

Colaço A, Martins I, Laranjo M, Pires L, Leal C, Prieto C, Costa V, Lopes H, Rosa D, Dando P (2006) Annual spawning of the hydrothermal vent mussel, *Bathymodiolus azoricus*, under controlled aquarium, conditions at atmospheric pressure. Journal of Experimental Marine Biology and Ecology 333(2): 166-171

Colaço A, Bettencourt R, Costa V, Lino S, Lopes H, Martins I, Pires L, Prieto C, Santos RS (2011) LabHorta: a controlled aquarium system for monitoring physiological characteristics of the hydrothermal vent mussel *Bathymodiolus azoricus*. ICES Journal of Marine Science: Journal Du Conseil 68(2): 349-356

Company J and Sardà F (1998) Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. Deep Sea Research Part I: Oceanographic Research Papers 45(11): 1861-1880

Company R, Serafim A, Bebianno M, Cosson R, Shillito B, Fiala-Medioni A (2004) Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. Marine Environmental Research 58(2): 377-381

Corliss JB and Ballard RD (1977) Oases of life in cold abyss. National Geographic 152(4): 441-453

Cottin D, Shillito B, Chertemps T, Thatje S, Léger N, Ravaux J (2010) Comparison of heat-shock responses between the hydrothermal vent shrimp *Rimicaris exoculata* and the related coastal shrimp *Palaemonetes varians*. Journal of Experimental Marine Biology and Ecology 393(1): 9-16

Cottin D, Brown A, Oliphant A, Mestre NC, Ravaux J, Shillito B, Thatje S (2012) Sustained hydrostatic pressure tolerance of the shallow water shrimp *Palaemonetes varians* at different temperatures: insights into the colonisation of the deep sea. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 162(4): 357-363

Cottin D, Ravaux J, Leger N, Halary S, Toullec JY, Sarradin PM, Gaill F, Shillito B (2008) Thermal biology of the deep-sea vent annelid *Paralvinella grasslei*: *in vivo* studies. Journal of Experimental Biology 211(14): 2196-2204

Dahl E (1954) The distribution of deep-sea crustacea. On the Distribution and Origin of the Deep Sea Bottom Fauna. International Union of Biological Sciences (B) 16: 43-46

Dattagupta S, Miles LL, Barnabei MS, Fisher CR (2006) The hydrocarbon seep tubeworm *Lamellibrachia luymesi* primarily eliminates sulfate and hydrogen ions across its roots to conserve energy and ensure sulfide supply. Journal of Experimental Biology 209(19): 3795-3805

Dayton PK, Newman WA, Oliver J (1982) The vertical zonation of the deep-sea Antarctic acorn barnacle, *Bathylasma corolliforme* (Hoek): experimental transplants from the shelf into shallow water. Journal of Biogeography 12: 95-109

Ding J, Chang Y, Wang Z, Song J (2007) Polyploidy induction by hydrostatic pressure shock and embryo development of sea cucumber *Apostichopus japonicus*. Chinese Journal of Oceanography and Limnology 25: 184-190

Distel DL, Baco AR, Chuang E, Morrill W, Cavanaugh C, Smith CR (2000) Marine ecology: do mussels take wooden steps to deep-sea vents? Nature 403(6771): 725-726

Dixon DR, Pruski AM, Dixon LR (2004) The effects of hydrostatic pressure change on DNA integrity in the hydrothermal-vent mussel *Bathymodiolus azoricus*: implications for future deep-sea mutagenicity studies. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 552(1): 235-246

Dixon DR, Dixon LR, Shillito B, Gwynn JP (2002) Background and induced levels of DNA damage in pacific deep-sea vent polychaetes: the case for avoidance. Cahiers De Biologie Marine 43(3/4): 333-336

Durand L, Zbinden M, Cueff-Gauchard V, Duperron S, Roussel EG, Shillito B, Cambon-Bonavita MA (2010) Microbial diversity associated with the hydrothermal shrimp *Rimicaris exoculata* gut and occurrence of a resident microbial community. FEMS Microbiology and Ecology 71(2): 291-303

Epifanio C, Perovich G, Dittel A, Cary S (1999) Development and behavior of megalopa larvae and juveniles of the hydrothermal vent crab *Bythograea thermydron*. Marine Ecology Progress Series 185: 147-154

Forbes E (1844) Report on the mollusca and radiata of the Aegan Sea, and on their distribution, considered as bearing on geology. Report of the British Association for the Advancement of Science for 1843: 129-193

Frederich M and Portner HO (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. American Journal of Physiology and Regulatory Integrative Comparative Physiology 279(5): R1531-8

Gage J and Tyler P (1981a) Non-viable seasonal settlement of larvae of the upper bathyal brittle star *Ophiocten gracilis* in the Rockall Trough abyssal. Marine Biology 64(2): 153-161

Gage J and Tyler P (1981b) Re-appraisal of age composition, growth and survivorship of the deep-sea brittle star *Ophiura ljungmani* from size structure in a sample time series from the Rockall Trough. Marine Biology 64(2): 163-172

Gage JD and Tyler PA. (1999) Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press

Gaill F, Shillito B, Ménard F, Goffinet G, Childress JJ (1997) Rate and process of tube production by the deep-sea hydrothermal vent tubeworm *Riftia pachyptila*. Marine Ecology Progress Series 148: 135-143

Galgani F, Chiffoleau J, Gall PL, Pichot Y, Andral B, Martin C (2005) Deep-sea caging of the mussel *Mytilus galloprovincialis*: potential application in ecotoxicological studies. Chemical Ecology 21(2): 133-141

George RY and Marum JP (1974) The effects of hydrostatic pressure on living aquatic organisms III. Behavior and tolerance of euplanktonic organisms to increased hydrostatic pressure. Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie 59(2): 175-186

Hamasaki K, Nakajima K, Tsuchida S, Kado R, Kitada S (2010) Number and duration of zoeal stages of the hydrothermal vent crab *Gandalfus yunohana* from laboratory reared specimens. Journal of Crustacean Biology 30(2): 236-240

Hamel J-F, Montgomery EM, Barnich R, Mercier A (2015) Range extension of the deep-sea polychaete worm *Neopolynoe acanellae* in Canada. Marine Biodiversity Records 8

Hamel J-F, Sun Z, Mercier A (2010) Influence of size and seasonal factors on the growth of the deep-sea coral *Flabellum alabastrum* in mesocosm. Coral Reefs 29(2): 521-525

Havenhand JN, Matsumoto GI, Seidel E (2006) *Megalodicopia hians* in the Monterey Submarine canyon: distribution, larval development, and culture. Deep Sea Research Part I: Oceanographic Research Papers 53(2): 215-222

Hayward BW (2001) Global deep-sea extinctions during the pleistocene ice ages. Geology 29(7): 599-602

Hessler RR and Sanders HL (1967) Faunal diversity in the deep-sea. 14(1): 65-78

Hessler RR, Wilson GD, Thistle D (1979) The deep-sea isopods: a biogeographic and phylogenetic overview. Sarsia 64(1-2): 67-75

Hessler R and Wilson G (1983) The origin and biogeography of malacostracan crustaceans in the deep sea. Evolution, Time and Space: The Emergence of the Biosphere : 227-254

Hessler R and Thistle D (1975) On the place of origin of deep-sea lsopods. Marine Biology 32(2): 155-165

Jablonski D (2005) Mass extinctions and macroevolution. Paleobiology 31(5): 192-210

Jablonski D, Sepkoski JJ,Jr, Bottjer DJ, Sheehan PM (1983) Onshore-offshore patterns in the evolution of phanerozoic shelf communities. Science 222(4628): 1123-1125

Jaenicke R (1983) Biochemical processes under high hydrostatic pressure. Naturwissenschaften 70(7): 332-341

Jain K (1994) High‐pressure neurological syndrome (HPNS). Acta Neurologica Scandinavica 90(1): 45-50

Jannasch HW and Taylor CD (1984) Deep-sea microbiology. Annual Reviews in Microbiology 38(1): 487-487

Kadar E, Tschuschke IG, Checa A (2008a) Post-capture hyperbaric simulations to study the mechanism of shell regeneration of the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* (Bivalvia: Mytilidae). Journal Experimental Marine Biology and Ecology 364(2): 80-90

Kadar E, Checa AG, Oliveira AN, Machado JP (2008b) Shell nacre ultrastructure and depressurisation dissolution in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus*. Journal of Comparative Physiology B 178(1): 123-130

Kádár E, Lobo-da-Cunha A, Santos RS, Dando P (2006) Spermatogenesis of *Bathymodiolus azoricus* in captivity matching reproductive behaviour at deep-sea hydrothermal vents. Journal of Experimental Marine Biology and Ecology 335(1): 19-26

Kádár E, Bettencourt R, Costa V, Santos RS, Lobo-da-Cunha A, Dando P (2005) Experimentally induced endosymbiont loss and re-acquirement in the hydrothermal vent bivalve *Bathymodiolus azoricus*. Journal of Experimental Marine Biology and Ecology 318(1): 99-110

Kiel S, Wiese F, Titus AL (2012) Shallow-water methane-seep faunas in the cenomanian western interior seaway: no evidence for onshore-offshore adaptations to deep-sea vents. Geology 40(9): 839-842

Kiel S and Little CT (2006) Cold-seep mollusks are older than the general marine mollusk fauna. Science 313(5792): 1429-1431

Kussakin O (1973) Peculiarities of the geographical and vertical distribution of marine isopods and the problem of deep-sea fauna origin. Marine Biology 23(1): 19-34

Lê S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. Journal of Statistical Software 25(1): 1-18

Lee RW (2003) Thermal tolerances of deep-sea hydrothermal vent animals from the Northeast Pacific. Biological Bulletin 205(2): 98-101

Lipps J and Hickman C (1982) Origin, age, and evolution of Antarctic and deep-sea faunas.

Locket NA (1977) Adaptations to the deep-sea environment. In *the visual system in vertebrates*. Springer Berlin Heidelberg 67-192

Macdonald A (1997) Hydrostatic pressure as an environmental factor in life processes. Comparative Biochemistry and Physiology Part A: Physiology 116(4): 291-297

Macdonald AG and Teal J (1975) Tolerance of oceanic and shallow water crustacea to high hydrostatic pressure. 22(3): 131-144

Marsh AG, Mullineaux LS, Young CM, Manahan DT (2001) Larval dispersal potential of the tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents. Nature 411(6833): 77-80

Martinez AS, Toullec JY, Shillito B, Charmantier-Daures M, Charmantier G (2001) Hydromineral regulation in the hydrothermal vent crab *Bythograea thermydron*. Biological Bulletin 201(2): 167-174

Martins E, Figueras A, Novoa B, Santos RS, Moreira R, Bettencourt R (2014) Comparative study of immune responses in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* and the shallow-water mussel *Mytilus galloprovincialis* challenged with vibrio bacteria. Fish Shellfish Immunology 40(2): 485-499

Matabos M, Cuvelier D, Brouard J, Shillito B, Ravaux J, Zbinden M, Barthelemy D, Sarradin P, Sarrazin J (2015) Behavioural study of two hydrothermal crustacean decapods: *Mirocaris fortunata* and *Segonzacia mesatlantica*, from the Lucky Strike vent field (Mid-Atlantic Ridge). Deep Sea Research Part II: Topical Studies in Oceanography 121: 146-158

Menzies R, George R, Rowe G (1973) Abyssal environment and ecology of the world OceansWiley–Interscience. New York, NY

Menzies RJ and Wilson JB (1961) Preliminary field experiments on the relative importance of pressure and temperature on the penetration of marine invertebrates into the deep sea. Oikos 12(2): 302-309

Mercier A and Hamel J-F (2009) Reproductive periodicity and host-specific settlement and growth of a deep-water symbiotic sea anemone. Canadian Journal of Zoology 87(11): 967-980

Mercier A and Hamel J-F (2008) Depth-related shift in life history strategies of a brooding and broadcasting deep-sea asteroid. Marine Biology 156(2): 205-223

Mercier A, Baillon S, Hamel J-F (2015) Life history and feeding biology of the deep-sea pycnogonid *Nymphon hirtipes*. Deep Sea Research Part I: Oceanographic Research Papers 106: 1-8

Mercier A, Baillon S, Hamel J-F (2014) Life history and seasonal breeding of the deep-sea annelid *Ophryotrocha sp.* (Polychaeta: Dorvelleidae). Deep Sea Research Part I: Oceanographic Research Papers 91: 27-35

Mercier A, Sewell MA, Hamel J-F (2013) Pelagic propagule duration and developmental mode: reassessment of a fading link. Global Ecology and Biogeography 22(5): 517-530

Mercier A, Sun Z, Hamel J-F (2011a) Reproductive periodicity, spawning and development of the deep-sea scleractinian coral *Flabellum angulare*. Marine Biology 158(2): 371-380

Mercier A, Baillon S, Daly M, Macrander J, Hamel J-F (2016) Biology of a deep-water sea anemone (Anthozoa: Actiniidae) from Eastern Canada: spawning, development, and growth. Deep Sea Research Part II: Topical Studies in Oceanography

Mercier A, Sun Z, Hamel JF (2011b) Internal brooding favours pre-metamorphic chimerism in a non-colonial cnidarian, the sea anemone *Urticina felina*. Proceedings of the Royal Society of London B: Biological Sciences 278(1724): 3517-3522

Mercier A, Sun Z, Baillon S, Hamel J-F (2011c) Lunar rhythms in the deep sea: evidence from the reproductive periodicity of several marine invertebrates. Journal of Biological Rhythms 26(1): 82-86

Mestre NC, Thatje S, Tyler PA (2009) The ocean is not deep enough: pressure tolerances during early ontogeny of the blue mussel *Mytilus edulis*. Proceedings of the Royal Society of London B: Biological Sciences 276(1657): 717-726

Mickel TJ and Childress JJ (1982a) Effects of pressure and temperature on the EKG and heart rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). Biological Bulletin 162(1): 70-82

Mickel TJ and Childress J (1982b) Effects of temperature, pressure, and oxygen concentration on the oxygen consumption rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). Physiological Zoology 36: 199-207

Miglietta MP, Faucci A, Santini F (2011) Speciation in the sea: overview of the symposium and discussion of future directions. Integrative and Comparative Biology 51(3): 449-455

Miyake H, Kitada M, Tsuchida S, Okuyama Y, Nakamura K (2007) Ecological aspects of hydrothermal vent animals in captivity at atmospheric pressure. Marine Ecology 28(1): 86-92

Miyake H, Lindsay DJ, Kitada M, Nemoto S, Miwa T, Itoh T. (2012) How to keep deep-sea animals. INTECH Open Access Publisher, Japan

Mora C, Tittensor DP, Adl S, Simpson AG, Worm B (2011) How many species are there on earth and in the ocean?

Morris J, Thatje S, Hauton C (2013) The use of stress‐70 proteins in physiology: A re‐appraisal. Molecular Ecology 22(6): 1494-1502

Morris J, Thatje S, Ravaux J, Shillito B, Fernando D, Hauton C (2015) Acute combined pressure and temperature exposures on a shallow-water crustacean: novel insights into the stress response and high pressure neurological syndrome. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 181: 9-17

Munro C, Morris JP, Brown A, Hauton C, Thatje S (2015) The role of ontogeny in physiological tolerance: decreasing hydrostatic pressure tolerance with development in the northern stone crab *Lithodes maja*. Proceedings of the Royal Society B Biological Sciences

New P, Brown A, Oliphant A, Burchell P, Smith A, Thatje S (2014) The effects of temperature and pressure acclimation on the temperature and pressure tolerance of the shallow-water shrimp *Palaemonetes varians*. Marine Biology 161(3): 697-709

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. Journal of Experimental Biology 214(7): 1109-1117

Orejas C, Gori A, Gili JM (2008) Growth rates of live *Lophelia pertusa* and *Madrepora oculata* from the Mediterranean Sea maintained in aquaria. Coral Reefs 27(2): 255-255

Pechenik J, Chang S, Lord A (1984) Encapsulated development of the marine prosobranch gastropod *Nucella lapillus*. Marine Biology 78(2): 223-229

Pradillon F and Gaill F (2007) Pressure and life: Some biological strategies. Reviews in Environmental Science and Bio/Technology 6(1-3): 181-195

Pradillon F, Shillito B, Young CM, Gaill F (2001) Deep-sea ecology: developmental arrest in vent worm embryos. Nature 413(6857): 698-699

Pradillon F, Shillito B, Chervin J, Hamel G, Gaill F (2004) Pressure vessels for *in vivo* studies of deep-sea fauna. High Pressure Research 24(2): 237-246

Pradillon F, Le Bris N, Shillito B, Young CM, Gaill F (2005) Influence of environmental conditions on early development of the hydrothermal vent polychaete *Alvinella pompejana*. Journal of Experimental Biology 208(8): 1551-1561

Pruski AM and Dixon DR (2003) Toxic vents and DNA damage: first evidence from a naturally contaminated deep-sea environment. Aquatic Toxicology 64(1): 1-13

Quetin LB and Childress JJ (1980) Observations on the swimming activity of two bathypelagic mysid species maintained at high hydrostatic pressures. Deep Sea Research Part A. Oceanographic Research Papers 27(5): 383-391

Ramirez-Llodra E, Tyler PA, Baker MC, Bergstad OA, Clark MR, Escobar E, Levin LA, Menot L, Rowden AA, Smith CR (2011) Man and the last great wilderness: human impact on the deep sea. PLoS One 6(7): e22588

Raupach MJ, Mayer C, Malyutina M, Wagele JW (2009) Multiple origins of deep-sea asellota (Crustacea: Isopoda) from shallow waters revealed by molecular data. Proceedings of the Royal Society of London B: Biological Sciences 276(1658): 799-808

Ravaux J, Cottin D, Chertemps T, Hamel G, Shillito B (2009) Hydrothermal shrimps display low expression of heat-inducible hsp70 gene in nature. Marine Ecology Progress Series 396: 153-156

Ravaux J, Hamel G, Zbinden M, Tasiemski AA, Boutet I, Léger N, Tanguy A, Jollivet D, Shillito B (2013) Thermal limit for metazoan life in question: *in vivo* heat tolerance of the pompeii worm. PLoS One 8(5): e64074

Ravaux J, Gaill F, Le Bris N, Sarradin PM, Jollivet D, Shillito B (2003) Heat-shock response and temperature resistance in the deep-sea vent shrimp *Rimicaris exoculata*. Journal of Experimental Biology 206(14): 2345-2354

Rex MA, McClain CR, Johnson NA, Etter RJ, Allen JA, Bouchet P, Warén A (2005) A source‐sink hypothesis for abyssal biodiversity. American Naturalist 165(2): 163-178

Rodríguez E, López-González PJ, Gili JM (2007) Biogeography of Antarctic sea anemones (Anthozoa, Actiniaria): what do they tell us about the origin of the antarctic benthic fauna? Deep Sea Research Part II: Topical Studies in Oceanography 54(16): 1876-1904

Sanders HL (1968) Marine benthic diversity: A comparative study. American Naturalist 23: 243-282

Seo M, Koyama S, Toyofuku T, Kojima S, Watanabe H (2013) Determination of extremely high pressure tolerance of brine shrimp larvae by using a new pressure chamber system. Zoological Sciences 30: 919-923

Shillito B, Bris NL, Gaill F, Rees J, Zal F (2004) First access to live alvinellas. High Pressure Research 24(1): 169-172

Shillito B, Ravaux J, Sarrazin J, Zbinden M, Sarradin P, Barthelemy D (2015) Long-term maintenance and public exhibition of deep-sea hydrothermal fauna: the AbyssBox project. Deep Sea Research Part II: Topical Studies in Oceanography 121: 137-145

Shillito B, Jollivet D, Sarradin P, Rodier P, Lallier F, Desbruyères D, Gaill F (2001) Temperature resistance of *Hesiolyra bergi*, a polychaetous annelid living on deep-sea vent smoker walls. Marine Ecology Progress Series 216: 141-149

Shillito B, Le Bris N, Hourdez S, Ravaux J, Cottin D, Caprais JC, Jollivet D, Gaill F (2006) Temperature resistance studies on the deep-sea vent shrimp *Mirocaris fortunata*. Journal of Experimental Biology 209(5): 945-955

Smith F, Brown A, Mestre NC, Reed AJ, Thatje S (2013) Thermal adaptations in deep-sea hydrothermal vent and shallow-water shrimp. Deep Sea Research Part II: Topical Studies in Oceanography 92: 234-239

Smith KE and Thatje S (2012) The secret to successful deep-sea invasion: Does low temperature hold the key? PLoS One 7(12): e51219

Smith KE, Brown A, Thatje S (2015) The metabolic cost of developing under hydrostatic pressure: experimental evidence supports macroecological pattern. Marine Ecology Progress Series 524: 71-82

Somero G (1992) Biochemical ecology of deep-sea animals. Experientia 48(6): 537-543

Sumida P, Tyler P, Lampitt R, Gage J (2000) Reproduction, dispersal and settlement of the bathyal ophiuroid *Ophiocten gracilis* in the NE Atlantic ocean. Marine Biology 137(4): 623-630

Sun Z, Hamel J-F, Mercier A (2009) Planulation of deep-sea octocorals in the Northwest Atlantic. Coral Reefs 28(3): 781

Sun Z, Hamel J-F, Edinger E, Mercier A (2010) Reproductive biology of the deep-sea octocoral *Drifa glomerata* in the northwest Atlantic. Marine Biology 157(4): 863-873

Thatje S, Casburn L, Calcagno JA (2010) Behavioural and respiratory response of the shallow-water hermit crab *Pagurus cuanensis* to hydrostatic pressure and temperature. Journal of Experimental Marine Biology and Ecology 390(1): 22-30

Thistle D and Hessler R (1976) Origin of a deep-sea family, the Ilyarachnidae (Crustacea: Isopoda). Systematics Biology 25(2): 110-116

Thistle D (2003) The deep-sea floor: an overview. Ecosystems of the World 23: 5-38

Tokuda G, Yamada A, Nakano K, Arita N, Yamasaki H (2006) Occurrence and recent long-distance dispersal of deep-sea hydrothermal vent shrimps. Biology Letters 2(2): 257-260

Tyler P and Dixon D (2000) Temperature/pressure tolerance of the first larval stage of *Mirocaris fortunata* from Lucky Strike hydrothermal vent field. Journal of the Marine Biological Association of the UK 80(4): 739-740

Tyler P and Young C (1998) Temperature and pressure tolerances in dispersal stages of the genus *Echinus* (Echinodermata: Echinoidea): prerequisites for deep-sea invasion and speciation. Deep Sea Research Part II: Topical Studies in Oceanography 45(1): 253-277

Tyler PA, Young CM, Clarke A (2000) Temperature and pressure tolerances of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri* (Echinodermata: Echinoidea): potential for deep-sea invasion from high latitudes. Marine Ecology Progress Series 192: 173-180

Verkaik K, Hamel J-F, Mercier A (2016) Impact of ocean acidification on reproductive output in the deep-sea annelid *Ophryotrocha sp.* (Polychaeta: Dorvilleidae). Deep Sea Research Part II: Topical Studies in Oceanography

Vevers WF, Dixon DR, Dixon LR (2010) The role of hydrostatic pressure on developmental stages of *Pomatoceros lamarcki* (Polychaeta: Serpulidae) exposed to water accommodated fractions of crude oil and positive genotoxins at simulated depths of 1000–3000 m. Environmental Pollution 158(5): 1702-1709

Villalobos FB, Tyler PA, Young CM (2006) Temperature and pressure tolerance of embryos and larvae of the Atlantic seastars *Asterias rubens* and *Marthasterias glacialis* (Echinodermata: Asteroidea): potential for deep-sea invasion. Marine Ecology Progress Series 314: 109-117

Wägele JW. (1989) Evolution und phylogenetisches system der isopoda: Stand der forschung und neue erkenntnisse. E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller),

Watanabe H, Kado R, Tsuchida S, Miyake H, Kyo M, Kojima S (2004) Larval development and intermoult period of the hydrothermal vent barnacle *Neoverruca sp.* Journal of the Marine Biological Association of the UK 84(04): 743-745

Watanabe YY, Ito M, Takahashi A (2014) Testing optimal foraging theory in a penguin-krill system. Proceedings of the Royal Society of London B: Biological Sciences 281(1779): 20132376

Watsuji T, Yamamoto A, Takaki Y, Ueda K, Kawagucci S, Takai K (2014) Diversity and methane oxidation of active epibiotic methanotrophs on live *Shinkaia crosnieri.* The ISME Journal 8(5): 1020-1031

Webb TJ, Vanden Berghe E, O'Dor R (2010) Biodiversity's big wet secret: the global distribution of marine biological records reveals chronic under-exploration of the deep pelagic ocean. PLoS One 5(8): e10223

Weinberg JR (1990) High rates of long-term survival of deep-sea infauna in the laboratory. Deep Sea Research Part A. Oceanographic Research Papers 37(8): 1375-1379

Wilcock S, Wann K, Macdonald A (1978) The motor activity of *Crangon crangon* subjected to high hydrostatic pressure. Marine Biology 45(1): 1-7

Wilson GD (1999) Some of the deep-sea fauna is ancient. Crustaceana 72(8): 1019-1030

Wilson N, Hunter R, Lockhart S, Halanych K (2007) Multiple lineages and absence of panmixia in the "circumpolar" crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. Marine Biology 152(4): 895-904

Wilson S, Yeh J, Korsmeyer KE, Drazen JC (2013) Metabolism of shallow and deep-sea benthic crustaceans and echinoderms in Hawaii. Marine Biology 160(9): 2363-2373

Wolff T (1960) The hadal community, an introduction. Deep Sea Research (1953) 6: 95-124

Woolley SN, Tittensor DP, Dunstan PK, Guillera-Arroita G, Lahoz-Monfort JJ, Wintle BA, Worm B, O'Hara TD (2016) Deep-sea diversity patterns are shaped by energy availability. Nature 533(7603): 393-396

Yoshiki T, Ono T, Shimizu A, Toda T (2011) Effect of hydrostatic pressure on eggs of *Neocalanus* copepods during spawning in the deep-layer. Marine Ecology Progress Series 430: 63-70

Yoshiki T, Toda T, Yoshida T, Shimizu A (2006) A new hydrostatic pressure apparatus for studies of marine zooplankton. Journal of Plankton Research 28(6): 563-570

Yoshiki T, Yamanoha B, Kikuchi T, Shimizu A, Toda T (2008) Hydrostatic pressure-induced apoptosis on nauplii of *Calanus sinicus*. Marine Biology 156(2): 97-106

Young C, Tyler P, Fenaux L (1997) Potential for deep sea invasion by Mediterranean shallow water echinoids: pressure and temperature as stage-specific dispersal barriers. Marine Ecology Progress Series 154: 197-209

Young CM and Tyler PA (1993) Embryos of the deep-sea echinoid *Echinus affinis* require high pressure for development. Limnology and Oceanography 38(1): 178-181

Young CM, Vázquez E, Metaxas A, Tyler PA (1996) Embryology of vestimentiferan tube worms from deep-sea methane/sulphide seeps. Nature 381(6582): 514-516

2.8 Tables

Table 2-1: List of variables collected from the literature with their definitions and the scales/categories that were compared.

Variable	Definition	Scale or Categories
Collection depth	Actual depth or range of depths from which specimens were collected.	0 – 4420 m
Depth stratum	Categories used for comparing general bathymetric origin.	Deep (≥200 m), shallow (<200 m)
Depth range	Categories used to refine the bathymetric analyses.	Intertidal (0 – 5 m), subtidal (>5 – 200 m), bathyal (>200 – 2000), abyssal (>2000 – 6000).
Climate zone (of origin)	Climate where shallow-water collections occurred. From a combination of geographic region and temperature data as per the methods described in Mercier et al. (2013).	Polar, temperate-cold, temperate, temperate-warm, tropical
Geographic region (of origin)	Regions where collections occurred and their acronyms.	Antartica (Antartica), Norwest Atlantic Ocean (NW Atl), Northeast Atlantic Ocean (NE Atl), Northeast Pacific Ocean (NE Pac), Northwest Pacific Ocean (NW Pac), Mediterranean Sea (Med), Tropical East Pacific Ocean (Trop E Pac), Tropical West Pacific Ocean (Trop W Pac), Indian Ocean (Ind Trop) Caribbean (Cari), Gulf of Mexico (GOM)

Habitat type (of origin)	Descriptor beyond depth that might refer to unique or endemic communities.	Chemosynthetic habitat, non-chemosynthetic habitats (which include: reef, seamount, salt marsh, canyons, mud volcano), lab culture
Initial life-history stage	The initial life stage tested from collection through experimental period.	Larva, juvenile, adult
Pressure	Pressure (MPa) that animals were exposed to during experiments.	0.1 – 50 MPa
Method-pressure	Protocol used to reach the experimental pressure level.	Incremental (stepwise), absolute (absolute)
Temperature	Temperature (°C) that animals were exposed to during experiments.	2 – 60°C
Method-temperature	Protocol used to reach the experimental temperature level.	Incremental (stepwise), absolute (absolute)
Experimental duration	Length of time (h) that individuals were exposed to experimental condition.	0 – 26280 h
Survival time	Length of time (hours, days, months, years) that deep-sea species survived while kept at atmospheric pressure.	0.5 – 1097 d
Survival	Percent surviving individuals (%) at the end of the experiment, relative to the initial number of individuals exposed to the condition.	0 – 100%
Maximum stage	Latest stage of development (larvae) immediately after exposure to pressure.	Species dependent (refer to Table A1)

Maximum stage of development	Percent (%) reaching the maximum stage (larvae) among the experimental group exposed to condition.	0.07 – 100%
Maximum age reached	Maximum length of time (d) survived (larvae) to the experimental condition as the latest developed stage.	0.1 – 199 d

Table 2-2: Summary of variables used in FAMD analyses and results from each test.

Response variable	Life stage	Environment	Deep Shallow	Pressure and duration (Yes/No)[a]	Phylum[b]	Geographic location[b]	Depth range	Results	
								Average of response variable	Significant group(s) (p<0.001)
Survival time (d)	Adult	Chemosynthetic	Deep	No	Ar Mo	NW Pac NE Atl GOM	Bathyal	235.1 d	Arthropoda survived for 310.7 d
Survival time (d)	Adult	Non-chemosynthetic	Deep	No	Ar Ch Cn Ec Mo Po	NE Pac NE Atl NW Atl GOM Med Trop W Pac	Bathyal	512.7 d	Arthropoda and Cnidaria from the Med survived for 7 d and 517 d, respectively
								512.7 d	Arthropoda from the NE Atl and GOM survived for 60 d and 639 d, respectively
								512.7 d	Arthropoda and Echinodermata from the Trop W Pac survived for 116.6 d and 120 d, respectively
								512.7 d	Arthropoda, Chordata, Cnidaria and Echinodermata from the NE Pac survived for 114 d, 730 d, 496 d and 300 d, respectively
								512.7 d	Cnidaria from the NW Atl survived for 730 d
			Deep	No	Ar Ch Cn Ec Mo Po	NE Pac NE Atl NW Atl GOM Med Trop W Pac	Bathyal Abyssal	530.3 d	Arthropoda and Cnidaria from the Med survived for 730 d and 730 d, respectively
								530.3 d	Arthropoda from the NE Atl and GOM survived for 639 d and 218.5 d, respectively
								530.3 d	Arthropoda and Echinodermata from the Trop W Pac survived for 291.5 d and 512.1 d, respectively
								530.3 d	Arthropoda, Chordata, Cnidaria and Echinodermata from the NE Pac survived for 730 d, 730 d, 730 d and 120 d, respectively
								530.3 d	Cnidaria from the NW Atl survived for 730 d
Survival (%)	Adult	Chemosynthetic	Deep	Yes	An Ar			Pressure 12.5 MPa	Arthropoda tested under experimental pressures

					Mo			Duration 2093.3 h, Survival 79.9%	for longer durations, 3781.8 h, survived 70.3%
Survival (%)	Adult	Chemosynthetic	Deep	No	An Ar Mo	NE Pac NW Pac NE Atl Trop E Pac	Abyssal Bathyal	79.9%	Arthropoda from the NE Atl collected at bathyal depths survived 62.3%
								79.9%	Mollusca from the NE Atl collected from bathyal depths survived 65.7%
								79.9%	Annelida from the NE Pac survived 84.3%
Survival (%)	Adult	Non-chemosynthetic	Deep Shallow	Yes	Ar Cn Ec Fo Mo Po			76.1%	Cnidaria collected from the deep-sea survived 80.5%
Survival (%)	Adult	Non-chemosynthetic	Deep Shallow	No	Ar Cn Ec Fo Mo Po	NE Atl Trop E Pac Trop Ind NW Atl NE Pac Med NW Atl	Subtidal Bathyal Abyssal	76.1%	Arthropoda from the Trop E Pac collected from shallow depths (subtidal) survived 32.1%
								76.1%	Cnidaria from the NW Atl collected from deep depths (bathyal) survived 78.3%
Survival (%)	Larvae	Chemosynthetic	Deep	Yes	An Ar Mo			Pressure 6.9 MPa Duration 215.5 h 73.1%	Annelida tested under high pressures, 11.5 MPa and short durations 73.1 h survived 56.9%
								Pressure 6.9 MPa 73.1%	Arthropoda tested under low pressures, 6.5 MPa, survived 84.6%
Survival (%)	Larvae	Chemosynthetic	Deep	No	An Ar Mo	NE Pac NE Atl NW Pac GOM	Bathyal Abyssal	73.1%	Annelida from the NE Pac collected from abyssal depths survived 56.9%
Survival (%)	Larvae	Non-chemosynthetic	Shallow Deep	Yes	An Ar Cn Ec Mo			Pressure 14.1 MPa duration 1581.7 h 69.7%	Cnidaria collected from deep depths tested under low pressures, 5.8 MPa, and long durations, 14334.7 h survived 73.6%
Survival (%)	Larvae	Non-chemosynthetic	Shallow Deep	No	An Ar Cn Ec Mo	NE Pac NW Pac NE Atl NW Atl GOM Trop W Pac Cari Antarctica	Intertidal Subtidal Bathyal	69.7%	Echinodermata from the NE Atl collected from shallow depths (subtidal) survived 94.7%
									Echinodermata from the Trop W Pac collected from shallow depths (subtidal) survived 15.4%
									Arthropoda from the

				NW Pac collected from shallow depths (subtidal) survived 89.8% Annelida from the shallow depths (intertidal) survived 63.1% Arthropoda from deep depths (bathyal) collected from the NW Pac and Cari survived 63.7% and 10%, respectively

[a] Pressure refers the experimental pressures applied to individuals during experiments and duration refers to the length of time individuals were tested under experimental pressures.
[b] Geographic locations where individuals were collected.

2.9 Figures

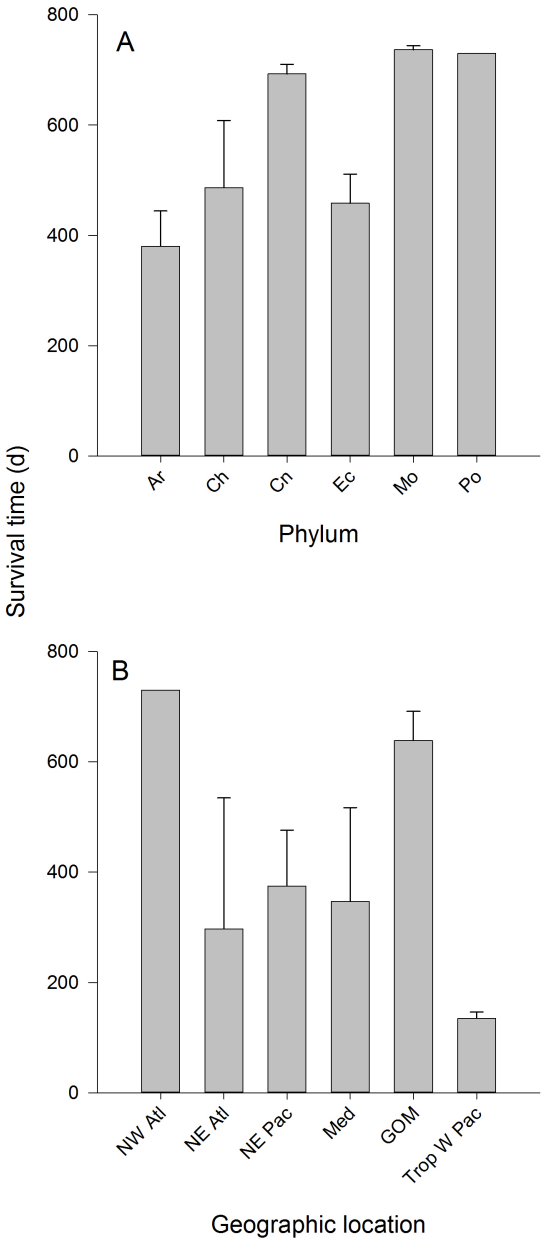

Figure 2-1: Survival time in the laboratory at atmospheric pressure for adult individuals (mean ± SE, *n* = 4-82) from chemosynthetic environments with regards to (A) phylum (Ar=Arthropoda; Ch=Chordata; Cn=Cnidaria; Ec=Echinodermata; Mo=Mollusca; Po=Porifera) and (B) geographic location (see Table 2-1 for outline of abbreviations).

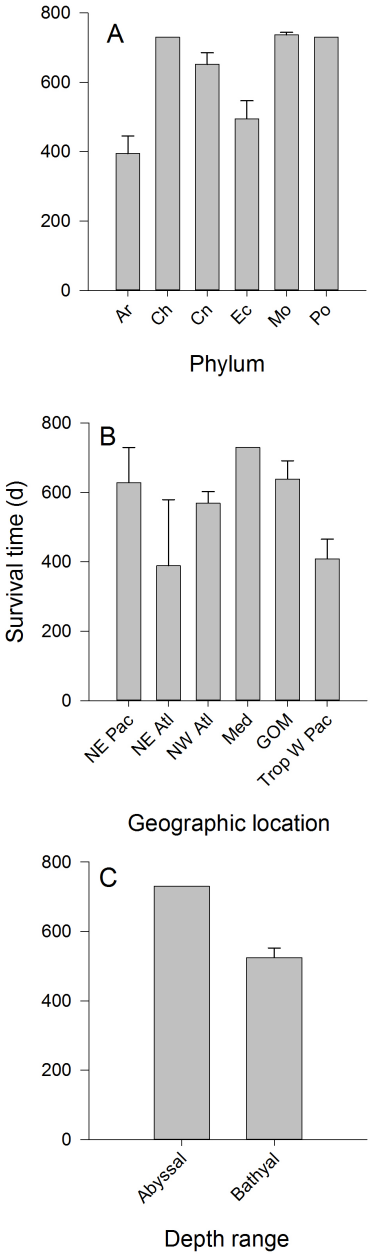

Figure 2-2: Minimum survival time in the laboratory at atmospheric pressure for adult individuals (mean ± SE, *n* = 3-35) from non-chemosynthetic environments with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Ch=Chordata; Cn=Cnidaria; Ec=Echinodermata; Mo=Mollusca; Po=Porifera), (B) geographic location (see Table 2-1 for outline of abbreviations) and (C) depth range.

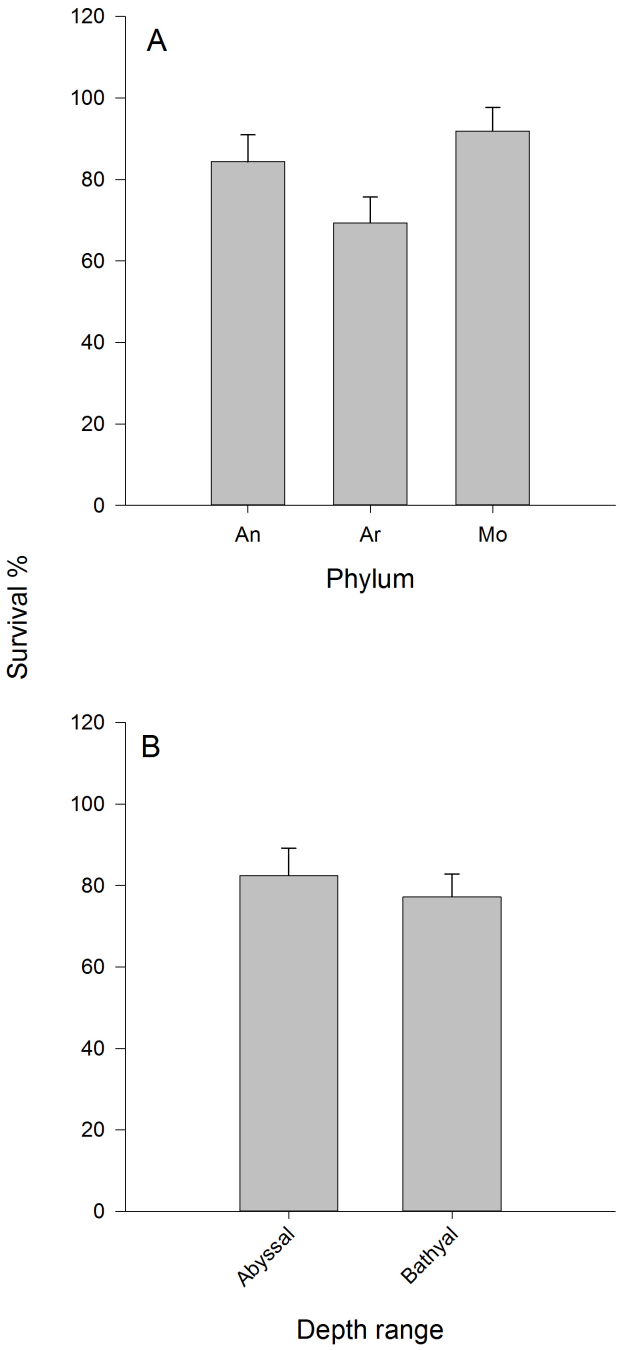

Figure 2-3: Survival (%) of adult individuals (mean ± SE, *n* =3-59) from chemosynthetic environments tested under experimental pressure conditions with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Mo=Mollusca), and (B) depth range.

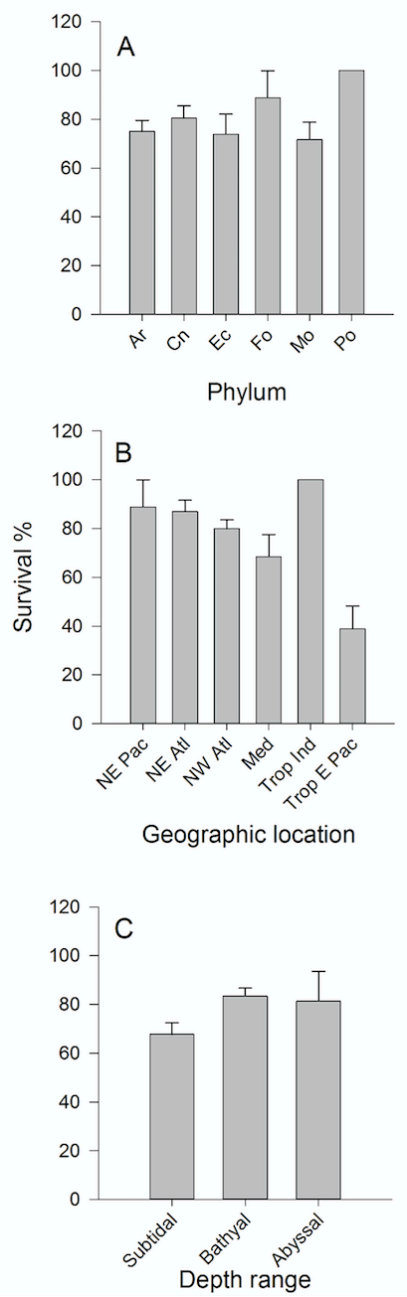

Figure 2-4: Survival (%) for adult individuals (mean ± SE, *n* =3-105) from non-chemosynthetic environments tested under experimental pressure conditions with regards to (A) phylum (Ar=Arthropoda; Cn=Cnidaria; Ec=Echinodermata; Fo= Foraminifera; Mo=Mollusca; Po=Porifera), (B) geographic location (Table 1 for outline of abbreviations) and (C) depth range.

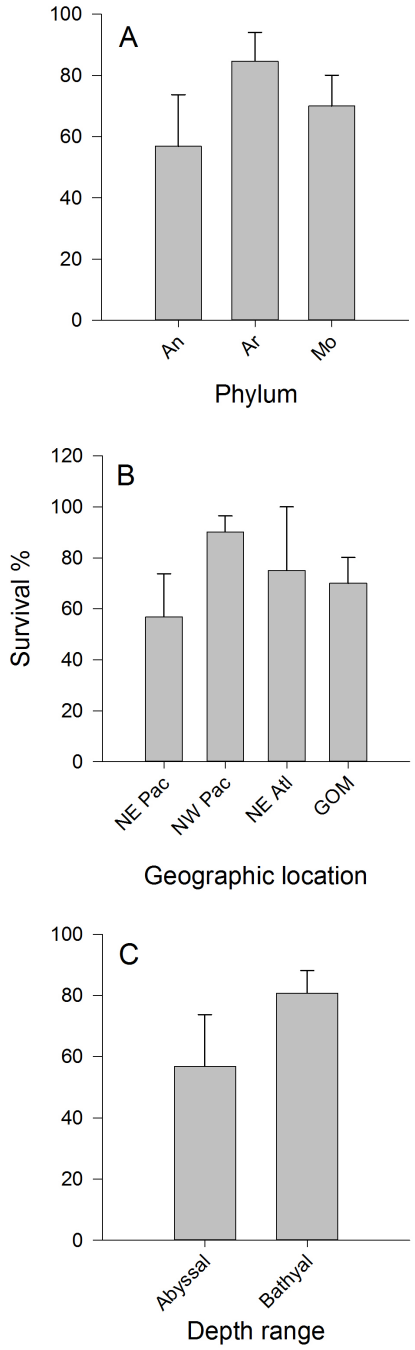

Figure 2-5: Survival (%) of larvae (mean ± SE, *n* = 4-15) from chemosynthetic environments with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Mo=Mollusca), (B) geographic location (see Table 2-1 for outline of abbreviations), and (C) depth range.

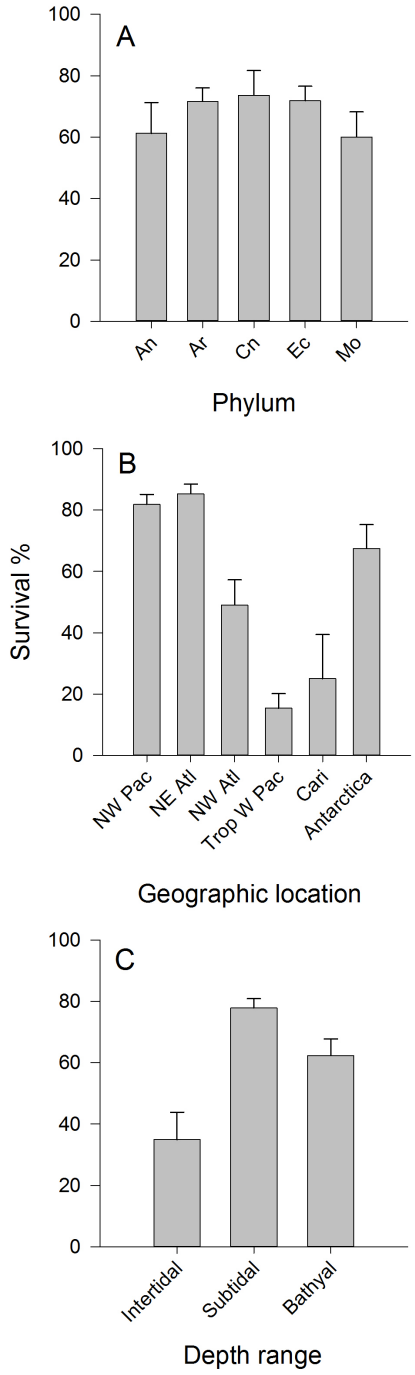

Figure 2-6: Survival (%) of larvae (mean ± SE, *n* = 7-111) from non-chemosynthetic environments with regards to (A) phylum (An=Annelida; Ar=Arthropoda; Cn=Cnidaria; Ec=Echinodermata; Mo=Mollusca), (B) geographic location (see Table 2-1 for outline of abbreviations), and (C) depth range.

CHAPTER 3

Life under pressure: an experimental study of behavioural responses to hydrostatic pressure and other stressors in echinoderms

3.1 Abstract

Although hydrostatic pressure is a key parameter in the definition of marine environments, our knowledge of its role on the physiology, behaviour and ecology of marine benthic organisms remains rudimentary. In an effort to develop a mechanistic understanding of depth distributions and vertical migrations, responses to pressure were tested in adults of echinoderms commonly occurring at shallow and upper bathyal depths in the North Atlantic, i.e. the sea urchin *Stronglyocentrotus droebachiensis*, the sea star *Leptasterias polaris* and the sea cucumber *Cucumaria frondosa*. Each species was exposed to pressures within and beyond its currently known bathymetric distribution, under ambient and low pH conditions (consistent with predicted ocean acidification), and for different durations (24 h, 72 h). Sea stars were additionally tested for up to 9 d. Measured responses included survival rates, feeding metrics, mobility levels, predator-prey interactions and post-trial recovery. Results showed that exposure to pressure atypical of their natural bathymetric distributions negatively affected the motor functions (time needed to anchor or right) of all species, irrespective of exposure duration. In trials ≤72 h under ambient pH, survival was reduced after exposure to the highest pressures, with sea urchins exhibiting the highest mortality. Feeding during or after pressure exposure was reduced in sea urchins and sea cucumbers, but was relatively unchanged in sea stars. Overall, species did not show any clear signs of adaptation to high pressure following longer periods of exposure. Sea cucumbers had reduced survival after 72-h exposure trials, and none of the sea stars survived high-pressure exposure for 9 d. In pH-pressure combination trials ≤72 h, sea urchins and sea stars typically fed less when exposed to low pH at atmospheric and high pressure levels, whereas the feeding response

in sea cucumbers was negatively impacted by pressure more than by pH. In terms of motor function, low pH appeared to counteract the negative effect of pressure on righting in sea stars and sea urchins, while it consistently worked to delay anchoring in sea cucumbers, irrespective of pressure. Taken together, findings provide insight into the constraints applied by hydrostatic pressure typical of depths within and beyond their current ranges (and by ocean acidification) on the life-sustaining behaviours of echinoderms. The potential of long-lived echinoderms to survive downward migration to greater depths is apparently species-specific, suggesting that there may be winners and losers in the face of near-future climate-driven migration patterns.

3.2 Introduction

Hydrostatic pressure is a fundamental mediator of biological processes (Macdonald 1997). It plays a critical role in defining oceanic environments as it forms a continuous linear gradient from the surface to the abyss (Pradillon and Gaill 2007). Specifically, pressure in the ocean increases by 0.1 MPa (1 MPa=10 bar~10 atm) for every 10 m of depth (Somero 1992). Since 95% of the oceans' volume lies at depths greater than 200 m, pressure is a prominent abiotic factor that the vast majority of marine life must cope with (Miyake et al. 2012; Pradillon and Gaill 2007).

The oceanic depth of 200 m generally marks the continental shelf break, establishing an arbitrary subdivision between shallow-water and deep-sea environments (Hessler 1974; Thistle 2003). In addition to greater pressure, factors that define the deep sea include: minimal nutrients from primary production, low temperature, darkness and relatively stable water chemistry (Carney 2005; Childress 1995; Pradillon et al. 2004). Although the bathymetric range of species is driven by a complex array of biotic and

abiotic variables, the suggested primary variables responsible for controlling distribution are pressure and temperature (Brown and Thatje 2014; Carney 2005). Tolerance to these factors is highly species-specific, thus the range of bathymetric distributions that different species occupy may vary from dozens to hundreds of meters (Tyler and Young 1998). Pressure is a critical abiotic parameter that has substantial effects on reaction rates, whereby it can either accelerate or reduce the speed of a reaction depending on whether reactant or product has the largest volume (Siebenaller and Somero 1989; Somero 1992; Swezey and Somero 1985). Although a considerable number of studies have examined the effects of combined thermal-pressure levels, our overall understanding of the biological effects of pressure remains rudimentary (Tyler et al. 2000b; New et al. 2014; Aquino-Souza et al. 2008; Tyler and Young 1998; Ravaux et al. 2003; 2009).

There is increasing incentive to study both the ecological and physiological pressure thresholds of marine species in light of impending climate changes. While the combination of anthropogenic and climatic factors are expected to impact both shallow-water and deep-sea environments, coastal ecosystems are anticipated to suffer the greatest changes, especially close to the poles (Brierley and Kingsford 2009; Harley et al. 2006; Hoegh-Guldberg and Bruno 2010). Among other ecological impacts, relatively rapid shifts in physico-chemical conditions (e.g. temperature, pH, UV radiation) over decadal scales are expected to drive the migration of marine species poleward and downward towards colder and/or more stable refuges (Doney et al. 2012; Graham et al. 2007; Harley et al. 2006; Perry et al. 2005). In this context, it becomes important to develop our understanding of the potential interaction between climate-related stress, such as ocean acidification, and pressure tolerance. To date few studies have looked at the synergistic

effects of pH and pressure within a controlled laboratory setting despite its obvious implications for ecosystem dynamics (Barry et al. 2004; Verkaik et al. 2016b).

Complex interactions between pressure and other abiotic factors are already modulating bathymetric distributions (Macdonald 1997), highlighting the need to tease out the exact pressure thresholds of marine species through laboratory studies. In general, the study of pressure is logistically challenged by the rarity of high-pressure vessels capable of maintaining large volumes of water under flow-through conditions and the difficulty in obtaining and testing deep-sea taxa under laboratory conditions (Miyake et al. 2007; Shillito et al. 2001; 2015). Rather than compromise sample sizes and to circumvent potential internal damage from collections, many investigators have *in lieu* used shallow-water species with close phylogenetic relationships to deep-sea species in an effort to infer effects on those of deep-sea relatives (Distel et al. 2000; Oliphant et al. 2011; Tokuda et al. 2006). This strategy has generated knowledge of how shallow-water taxa cope with pressure in a bid to identify underlying mechanisms of piezophily (barophily) in marine species (Oliphant et al. 2011; Robinson et al. 2009).

Fundamentally, high pressure affects the biochemical and physiological activities of biological systems by directly altering their volume (Somero 1992), thus limiting the speed of the reaction responsible for converting reactants to products (i.e. generation of enzymes and lipid membranes). Inversely, low pressure accelerates the rate of the reaction (Hochachka and Somero 1984; Somero 1992). Shallow-water species can reduce their membrane fluidity, whereas deep-species maintain fluidity by modifying the homeoviscous structure (Somero 1992). Thus, the inability to maintain reaction rate

equilibrium can manifest as impairment in neural and muscular functionality in metazoans (Macdonald 1984).

A number of laboratory studies have been conducted on how shallow-water species cope with pressure that exceeds their natural bathymetric distributions (see Chapter 2 and review by Brown and Thatje 2014). Studies have shown that the response of species to pressure exposure occurs on an extensive physiological level. High pressure exposure experiments on crustaceans have induced mechanistic challenges such as: dramatic decreases in heart rate from cardiac stress (Robinson et al. 2009); depression in metabolic rate as a result of poor membrane functionality (Thatje et al. 2010); and higher oxygen consumption relative to atmospheric rates, indicative of internal stress (Thatje and Robinson 2011). Such problems can trigger the high-pressure neurological syndrome (HPNS), which was originally described in vertebrates (Bowser-Riley 1984) and later observed in invertebrates exposed to pressure conditions beyond their natural bathymetric range (Somero 1992). HPNS causes chronic stress symptoms like paralysis, spasms and uncoordinated movements (Morris et al. 2015; Oliphant et al. 2011; Wilcock et al. 1978). Overall, pressure negatively impacts the neurological and muscular pathways of many organisms (Morris et al. 2015). Exposure to pressures exceeding the natural range of invertebrates has been found to elicit complications that hinder inhibitory or excitatory motor activity (Wilcock et al. 1978). In the early stages of exposure to such pressures, motor activity undergoes a stage of hyper-excitability (spasms, convulsions and twitches) but is immediately followed by a reduction in coordination of movements and mobility (Macdonald and Gilchrist 1978; Macdonald 1997; Wilcock et al. 1978). The term "pressure-paralysis" has been used to describe the point at which convulsive thresholds

are met and there is a subsequent absence of motor activity (Macdonald and Gilchrist 1978). Therefore, examining the behavioural responses of species is an effective means of determining their ability to maintain internal homeostasis (Thatje et al. 2010). The efficiency of critical behaviours such as feeding were also compromised under such conditions in the shallow-water spider crab *Maja brachydactyla*, inferred by unsustainable long-term survival (Thatje and Robinson 2011). Other investigations have reported that stressful pressure conditions induce a "loss of equilibrium" in which individuals are reduced to a moribund state, notably in species of shrimp and crabs (Morris et al. 2015; Oliphant et al. 2011; Shillito et al. 2004; 2006). Although survival has been noted for individuals during 24-h exposures to various pressures, whether they are capable of surviving in an ecological context, i.e. by maintaining the ability to escape predators or forage to exploit food sources, has not been fully explored.

The phylum Echinodermata has commonly been used in pressure studies because its members are ubiquitous in marine benthic ecosystems across depths, exhibit a diversity of complex life histories, and many of them act as keystone species (Gage and Tyler 1999; Aquino-Souza et al. 2008; Villalobos et al. 2006; Young and Tyler 1993; Young et al. 1996; 1997). Many echinoderms are also suspected to be extremely long-lived (Ebert and Southon 2003), and therefore susceptible to experience decadal-scale changes in ocean conditions resulting from anthropogenic and climate-related stressors. Tyler and colleagues have done an impressive amount of work on early stages of shallow-water echinoderms across a wide latitudinal gradient, generally supporting the ability of echinoderm larvae to survive pressures that are beyond the natural bathymetric range of adults (Young and Tyler 1993; Young et al. 1996; 1997; Aquino-Souza et al.

2008; Villalobos et al. 2006; Tyler and Young 1998). It has been suggested that larvae could successfully invade deeper waters and establish populations within one generation (Tyler and Young 1998; Tyler and Dixon 2000). However, most of the current knowledge about the interactions of marine invertebrates with pressure is limited to pre-metamorphic life stages (before settlement). Why adults of these species have not been found at such depths in nature remains unclear (Morris et al. 2015). Until recently, studies were limited to testing adults under static water volumes for short periods of time (<24 h), which were not representative of natural ecological conditions (Company and Sardà 1998; Mickel and Childress 1982a; Mickel and Childress 1982b). The study of larvae was favoured since adult body sizes and more developed metabolisms required larger vessels capable of maintain flow-through conditions (Brooke and Young 2009; Shillito et al. 2015).

The present study aims to address knowledge gaps in our understanding of how marine organisms cope with pressure by examining the tolerance of adult echinoderms to hydrostatic pressure using a suite of indicators over short and long periods, and by studying their responses to combined stressors. The interactions of pressure with abiotic stressors typical of climate change other than temperature have rarely been examined; to date it is not known whether shifting pH levels will be exacerbated by the effects of pressure on species (Pradillon and Gaill 2007). Given that climate-driven changes (rising seawater temperatures and ocean acidification) may drive vertical migrations, it is important to assess the ability of species to colonize and thrive in increasingly deeper waters. Focal species were selected based on their broad distribution in temperate, cold and polar subtidal environments: the sea urchin *Strongylocentrotus droebachiensis,* the

sea star *Leptasterias polaris* and the sea cucumber *Cucumaria frondosa*. These species also exemplify three of the main echinoderm classes (Echinoidea, Asteroidea, Holothuroidea, respectively), three feeding modes (omnivory, carnivory, herbivory, respectively) and very different body forms that contribute to different modes of locomotion (test/exoskeleton, rigid calcareous body, weakly calcareous soft body, respectively).

The study specifically tested the hypotheses that if shallow-water species are limited in their spatial distribution by pressure, behaviour will vary upon exposure to different pressures (depths). Secondly, if species can adapt relatively quickly (within a generation) to increased pressure, responses measured after long exposure (72 to 216 h) will be weaker than those measured after acute 24-h exposure. Lastly, if pressure and pH interact to impose selective pressures on echinoderms, treatments with high levels of these stressors will have more deleterious effects on the measured responses.

3.3 Methods

3.3.1 Collection and maintenance

Adult specimens of the green sea urchin *S. droebachiensis* (40-102 g; 33.1-66.6 mm test diameter), the polar sea star *L. polaris* (23-114 g; 6.0-11.2 mm major axis), the northern sea cucumber *C. frondosa* (1-3 g, immersed weight; 81-171 mm) and the purple sunstar *Solaster endeca* were collected via scuba diving at depths of 10-15 m between May 2013 and November 2014 off the coast of Newfoundland, eastern Canada (47.0833°N, 52.9500°W). All species were transferred to laboratory holding facilities and maintained in tanks (20-2000 L) under a flow-through system of unfiltered seawater (150 L h^{-1}). Individuals were kept close to large windows with naturally fluctuating light

(maximum of 25-40 lx). A chilling unit (Universal Marine Industries, 5 hp) kept water suitably cold during the summer-fall months from July to October (<10°C). A light-temperature HOBO Pendant (UA-002-64) water temperature recorded fluctuations throughout the day. At least 1-3 weeks prior to experiments, individuals were transferred to holding tanks held constant at 4-6°C. Starvation was implemented during this period to decrease metabolic variability among individuals of a given species and among the three species tested (New et al. 2014). Only healthy individuals without obvious damage to tissues, spines or arms were selected for the experiments.

3.3.2 Equipment and experimental conditions

Two flow-through stainless steel incubators of 19 L (IPOCAMP^{TM}, Autoclave, France) that can be pressurized to a maximum of 30 MPa (or 300 bar), equivalent to ~3000 m depth, were used (Shillito et al. 2001). They were maintained under flow though at 20 L h^{-1} for the duration of each trial. Temperature was controlled and measured by probes (Huber CC 240, Offenburg, Germany) situated at the inlet and outlet of the IPOCAMP (±1°C). Sophisticated hyperbaric vessels such as the IPOCAMP are rare commodities (only 8 such units exist in the world, two of which were used here). They are also more challenging to use than regular tanks. Therefore, the duration of the trials and replication designs outlined below had to be adjusted within the boundaries of several technical constraints.

Prior to each trial, the IPOCAMP was run for at least 2 hours to allow for the desired temperature to be reached before inserting the individuals in the vessel (Oliphant et al. 2011). Fiber optic light guides (KL 1500 LCD, Schott, USA) were placed in two of the view-ports to uniformly illuminate the interior (~100 lux), as confirmed from

verification with an endoscope (Fort Dourban, France) placed in the third view-port. When required, visual observations were recorded with a microscope camera (AxioCam ERc 5S, Zeiss, Germany) fitted to the endoscope.

Rather than mimicking the deep-sea environment, the experiments were designed to test the effect of pressure alone (or in combination with pH) while keeping the other parameters as close to natural conditions as possible. Temperature was adjusted to match holding conditions during acclimation (6°C), which are representative of natural subtidal (and deep-sea) oceanic temperatures at the initial time of experimentation (DFO 2009). A 12:12 photoperiod was selected to allow for equal levels of diurnal/nocturnal activity. A multi-probe system (556 MPS, YSI Environmental, USA) was used to measure environmental parameters in the vessels immediately before and after each trial run. These include dissolved oxygen (DO; mg/L), salinity (psu) total conductivity (g/%; m/s/cm), inorganic carbon (DIC), pH, oxidation-reduction potential (ORP) and temperature (°C). Total alkalinity (TA) was measured periodically with a test kit (Orion 700010, Thermo Fisher Scientific, USA).

3.3.3 Response to pressure

Based on known bathymetric ranges for each species, pressures levels were separated into: atmospheric pressure (sea surface), medium pressure (middle of known bathymetric distribution) and high pressure (twice the known maximum depth of occurrence; Table 3-1). All pressures selected were rounded to the nearest integer (0.1 MPa=0 MPa). Bathymetric ranges of occurrence within North Atlantic Canadian waters were determined from records accessed on the World Register of Marine Species (WoRMS Editorial Board 2014). Searches for maximum and minimum depth

distributions only considered records of >3 samples collected and identified post 1980, or posterior to any major taxonomic revision (Brodie et al. 2013; Smithsonian Institution 1974). A transition period was allowed by manually setting the experimental pressure following increments of 2 MPa every 6 min; the same method was used to depressurize the vessels. This rate was selected in an effort to reduce stress to individuals as drastic changes in pressure over short intervals (commonly used in previously published studies of a similar nature: New et al. 2014; Yoshiki et al. 2008) have been found to cause DNA damage in hydrothermal vent worms, *Paralvinella grasslei* (Dixon et al. 2002).

Responses of the three focal species were first tested in the context of 24-h exposure to the three pressure levels (Table 3-1). Based on biomass constraints, each 24-h trial with *S. droebachiensis* and *L. polaris* consisted of 6 individuals (run twice with a total of n=12 per treatment, all different individuals), whereas each 24-h trial testing *C. frondosa* consisted of 3 individuals (run twice with a total of n=6 per treatment). Due to pressurization-depressurization time (see below), 24-h trials could not be conducted every day, but only every other day. Duplicate runs were thus carried out either simultaneously or within 48 h, once in each of the two IPOCAMP vessels, to minimize any tank effects. In order to confirm the absence of any temporal variation, control treatments were repeated whenever a full set of experiments (for a species and a factor) could not be completed within 8 days (for technical reasons). Inside the vessel, *S. droebachiensis* and *L. polaris* were individually housed in perforated meshed clear containers (760 ml; 100-µm nylon mesh), which were stacked vertically at random. Individuals of *C. frondosa* were uncontained and placed on 3 separate levels of the holder designed for the IPOCAMP (Ravaux et al. 2003).

Following 24-h experiments, longer experiments were conducted to test exposure to atmospheric and high-pressure levels on *S. droebachiensis*, *L. polaris* and *C. frondosa* for a continuous period of 72 h (3 days). All three species were tested together as they do not pose predatory threats to each other and can be found in abundance in the same locations in eastern Newfoundland. Each of the two IPOCAMP vessels contained two individuals of each species on different levels of the holder (for vessel design, refer to Ravaux et al. 2003). Control and experimental conditions were tested simultaneously using the two vessels: one at atmospheric pressure (0 MPa) and the other at high pressure (24 MPa; i.e. the average of the high pressures tested in the 24-h experiments). The trials were replicated three times, for a total of 6 individuals of each species per treatment. Placement of the species on the levels was mirrored in the control and experimental vessels, and was flipped across the runs to account for putative vertical variability in conditions (water flow in the vessels).

A longer-term behavioural and feeding experiment was conducted on *L. polaris*, during which individuals were maintained under continuous pressure for a period of 216 h (9 days). The IPOCAMP was run in flow-through mode so that there was a constant supply of fresh seawater in the header tank. The protocols for experimental pressurization followed those outlined for 24-h trials. The 216-h experimental period tested the control (atmospheric pressure) and the species-specific high pressure in order to determine if/how individuals are able to acclimate to pressures atypical of their distributions (Table 3-2). In each vessel, feeding was quantified from individuals (n=4) that were randomly selected and placed within separate containers stacked in the IPOCAMP. Another group of individuals (n=3) were placed on a flat platform situated directly on top of the stacked

containers (for vessel design, refer to Ravaux et al., 2003). These individuals were not contained in order to monitor behaviour throughout the experiment using the endoscope camera setup described earlier.

Response metrics (dependent variables) were measured, including various morphometrics, indices and behaviours, at different times for the various pressure levels and durations tested (Table 3-2; Section 3.3.5). Pre-trial responses were tested at least 2 hours before the start of the trials, post-trial monitoring was done immediately after removal from the IPOCAMP, while post-recovery responses were measured 7 days post trial.

3.3.4 Combined response to pressure and pH

Acidification experiments modified the pH of seawater by injecting CO_2 from a gas cylinder and solenoid (Milwaukee, MA957, USA) into the IPOCAMP supply tank. The ambient pH level of incoming seawater was measured and either left unchanged (ambient pH) or reduced by 0.4 units (low pH; ~7.55 ± 0.05), following projected oceanic pH decrease by 2100 from the IPCC (2007). This level was constantly regulated in the supply tank with a pH controller (270002, Aquatic Life, USA). The cumulative effects of pressure (atmospheric, high) and pH (ambient, low) were tested in a two-by-two factorial design. This series of trials was conducted on the same three species. The experimental design followed the design described above for pressure experiments, and the same response metrics were used as dependent variables (see below).

3.3.5 Response metrics

3.3.5.1 Body metrics and weight variations in all species

The sizes of all individuals were measured before the trials to ensure they were within a similar range across experimental and control treatments. Wet (blotted) weights were recorded for *S. droebachiensis* and *L. polaris*, and immersed weights for *C. frondosa* (as sea cucumbers retain proportionally much more water). Test diameter was used for *S. droebachiensis*, major radius (length from the central disc to the tip of the longest arm) for *L. polaris* and contracted length (mouth-anus) for *C. frondosa*. Lastly, the extracellular coelomic pH was assessed only for *S. droebachiensis* after certain treatments; this experiment is described in Appendix B.

The initial and final wet weights of *S. droebachiensis* and *L. polaris* were also used to determine the net weight loss/gain associated with experimental conditions, if any. A change in weight is considered a stress indicator that measures variations in fluid volume (Ferguson 1992). Wet weights before and after each trial were averaged to obtain mean wet weight used to calculate the feeding indices for these species. Since they were not required for any feeding indices, the final weights of *C. frondosa* were not obtained to avoid delaying measurement of post-trial dependent variables (due to slightly more complex method required for weighing immersed individuals).

3.3.5.2 Righting times in S. droebachiensis and L. polaris

Righting times were tested at atmospheric pressure in sea urchins and sea stars to assess the condition of the individuals post-trial, since this response is indicative of health (Taylor et al. 2014). Immediately after removal from the vessels, individuals were placed in separate 20-L tanks (34.3 X 39.4 X 20.3 cm) with seawater maintained at ~6-8°C with

ice packs. There was no water flow in the tanks to avoid disturbance. After a 10-minute acclimation period to the new environment, individuals were tested for their righting response by flipping them 180º onto their aboral surface. The following were recorded: time to 90° righting and time to 180º righting, equivalent to full recovery to original upright position. For *L. polaris* the 180° orientation of central disk to the bottom was considered when all arms were lying flat on the bottom (without any contortions). Experiments were ended after 2 hours; if the response was incomplete the time was considered to be 120 minutes. The proportion of individuals that did not complete the righting motion within 2 h was also noted.

For 216-h exposure the possible post-trial effects of containment on individuals from the experimental design were taken into account, and the righting responses of the two groups (contained and free-moving within the vessel) were tested for significant differences. As there was no significance between the two groups at either pressure for righting immediately after exposure or after a 7-day recovery period, the data were pooled for analysis (n=7).

To account for the possible effect of post-trial experimental seawater pH following pressure exposures conducted at low pH, the individuals from each vessel were divided into two groups of six. One group was tested in ambient seawater and the other in acidified seawater. As there was no significant effect of post-trial pH condition on the righting times tested, data were pooled together for analysis (n=12).

3.3.5.3 Ingestion index in S. droebachiensis

At the onset of a trial, each individual was placed directly on piece of kelp *Laminaria digitalis*, a typical food source for this species (Meidel and Scheibling 1999).

The kelp fragment had been thoroughly blotted and cut to obtain an initial wet weight of 10 g. At the end of the trial, residual food was removed, blotted and weighed again. The ingestion index was calculated as the weight of food consumed (mg) on the average weight of the individual (g) per day (24 h). Preliminary control experiments had been conducted to determine whether kelp weight was affected by experimental conditions. Exposure to high pressure for 24 h had no significant effect on initial versus final kelp weights.

3.3.5.4 Ingestion index in L. polaris

In 24-h experiments a single mussel *Mytilus edulis* (4-5 cm) was offered to each sea star (Rochette et al. 1994). As the study objective was to examine consumption without foraging effort, the mussels were opened and the tissue attached to the abductor muscles offered on one shell. All loose fragments were removed so only one intact piece of flesh was present (initial mussel tissue 1.28-10.39 g). Mussels were thoroughly blotted to obtain initial weight. Following the completion of the experiments, the residual food was removed blotted and weighed to determine the amount of food ingested. The ingestion index was calculated as the weight of food consumed (mg) on the average weight of the individual (g) per day (24 h).

A series of preliminary experiments had determined that the weight of mussel flesh was affected by exposure to pressure. Therefore, a standard error was determined from exposure of mussel tissues to different pressures, under both ambient and acidified conditions. In total, 12 pre-weighed mussel halves were placed inside a vessel filled with ambient seawater at atmospheric, medium and high pressure for 24 h. The same was done with acidified water at atmospheric and medium pressure. The difference between the

initial and final weights of the tissues was determined; this value was divided by the initial weight to find the proportion of error. These percent inherent variations in mussel weight under different conditions were used to correct feeding indices following the experimental treatments.

For 216-h exposure, feeding was measured according to the techniques outlined for 24-h trials, except two mussels were provided and the quantity always exceeded that of the shorter trials. Contained individuals were each provided 2 mussels with the combined initial tissue weight ranging from 11.7-19.2 g. Feeding was observed *in vivo* using an endoscope to monitor behaviour throughout the experiment. The 3 uncontained individuals were offered a total of 4 live (shelled) mussels so that qualitative observations could be made about foraging behaviour. The initial weight of mussel tissue could not be accurately determined under these circumstances; so the combined total weight (shells and tissue) ranged from 190.4-223.5 g. Observations were made twice every 24 h (10:00, 16:00) over the trial period starting from the initial time of pressurization. They consisted of recordings of 30 sec video feed at intervals of 30 sec for 10 min (for a total of 5 min twice daily in each treatment). The videos were long enough to determine the movements and activity of the individuals and whether they were feeding on the mussels.

3.3.5.5 Final feeding position in L. polaris

Upon depressurization and removal from the vessel after the 24-h (ambient and acidified pH) and 72-h trials, each container was immediately observed to determine the position of the individuals relative to the food item and whether active feeding could be scored. These observations were only possible for *L. polaris* because of their conspicuous feeding mechanism of everting their stomach (unlike the much less obvious grazing of *S.*

droebachiensis). In total, 4 different positions were noted to describe feeding: (1) Stomach everted and arms wrapped around the mussel shell. (2) No everted stomach and arms wrapped around the mussel shell. (3) Stomach everted and arms not wrapped around the mussel shell. (4) No everted stomach and arms not wrapped around the mussel shell.

3.3.5.6 Post-trial and post-recovery in L. polaris

All sea stars were tested to determine experimental recovery. After experiments, all sea stars from the different treatments were maintained in 20-L tanks under the previously described holding conditions. Exactly 168 h (7 days) post-trial, they were placed in separate tanks and their righting response was re-evaluated following the methods described previously. Post-trial righting recovery periods have been found to be a useful means of assessing physiological health in the sea star *Asterias rubens* (Appelhans et al. 2014).

3.3.5.7 Health indices in C. frondosa

The following health indices for *C. frondosa* were adapted from previous studies (Gianasi et al. 2015; Verkaik et al. 2016a): feeding (i.e. when all ten oral tentacles are fully extended to capture food and one tentacle is introduced into the mouth), cloacal openings (i.e. cloacal respiration, the number of times the cloaca opens and closes in a given time interval as water enters/exits the respiratory tree), escape response (i.e. initiation of reactions such as contraction, elongation and swelling in the presence of a predator) and anchorage (i.e. time to firm attachment of podia to a substrate as determined when individual cannot be dislodged with gentle poking). Cloacal opening

and anchorage were assessed pre-trial to determine baseline health status of individuals with minimum disturbance. All health indices were measured post-trial.

Pre-trial anchorage time was measured while the individuals were held individually in 9-L tanks matching conditions in the experimental vessels. Individuals were first introduced to the center of the tank and the time for anchorage was determined to the nearest 2 min. Individuals that were not capable of anchoring after 30 minutes before the trial were replaced. In post-trial measurements, individuals that had not anchored after this delay were scored as 30 minutes (after exposure to 0 MPa anchorage took 1.43 min). Once all of the individuals were successfully anchored, or following the maximum delay mentioned above, cloacal opening was measured for a period of 5 min. This measure is a proxy of respiration rate as it indicates the rate of water exchange in the respiratory tree (Doyle and McNiell 1964; Gianasi et al. 2015).

The two previous indices were measured both pre-trial and post-trial, whereas the following indices were only measured post trial. The water flow to the tanks was interrupted briefly to measure the feeding index, in order to maintain high food concentrations (Gianasi et al. 2015). The food consisted of 3 ml of a commercial algal feed (Phytofeast-Feast® Live) comprised of six phytoplankton species (*Pavlova* sp., *Isochrysis* sp., *Thalassiosira weissflogii, Tetraselmis* sp., *Nannochloropsis* sp., and *Synechococcus* sp.) with cell sizes ranging from 1 to 15 μm in diameter. The food mixture was added to each tank in a uniform fashion to ensure equal mixing. Time required for each individual to open its tentacles and begin feeding was recorded. Individuals that had not started feeding after 60 min were scored as 60 min (after exposure to 0 MPa, feeding response took <6 min).

Following the feeding experiment, individuals were transferred to a tank with recirculating fresh seawater before testing the predator response. The known predator of the *C. frondosa*, the sea star *Solaster endeca*, was placed directly on the sea cucumber (So et al. 2010). Time needed to initiate the escape response and its various stages, i.e. swelling, elongation and contraction, was monitored for a total of 15 minutes (Gianasi et al. 2015).

3.3.6 Statistical analysis

The comparison of means was carried out using Student's t-tests, one-way or two-way analyses of variance (ANOVAs). When statistically significant differences occurred, post-hoc multiple comparisons tests were conducted using the Holm-Sidak method. Where equal variance was violated, ANOVA on ranks were used, followed by Dunn's post-hoc tests as appropriate. Where interactions were found between pH and pressure, independent tests were carried out on possible combinations using either t-tests or Mann-Whitney U tests, as appropriate. If the results for righting to 90° and to 180° were not statistically significant, only the results for 180° were graphically presented (while all results were reported in 3.4.1 and 3.4.2). Significance levels were considered at $p<0.05$ and all analyses were carried out using Sigma Plot version 11.0 (Systat Software, USA). Full results are summarized in Appendix C (Tables C1-C3).

3.4 Results

3.4.1 Response to pressure

3.4.1.1 Strongylocentrotus droebachiensis

The 24-h experimental exposure to pressure had deleterious effects on behaviours in *S. droebachiensis* during and after exposure (Table 3-3; 3-4). Feeding significantly decreased as pressure treatments increased ($H=26.56$, $df=2$, $p<0.001$) whereby feeding indices were lower at high than at either atmospheric or medium pressure ($p<0.05$). Feeding did not vary significantly between the latter ($p>0.05$), which are within the known depth range of the species (Figure 3-1A). Post-trial righting was significantly impacted (90°: $H=6.26$, $df=2$, $p=0.044$; 180°: $H=7.80$, $df=2$, $p=0.020$). Specifically righting times to 90° were significantly longer as pressure increased (atmospheric < medium < high pressure; $p<0.05$; Figure 3-1B), and righting times to 180° were significantly longer for the high-pressure treatment than the two other treatments ($p<0.05$; Figure 3-1C). While all individuals survived 24-h exposure to atmospheric and medium pressures, only 50% of them survived high-pressure beyond the known bathymetric distribution (Table 3-5). Following exposure to high-pressure, spines were brittle and easily broke upon light handling. However, pressure did not significantly affect body weight; the difference in mean weight after exposure to high, medium and atmospheric pressures were -0.33 g, -0.22 g and -0.02 g, respectively ($H=10.89$, $df=2$, $p=0.004$).

The results of the longer 72-h exposure essentially followed those of the 24-h trial. Because there was a significant interaction between pressure levels and time (pre-trial, post-trial, post-recovery) on time to right to 90° ($F_{2, 35}=4.71$, $p=0.017$; Figure 3-1D), independent analyses were conducted at each level of each factor. Righting post-trial took

significantly longer after exposure to high than to atmospheric pressure (t=-3.86, df=10 p=0.003; Figure 3-2A); after high-pressure exposure post-trial righting was longer than the pre-trial (t=5.72, df=10, p=<0.001) and post-recovery values (t=2.42, df=10, p=0.036). Individuals were slower to right post-recovery relative to pre-trial, showing an inability to recover from high pressure (U=2.00, df=10, p=0.009). A similar interaction between time and pressure was also found for time to right to 180° ($F_{2, 35}$ =4.71, p=0.017; Figure 3-1E). After exposure to high pressure, the same trends as righting to 90° persisted except the difference between post-trial and post-recovery values fell short of significant by a narrow margin (t=2.21, df=10, p=0.052), indicating weak if not absent recovery after a week.

The results of a two-way ANOVA on the effect of pressure levels (atmospheric, high) and trial duration (24, 72 h) on the post-trial time to right to 180° showed slower responses after exposure to high than atmospheric pressure ($F_{1, 47}$ =8.40, p=0.006); but exposure duration had no effect ($F_{1, 47}$ =3.36, p=0.074). Survival was still lower following exposure to high pressure (83%) than atmospheric pressure (100%) but non-significantly so.

3.4.1.2 Leptasterias polaris

The 24-h exposure to pressure had a significant effect on weight of sea stars (H=10.89, df=2, p=0.004; Table 3-3; 3-4), whereby weight loss was greater at high pressure than either atmospheric or medium pressure (p<0.05); but not between the latter (p>0.05). Mean difference in weights following pressure exposures were -3.97 g, -1.09 g and -0.24 g, respectively. By contrast, feeding was not significantly affected by pressure over 24 h ($F_{2,45}$ =0.40, p=0.669; Figure 3-3A.). Generally, individuals under pressure did

not assume feeding positions, but they were not in obvious feeding positions under atmospheric pressure either (Table 3-6). The 24-h exposure to pressure significantly affected the righting times to 180° (H=22.98, df=2, p=<0.001), which were slower after high than medium or atmospheric pressure exposures (p<0.05), but responses did not vary between the latter (p>0.05; Figure 3-3B). Post-trial survival of *L. polaris* for 24-h high pressure treatments was 92% and among those exposed, 17% were unable to right and displayed uncoordinated tube feet movements. Survival after a week was 83% for the high-pressure treatment and 100% for the others (Table 3-5). Among the survivors, post-recovery righting time to 180° was not significantly different across pressure treatments (H=1.09, df=2, p=0.579; Figure 3-3B), and individuals exposed to 22 MPa took less time to right post-recovery than post-trial (H=31.91, df=1, p=<0.001), indicative of improved condition after a week.

There was 100% survival of individuals exposed to high and atmospheric pressure for 72 h, both immediately after exposure and post-recovery (Table 3-5). However, the motor functions were negatively impacted. There was a significant interaction between the effects of time and pressure on righting times to 180° ($F_{2, 35}$ =5.73, p=0.008; Figure 3-3C), which led to independent analyses within each factor. Based on the latter, it took longer to right after exposure to high than atmospheric pressure post-trial (t=-6.20, df=10 p=<0.001) but no difference between pressure levels occurred for either pre-trial (t=-0.39, df=10, p=0.70) or post-recovery values (t=0.71, df=10, p=0.493; Figure 3-2B-C). There was no effect of high pressure on the time of righting to 180° by post-recovery individuals (U=6.00, df=10, p=0.065). In two-way ANOVAs examining pressure levels (atmospheric, high) and exposure duration (24, 72 h), post-trial righting times to 180°

were affected by pressure ($F_{1, 47}$=6.26, p=0.016) but not time ($F_{1, 47}$=1.69, p=0.201). Individuals took longer to right after exposure to high pressure than atmospheric pressure for both 240h and 72-h experiments ($p<0.05$).

Feeding was not significantly affected by pressure during the 216-h exposure ($F_{1, 43}$=2.63, p=0.113; Table 3-7). All individuals displayed some level of activity over the first 3 days but, by day 6, all of them were on the bottom, scattered among the mussels, and no further movement was detected for the remainder of the experiment (Figure 3-2D). Post-trial, the arms and central disks of both the free-moving and contained individuals from the high-pressure treatments were softened to the point of fragmentation upon handling. In several instances, arms were completely detached. None of the individuals exposed to high pressure for 216 h were able to successfully right (to either 90° or 180°). There was 100% survival for individuals exposed to atmospheric pressure but only 57% survival for those exposed to high pressure. Following 7-d recovery, survival was still 100% in individuals exposed to atmospheric pressure and 86% of them were able to right, whereas individuals that had been exposed to high pressure exhibited 100% mortality.

3.4.1.3 Cucumaria frondosa

All individuals survived the 24-h trials and recovery period, irrespective of pressure level, but post-trial feeding response was significantly affected by pressure level (H=9.96, df=2, p=0.007; Figure 3-4A; Table 3-3; 3-4). Specifically, feeding was delayed after exposure to medium compared to atmospheric pressure as it was when comparing high and atmospheric pressure ($p<0.05$); there was no difference between medium and high pressure ($p>0.05$). Following the 72-h trials, there also was 100% immediate

survival, but post-recovery survival was down to 83% for individuals exposed to high pressure (Table 3-5; Figure 3-2E). Pressure level did not have any significant effect on the feeding response after 72-h exposure ($p>0.05$; Figure 3-5A). When examining the combined effect of trial duration (24, 72 h) and pressure level (atmospheric, high) on feeding response, there was no effect of the former ($F_{1, 23}=1.91$, $p=0.182$) but significant influence of the latter ($F_{1, 23}=9.97$, $p=0.005$). After 24-h exposure, the feeding response was faster for individuals exposed to atmospheric than high pressure ($p<0.05$), but there was no difference after 72-h exposure ($p>0.05$). Since there was no difference in feeding between either of the tested times, there is no direct evidence for acclimation to pressure.

The frequency of cloacal movement (cloacal respiration) following 24-h trials was also significantly affected by pressure level ($F_{2, 53}= 7.15$, $p= 0.002$; Figure 3-4B) and time (pre-trial, post-trial, post-recovery) ($F_{2, 53}= 15.03$, $p<0.001$), with a significant interaction between these factors ($F_{2, 53}= 7.05$, $p= <0.001$). Cloacal movement post-trial was higher after exposure to atmospheric than high pressure ($t=8.17$, $d=10$, $p=<0.001$) and for individuals exposed to medium than high pressure ($U=5.50$, $d=10$, $p=0.041$) but not between atmospheric and medium pressure ($U=13.00$, $d=10$, $p=0.466$). Time relative to high-pressure exposure had a significant effect on cloacal movement; there was significantly less movement at post-trial than at both pre-trial ($t=8.97$, $df=10$, $p=0.017$) and post-recovery ($t=6.82$, $df=10$, $p=0.025$); but not between pre-trial and post-trial ($t=2.15$, $df=10$, $p=0.05$). Findings from the 72-h trials were similar to those from 24-h trials. Cloacal movement was influenced by pressure ($F_{1, 35}=4.60$, $p=0.040$) and time ($F_{2, 35}=6.08$, $p=0.006$), but with an interaction between the two ($F_{2, 35}=4.21$, $p=0.025$; Figure 3-5B). Independent analyses showed that cloacal movement was more frequent after

exposure to low than to high pressure but only post-trial (t=5.41, df=10 p=<0.001); values did not differ between pre-trial pressure levels (t=-1.57, df=10, p=0.147) or post-recovery (U=13.00, df=10, p=0.485), indicating a dissipation of high pressure impacts on cloacal respiration after a week. For high-pressure trials, there was more frequent cloacal movement pre-trial than post-trial (t=-7.94, df=10, p=<0.001) or post-recovery (U=2.00, df=10, p=0.009); but no difference between post-trial and post-recovery (U=12.00 df=10, p=0.394; Figure 3-5B), indicating a persistence of the effects of high pressure after a week. A two-way ANOVA showed that pressure level had a significant effect on cloacal movement ($F_{1, 24}$ =79.32, p<0.001) but trial duration did not (24 vs. 72 h; $F_{1, 24}$ =3.70, p=0.069). Cloacal movement was less frequent for individuals exposed to high than atmospheric pressure after both 24 h and 72 h (p<0.05).

Pressure did not have any significant effect on the predator-escape response following the 24-h ($F_{2, 17}$=2.89, p=0.087; Figure 3-4C) or 72-h (t=-0.571, df=10, p=0.580; Figure 3-5C) trials. A two-way ANOVA confirmed that trial duration (24, 72 h) and pressure (atmospheric, high) did not have any effect on the time to escape predators ($F_{1, 23}$=0.003, p=0.952; $F_{1, 23}$=3.150, p=0.091).

By contrast, after 24-h exposure, pressure had a significant effect on the time needed to anchor to the substrate ($F_{2, 23}$=3.94, p=0.027) but there was no effect of time (pre-trial, post-trial, post-recovery) ($F_{2, 23}$=2.54, p=0.090; Figure 3-4D). Anchoring times were only slower following exposure to high vs. atmospheric pressure (p<0.05). Following the 72-h exposure, anchorage time was influenced by pressure ($F_{1, 35}$ =4.55, p=0.041) but not time ($F_{2, 35}$ =1.96, p=0.159); there was no interaction between the two ($F_{2, 35}$ =1.64, p=0.210; Figure 3-5D). Individuals took longer to anchor following

exposure to high than atmospheric pressure, but immediately post-trial only ($p<0.05$). A two-way ANOVA showed that anchor time was significantly affected by pressure (atmospheric, high) ($F_{1, 23}=8.40$, $p=0.009$) but not trial duration (24, 72 h) ($F_{1, 23}=0.065$, $p=0.801$). Individuals were slower to attach to the substrate after exposure to high than atmospheric pressure in both durations ($p<0.05$). Generally, individuals took longer to anchor, both post-trial and post-recovery, following the 72-h than 24-h exposure to high pressure, suggesting an inability to acclimate.

3.4.2 Combined response to pressure and pH

3.4.2.1 Strongylocentrotus droebachiensis

There was no effect of pressure or pH on the weight of individuals; mean difference in weights following exposure to low pH conditions under atmospheric and medium pressure were -0.28 g and 0.26 g, respectively (for ambient pH weight differences see 3.4.1.1; Table 3-4). No post-trial mortality was observed. Both pH and pressure interactively affected feeding ($F_{1,56} = 4.25$, $p=0.044$); independent analyses showed that feeding was greater during exposure to atmospheric than medium pressure under low pH ($t=2.19$, $df=22$, $p=0.039$) but did not differ between pressure levels under ambient pH ($t=-1.03$, $df=34$, $p=0.312$). Moreover, feeding was higher under ambient than acidified conditions at medium pressure ($t=-3.18$, $df=22$, $p=0.004$), whereas there was no effect of pH at atmospheric pressure ($U=219.0$, $df=34$, $p=0.933$; Figure 3-6A).

Similarly, time to right to 180° post-trial was consistently faster under low than under ambient pH ($F_{1, 56}=1.09$, $p=0.301$). Righting was non-significantly faster after exposure to atmospheric than medium pressure, irrespective of pH level ($F_{1, 56}=1.52$,

p=0.223), which was consistent with the significant trend detected previously (Figure 3-6B).

3.4.2.2 Leptasterias polaris

There was no significant effect of pressure on *L. polaris* weight between pressure levels under ambient pH conditions ($p>0.05$; for mean difference in weight see 3.4.1.2; Table 3-4), but under low pH conditions greater weight loss occurred for individuals exposed to medium than atmospheric pressure (-2.16 g and 0.69 g, respectively; $p<0.05$). All individuals survived the trials but feeding during exposure was significantly influenced by both pressure ($F_{1, 56}=5.09$, $p=0.028$) and pH ($F_{1, 56}=9.36$, $p=0.003$), with no interaction between the two ($F_{1, 56}=1.33$, $p=0.254$). Feeding was higher at medium than atmospheric pressure under acidified conditions ($p<0.05$). More feeding occurred under ambient than acidified conditions at atmospheric pressure ($p<0.05$; Figure 3-7A). Unlike under ambient pH conditions, a generally higher proportion of individuals had their arms wrapped around the mussel with everted stomach after exposure to high than to atmospheric pressure (Table 3-7).

Exposure to pressure that was typical of the species depth range showed that there was no effect of pressure ($F_{1, 56} = 2.20$, $p=0.143$) or pH levels on the time to right to 180° immediately post-trial ($F_{1, 56} =0.50$, $p=0.482$; Figure 3-7B). In post-recovery 7 d later, the ability to right to 90° was not influenced by pressure treatments ($F_{1, 56} =1.97$, $p=0.166$) but was effected by pH levels ($F_{1, 56} =1.36$, $p=0.007$). Specifically, post-recovery time to right to 90° was faster in individuals exposed to low-pH than ambient-pH conditions ($p>0.05$) under medium pressure, but was not significantly affected by pH at atmospheric

pressure ($p>0.05$; Figure 3-7C). The post-recovery time to right to 180° was not influenced by pressure ($F_{1, 56}=1.36$, $p=0.248$) or pH ($F_{1, 56}=2.67$, $p=0.108$; Figure 3-7D).

3.4.2.3 Cucumaria frondosa

Cloacal movement after the trial was not affected by pressure ($F_{1, 23}=3.62$, $p=0.072$) or pH level ($F_{1, 23}=0.003$, $p=0.952$), and there was no significant interaction between the factors ($F_{1, 23}=0.04$, $p=0.856$; Table 3-4). By contrast, the combination of trial duration and pH showed that former had a significant effect on cloacal movement ($F_{2, 66} = 3.54$, $p= 0.035$) but not the latter ($F_{1, 66} = 1.73$, $p= 0.193$); and there was no interaction between these factors ($F_{2, 66} = 0.363$, $p= 0.697$). Specifically, cloacal movement was more frequent pre-trial than post-recovery ($p<0.05$) but was not different between other conditions ($p>0.05$; Figure 3-8A-C).

Anchorage time post-trial was not affected by pressure ($F_{1, 23}=0.32$, $p=0.576$) but was influenced by pH ($F_{1, 23}=5.33$, $p=0.032$), with no significant interaction between the two factors ($F_{1, 23}=1.92$, $p=0.181$). Specifically, time to anchor at atmospheric pressure was faster under ambient than low pH ($p<0.05$), but there was no effect of pH at medium pressure ($p>0.05$). The time to anchor was affected by both trial duration ($F_{2, 63}=3.55$, $p=0.035$) and pH level ($F_{1, 63}=6.67$, $p=0.012$) but there was significant interaction between the factors ($F_{2, 63}=5.09$, $p=0.009$; Figure 3-8.D-F). In independent tests, under acidified pH conditions post-trial times to anchor were longer than pre-trial times ($F_{2, 21}=4.50$, $p=0.047$) and post-recovery times ($F_{2, 21}=5.91$, $p=0.025$); there was no difference between pre-trial and post-recovery times ($F_{2, 21}=0.11$, $p=0.748$). The time to anchor was not different among time points under ambient pH conditions ($H=4.24$, $df=2$, $p=0.120$). There was no difference between anchor times for exposure to acidified vs. ambient pH

conditions for either pre-trial (H=2.77, df=1, p=0.096) or post-recovery (H=3.56, df=1, p=0.551), but exposure to acidified pH resulted in longer post-trial anchor times than in ambient pH (H=4.02, df=1, p=0.045).

A significant interaction between the effects of pressure and pH on post-trial feeding activity in *C. frondosa* was found ($F_{1, 23}$ = 7.28, p=0.014; Figure 3-9A). Feeding occurred significantly faster after exposure to atmospheric than to medium pressure under ambient pH (t=-5.72, df=10, p=<0.001) but not under low pH (U=17.0, df=10, p=0.937). The onset of feeding was not significantly affected by pH treatment at medium (t=-2.11, df=10, p=0.061) or atmospheric pressure (U=10.0, df=10, p=0.240).

Finally, the predator escape response was significantly affected by pressure ($F_{1, 23}$ =4.75, p=0.041; Figure 3-8B) but not pH ($F_{1, 23}$ =1.03, p=0.322), and there was no interaction ($F_{2, 17}$=2.89, p=0.087). Specifically, *C. frondosa* escaped faster after exposure to atmospheric than medium pressure under ambient pH ($p<0.05$) but there was no difference between responses in the two pressures treatments under low pH conditions ($p>0.05$).

3.5 Discussion

3.5.1 Response to pressure

The results of the present study support the main hypothesis that behaviour in adult echinoderms is more strongly impacted by exposure to pressures beyond than within their natural bathymetric ranges. The highest hydrostatic pressure generally reduced the ability of the focal species to feed, move, right and/or anchor, which could have impacts on their health, survival and reproduction. In addition, for trials ≤72 h, mortalities were only elicited by exposure to the highest pressure. There was no

consistent weakening of the negative responses after longer exposures to support the hypothesis of rapid acclimation in the three species under study. Instead, all of them exhibited variable responses to longer exposures (i.e. 24 h vs 72/216 h). Generally, survival was lower post-recovery than immediately post-trial, suggesting that non-lethal physiological damage sometimes led to post-traumatic complications. The lowest survival among the tested species for trial duration ≤72 h was with the sea urchin *S. droebachiensis*, suggesting calcification or morphological drivers of the contrasting species-specific pressure tolerances. Overall, the results imply that the focal species would not likely be able to sustain short-term displacements (hours to days) across depths (~500 m), especially not beyond their current bathymetric current range of occurrence.

Because the echinoderm species studied here occupy continental slopes that are characterized by vertical drops and high abundances of predators, the likelihood of toppling down cliff walls during storm events or to escape predators exists. This type of behaviour has recently been observed in laboratory studies, where individuals of *C. frondosa* actively moved off elevated platforms and fell to the bottom of a large mesocosm (J. Sun per. comm., 2016). Similarly, it has been shown that black turban snails (*Tegula funebralis*) can "tumble" down a "steep slope" after encountering a predator (Feder 1963; 1972). Based on the present study, abrupt relocation to deep habitats beyond their current range would not allow adult echinoderms to readily resume feeding. For instance, feeding activity in *S. droebachiensis* and feeding response in *C. frondosa* were slower and less defined during or following exposure to the highest pressure, respectively. Similarly, Thatje and Robinson (2011) found that, when the

shallow-water crab *Maja brachydactyla* was exposed to pressure atypical of its depth distribution, it did not feed.

On the other hand, limited vertical movement appears to have minimal effects on feeding. During pressure exposure that was characteristic of mid-depth distributions, *S. droebachiensis* and *L. polaris* consumed more food than at atmospheric pressure. Pressure has been shown to induce metabolic demands and cause temporary increase in basal activity of many marine species (Schlieper 1968), thus, a benign increase in pressure may create a demand for nutrients to cope with the stress or fulfill metabolic demands under the new conditions. Similarly, when exposed to slightly increased pressures within natural distributions (<6 MPa) *M. brachydactyla* showed increased feeding (Thatje and Robinson 2011) and the shallow-water shrimp *Palaemonetes varians* displayed increased respiration rates (Oliphant et al. 2011).

Unlike feeding, it was evident that post-trial motor functions of individuals with more rigid bodies was severely compromised with increasing pressure, regardless of exposure duration. After exposure to high pressure, individuals lacked coordination and their tube feet appeared more fragile (readily breaking off in *S. droebachiensis* and *L. polaris*). Since *S. droebachiensis* has a calcareous skeleton (test) it was limited in its ability to deform and adapt to the forces of compression and thus may be susceptible to physical damage, as evidenced by the easy breaking of spines after exposure to high pressure. Similarly, *L. polaris* was not able to successfully attach to the substrate after high-pressure trials and its attempts to right were impeded by uncoordinated arm movements. These responses may be the result of internal damage caused by pressure, in line with the finding that chondrocyte-like cell lines exposed to elevated pressures of 10-

50 MPa for short periods of time (<24 h) displayed damage to cell cytoskeletons and dissociation of protein structures (Balny et al. 1997; Swezey and Somero 1985; Takahashi et al. 1998). These cellular and molecular changes have been observed in adult crustaceans manifesting convulsions, spasms and/or paralysis (e.g. Cottin et al. 2012; Macdonald and Gilchrist 1978; Oliphant et al. 2011; Wilcock et al. 1978). The atypical behaviours recorded in the present study are consistent with evidence that links spasms from high-pressure exposure to HPNS in other invertebrate phyla (e.g. Macdonald 1972; Oliphant et al. 2011; Thatje and Robinson 2011).

The effects of prolonged exposure to elevated pressure confirmed that, similar to the 24-h trials, the more calcified (hard-bodied) echinoderms were unable to acclimate to pressurized conditions over longer time period. In the case of motor responses in *S. droebachiensis*, individuals took three times longer to right to 180° after 72-h trials then after 24-h trials, emphasizing the building effects of pressure exposure. Sea urchins exposed to high pressure retracted their podia, which reportedly increases the internal pressure of the coelomic space through shifts in the hydrovascular system (Ellers and Telford 1992). The reduced coelomic pressure may negatively affect the ability for sea urchins to deploy podia or cause severe podia damage resulting in immobility (Ellers and Telford 1992). Although coelomic pressure was not evaluated in the present study, it is likely that urchins under high pressure experienced the effects described above as the podia were noted to weaken and lack coordination post trial. Given that *S. droebachiensis* was not able to maintain motor functions, it is unlikely that it would survive at this pressure for longer periods of time. Impaired mobility in a natural setting would disrupt foraging and predator evasion, resulting in low survival. In sea stars (*L. polaris*),

increased exposure time to high pressure did not necessarily intensify the negative effects of pressure on the time to right even though righting took longer after high-pressure than atmospheric treatments. It is possible that longer exposure to pressure could result in temporary tolerance to these conditions but not complete adaptation, as revealed by the 216-h experiments (see below). Our results therefore indicate that adult sea stars exhibit complex responses to pressure. In larvae, tolerance (measured as survival) was found to be inversely related to exposure duration in *Asterias rubens* and *Marthasterias glacialis* exposed to 20 MPa (~2000 m) (Villalobos et al. 2006).

Interestingly, feeding, motor functions and other health proxies were not permanently disrupted by exposure to high pressure in most of the species and treatments. Although *L. polaris* was more sluggish after exposure to high pressure relative to atmospheric pressure for the two exposure durations, righting times were back to pre-trial values after the weeklong post-trial recovery at atmospheric pressure. By contrast, after 72-h pressure exposure, *S. droebachiensis* was the most severely compromised; its post-recovery righting times improved from post-trial times but these were still longer than the pre-trial times. Evidently post-recovery times for the motor tests of *L. polaris* showed that individuals can recover from the deleterious effects of high pressure but this was not as clear for *S. droebachiensis.* In Asteroidea muscle cells receive sensory information about whole-body movement because of internal pressure changes in the coelomic cavity. Environmental hydrostatic pressure can distort such messages and inhibit activity (Gardiner and Rieger 1980). Thus, sea stars may have regained function of their muscle cells and their mobility under atmospheric pressure. In the third species, *C. frondosa*, not all health proxies were as severely impacted as in the other two, although anchorage and

cloacal respiration were both negatively affected by high pressure, irrespective of the exposure duration.

While the sea star *L. polaris* might acclimate to high pressure exposures to some degree, based on results of the 72-h exposure and on post-recovery trials, the result of the 216-h exposures to high pressure revealed that individuals were unable to right immediately post trial, and that many individuals displayed softened tissues and peeling epidermis. Furthermore, all sea stars were moribund/dead a week after this long-term trial. Internal damage was presumably extensive given that *L. polaris* lacks any defined skeleton (rather possessing hundreds of minute calcareous ossicles), so its ability to maintain internal rigidity/stability is minimal (Cavey and Märkel 1994; LeClair 1993). This experiment could not be replicated in a suitable time frame due to technical difficulties with the IPOCAMP system; however, these preliminary findings warrant further investigation as they contrast with the findings of the 72-h experiment.

Contrary to the low tolerance for high-pressure over prolonged exposure seen in the other species, *C. frondosa* exhibited variable responses across behaviours, most of which did not fully support the acclimation hypothesis. *C. frondosa* took less time to initiate feeding and escape from predators after the 72-h than 24-h exposure, indicative of acclimation to pressure. On the other hand, elevated pressure severely decreased cloacal movement regardless of exposure duration, yet the potential for recovery differed based on duration of exposure. All individuals exposed for 24 h had returned to basal cloacal respiration levels after a week of recovery, whereas those exposed for 72 h still had slower rates of cloacal opening post recovery. Typically, cloacal respiration increases with stress in *C. frondosa* (Gianasi et al. 2015) so the reduction of water circulation may

indicate internal damage to the respiratory tree, where oxygenation occurs (Doyle and McNiell 1964; Gianasi et al. 2015) or a weakening of the cloacal muscles. Internal pressure increases with body wall contractions, required for movement, and external pressure from the environment; if the pressure reaches the maximum threshold then the opening of the cloaca can result in the rupture of the cloacal wall (VandenSpiegel and Jangoux 1987). Similarly, time to anchor post-trial was significantly longer than pre-trial and post-recovery following the high-pressure exposure for 72 h but not 24 h. Post-recovery anchoring delays were still relatively longer than pre-trial values, demonstrating an intensification of the effects of pressure on tube feet activity over longer exposures.

3.5.2 Combined response to pressure and pH

Our findings show that the behavioural responses to pressure exposure within the natural bathymetric ranges under the ocean acidification scenario varied both within and among species, not fully supporting the hypothesis that the interaction between pressure and pH would systematically result in more deleterious responses. Some health proxies (feeding and anchoring) were negatively affected by low pH and high pressure but other responses such as righting, predator escape and cloacal respiration showed no evident additive effects following exposure to both factors. No mortality was observed either post-trial or post-recovery following any of these acute experiments. The combination of acidity and pressure did not have any different effect on the survival of individuals than pressure alone, although prolonged exposure was not investigated in this segment. Nevertheless, our study managed to show that pressure and acidity do interact to some degree to impose stressful conditions.

In the case of feeding, it was evident that most species were negatively affected by the interaction of pH and pressure. This finding provides novel insight for the potential effects of ocean acidification and adds to the existing literature on the interactive effects of factors related to climate change such as ocean acidification and warming (Byrne and Przeslawski 2013; Kroeker et al. 2013). When kept under low pH for >10 days at atmospheric pressure, *S. droebachiensis* was previously found to consume significantly less food than under ambient pH conditions (Siikavuopio et al. 2007; Stumpp et al. 2011). Here, there was no significant impact of pH alone over the first 24 h at atmospheric pressure. However, in the medium pressure treatment, low pH decreased feeding significantly. Given that the pressure used was within the reported natural tolerance of *S. droebachiensis* (~600 m), decreasing pH due to ocean acidification might have even greater impacts on sea urchin populations at the extreme of their bathymetric range. Contrary to *S. droebachiensis,* feeding in *L. polaris* seemed to be impacted directly by pH without any additive effect of pressure. In fact the lowest feeding indices were obtained under acidified conditions at atmospheric pressure, whereas the food ingested at medium pressure (~500 m) under low pH was only marginally less than at ambient pH. Low pH has been shown to reduce the functionality of digestive enzymes of sea stars, thus limiting the rate of consumption (Appelhans et al. 2012). The combination of pressure and pH could have enhanced metabolic demands in *L. polaris,* explaining the slightly higher feeding rates relative to atmospheric pressure. Similarly, the shrimp *Palaemonetes varians* increased food consumption under high pressure and high temperature conditions (Cottin et al. 2012).

The mobility of the more calcified species in this study (*S. droebachiensis* and *L. polaris*) were not explicitly reduced by exposure to acidity. Righting in *S. droebachiensis* generally took longer under acidified conditions relative to ambient pH (for both pressure treatments) but there was no significant effects on movement. The absence of any clear motor response to acidity might be related to the fact that pH had no effect on the internal physiology or coelomic pH levels. Similarly, in *L. polaris*, righting was neither affected by acidity nor pressure. At medium pressure, individuals exposed to low pH even righted faster than those exposed to ambient pH. An increase in metabolic activity due to stress from the combination of abiotic factors could be responsible for the accelerated motor response. Stumpp et al. (2011) have shown that acidification can result in re-allocation of energy for certain activities in adult echinoderms. Generally, individuals of *L. polaris* that had been exposed to low pH conditions still took longer to right in post-recovery tests, although the only significant difference among pH treatments occurred for righting to 90° after exposure to medium pressure, and not for complete righting to 180°.

Feeding times in *C. frondosa* were slightly delayed (non-significantly) after exposure to medium pressure relative to atmospheric pressure under low pH conditions. The most sluggish response occurred after the ambient pH and high-pressure treatment. This indicates that the stress of pressure seems to be a greater effector of slower feeding than low pH; or that sea cucumbers recuperate more quickly (~instantaneously) from the latter. For the escape response, delays were more uniform across the treatments. Only the ambient pH and high-pressure combination yielded more sluggish responses to the predator. This supports that sea cucumbers are more readily impacted by pressure than by pH and that, somehow, low pH counteracts the negative effects of high pressure. It

should be noted however that this experiment was relatively short (24 h) compared to most ocean acidification studies, and that the effects of ocean acidification on *C. frondosa* seem to be acting over longer scales, e.g. seasonally on gametogenesis (Verkaik et al. 2016a). Incidentally, unlike locomotion, time to anchor was mainly affected by pH rather than pressure. Following exposure to low pH at both pressures (atmospheric and medium) time to anchor took three times longer than under ambient pH. In the sea cucumber *Stichopus moebii*, the combination of elevated pressure (50 MPa) and low pH (6.8-7.0) inhibited volume changes during ATP reactions that are associated with low mobility, whereas higher pH (8.0) was found to activate reaction rates (Guthe 1969). It is possible that low pH reduced tube feet activity required for successful anchorage, which is among the basic descriptors of health in *C. frondosa* (Gianasi et al. 2015). In post-recovery trials, time to anchor had reverted back to pre-trial values, suggesting a non-permanent effect. Interestingly, those individuals subjected to low pH conditions and medium pressure were slimy in texture due to mucus production. Echinoderms are known to secrete mucus as a means of defense and protection evoking a stress response (Lawrence 1987; Nance 1981). Different levels of pressure or pH did not affect the cloacal movement and there was also no variation between pre-trial, post-trial and post-recovery values. Generally the behaviours of *C. frondosa* were more readily affected by pressure than acidity, but the variability of the responses warrants further investigation.

Overall our findings reveal that pressure and acidity have a complex effect on the behavioural responses of echinoderms. Because echinoderms can be long-lived, in the order of decades and even centuries (Ebert and Southon 2003), it can be predicted that they will experience significant climate-driven changes within their lifetime.

Furthermore, as shallow-water areas around the continental shelf are experiencing more rapid changes (increasing temperature and acidity) vertical movements of subtidal communities to more stable deeper-water regions can be expected (Brown and Thatje 2014). Therefore it is important to assess the ability of shallow-water species to colonize and thrive in deeper waters characterized by high pressure. Given the growing threat of ocean acidification, it would be advisable (but not necessarily easily achievable) for future investigations to test combined exposure for longer durations to determine whether the trends intensify over time.

3.6 Acknowledgements

We would like to thank the Ocean Sciences Centre Field Services (Memorial University) for the animal collections. Also we would like to extend our thanks to G. Nash and S. Hills for technical help with the IPOCAMP systems. Thanks to E. Montgomery, C. Parzanini and M. Osse for their invaluable support. This research was supported by grants from the Natural Science and Engineering Research Council (NSERC), the Canadian Foundation for Innovation (CFI) and the Research and Development Corporation (RDC) of Newfoundland and Labrador to A. Mercier.

3.7 References

Appelhans Y, Thomsen J, Pansch C, Melzner F, Wahl M (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid–base status of *Asterias rubens* and *Carcinus maenas*. Marine Ecology Progress Series 459: 85-98

Appelhans YS, Thomsen J, Opitz S, Pansch C, Melzner F, Wahl M (2014) Juvenile sea stars exposed to acidification decrease feeding and growth with no acclimation potential. Marine Ecology Progress Series 509: 227-239

Aquino-Souza R, Hawkins S, Tyler P (2008) Early development and larval survival of *Psammechinus miliaris* under deep-sea temperature and pressure conditions. Journal of the Marine Biological Association of the UK 88(3): 453-461

Balny C, Mozhaev VV, Lange R (1997) Hydrostatic pressure and proteins: basic concepts and new data. Comparative Biochemistry and Physiology Part A: Physiology 116(4): 299-304

Barry JP, Buck KR, Lovera CF, Kuhnz L, Whaling PJ, Peltzer ET, Walz P, Brewer PG (2004) Effects of direct ocean CO_2 injection on deep-sea meiofauna. Journal of Oceanography 60(4): 759-766

Bowser-Riley F (1984) Mechanistic studies on the high pressure neurological syndrome. Philosophical Transactions of the Royal Society of London B: Biological Sciences 304(1118): 31-41

Brierley AS and Kingsford MJ (2009) Impacts of climate change on marine organisms and ecosystems. Current Biology 19(14): 602-614

Brodie B, Mowbray F, Power D DFO Newfoundland and Labrador region ecosystem trawl surveys. Version 1 in OBIS Canada digital collections. Bedford Institute of Oceanography, Dartmouth, NS, Canada. Published by OBIS, digital http://www.iobis.org/. Accessed on 2017-07-01

Brooke SD and Young CM (2009) Where do the embryos of *Riftia pachyptila* develop? Pressure tolerances, temperature tolerances, and buoyancy during prolonged embryonic dispersal. Deep Sea Research Part II: Topical Studies in Oceanography 56(19): 1599-1606

Brown A and Thatje S (2014) Explaining bathymetric diversity patterns in marine benthic invertebrates and demersal fishes: physiological contributions to adaptation of life at depth. Biological Reviews 89(2): 406-426

Byrne M and Przeslawski R (2013) Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. Integrative and Comparative Biology 53(4): 582-596

Carney RS (2005) Zonation of deep biota on continental margins. Oceanography and Marine Biology: An Annual Review 43: 211–227

Cavey MJ and Märkel K (1994) Echinoidea. Microscopic Anatomy of Invertebrates 14: 345-400

Childress JJ (1995) Are there physiological and biochemical adaptations of metabolism in deep-sea animals? Trends in Ecology & Evolution 10(1): 30-36

Company J and Sardà F (1998) Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. Deep Sea Research Part I: Oceanographic Research Papers 45(11): 1861-1880

Cottin D, Brown A, Oliphant A, Mestre NC, Ravaux J, Shillito B, Thatje S (2012) Sustained hydrostatic pressure tolerance of the shallow water shrimp *Palaemonetes varians* at different temperatures: insights into the colonisation of the deep sea. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 162(4): 357-363

DFO (2009) State of the ocean: Physical oceanographic conditions in the Newfoundland and Labrador region DFO (Canadian Science Advisory Secretariat Science Advisory Report 2009/057).

Distel DL, Baco AR, Chuang E, Morrill W, Cavanaugh C, Smith CR (2000) Marine ecology: do mussels take wooden steps to deep-sea vents? Nature 403(6771): 725-726

Dixon DR, Dixon LR, Shillito B, Gwynn JP (2002) Background and induced levels of DNA damage in pacific deep-sea vent polychaetes: the case for avoidance. Cahiers De Biologie Marine 43(3/4): 333-336

Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N (2012) Climate change impacts on marine ecosystems. Marine Science 4; 45-53

Doyle WL and McNiell GF (1964) The fine structure of the respiratory tree in *Cucumaria*. Quarterly Journal of Microscopical Science 3(69): 7-11

Ebert TA and Southon JR (2003) Red sea urchins (*Strongylocentrotus franciscanus*) can live over 100 years: confirmation with A-bomb ^{14}carbon. Fishery Bulletin National Oceanic and Atmospheric Administration 101(4): 915-922

Ellers O and Telford M (1992) Causes and consequences of fluctuating coelomic pressure in sea urchins. Biological Bulletin 182(3): 424-434

Feder HM (1963) Gastropod defensive responses and their effectiveness in reducing predation by starfishes. Ecology 44(3): 505-512

Feder HM (1972) Escape responses in marine invertebrates. Scientific America 227: 92-100

Ferguson JC (1992) The function of the madreporite in body fluid volume maintenance by an intertidal starfish, *Pisaster ochraceus*. Biological Bulletin 183(3): 482-489

Gage JD and Tyler PA. (1991) Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press

Gardiner SL and Rieger RM (1980) Rudimentary cilia in muscle cells of annelids and echinoderms. Cell and Tissue Research 213(2): 247-252

Gianasi BL, Verkaik K, Hamel J-F, Mercier A (2015) Novel use of PIT tags in sea cucumbers: promising results with the commercial species *Cucumaria frondosa*. PloS One 10(5): e0127884

Graham MH, Kinlan BP, Druehl LD, Garske LE, Banks S (2007) Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. Proceedings of the National Academy of Sciences 104(42): 16576-16580

Guthe KF (1969) Hydrostatic pressure effects on rabbit and echinoderm myosin ATPase. Archives of Biochemistry and Biophysics 132(1): 294-298

Harley CD, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJ, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. Ecology Letters 9(2): 228-241

Hessler RR (1974) The structure of deep benthic communities from central oceanic waters. The Biology of the Oceanic Pacific 23: 79-93

Hochachka P and Somero G (1984) Temperature adaptation. Biochemical Adaptation 45: 355-449

Hoegh-Guldberg O and Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328(5985): 1523-1528

IPCC (2007) The fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso J (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology 19(6): 1884-1896

Lawrence JM. (1987) Functional biology of echinoderms. Croom Helm, London

LeClair EE (1993) Effects of anatomy and environment on the relative preservability of asteroids: a biomechanical comparison. Palaios (8): 233-243

Macdonald A (1997) Hydrostatic pressure as an environmental factor in life processes. Comparative Biochemistry and Physiology Part A: Physiology 116(4): 291-297

Macdonald A and Gilchrist I (1978) Further studies on the pressure tolerance of deep-sea crustacea, with observations using a new high-pressure trap. Marine Biology 45(1): 9-21

Macdonald AG (1984) The effects of pressure on the molecular structure and physiological functions of cell membranes. Philosophical Transactions of the Royal Society of London B: Biological Sciences 304(1118): 47-68

Macdonald AG (1972) The role of high hydrostatic pressure in the physiology of marine animals. Symposia of the Society for Experimental Biology 26: 209-231

Meidel S and Scheibling RE (1999) Effects of food type and ration on reproductive maturation and growth of the sea urchin *Strongylocentrotus droebachiensis*. Marine Biology 134(1): 155-166

Mickel TJ and Childress J (1982a) Effects of pressure and pressure acclimation on activity and oxygen consumption in the bathypelagic mysid *Gnathophausia ingens*. Deep Sea Research Part A Oceanographic Research Papers 29(11): 1293-1301

Mickel TJ and Childress JJ (1982b) Effects of pressure and temperature on the EKG and heart rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). The Biological Bulletin 162(1): 70-82

Miyake H, Kitada M, Tsuchida S, Okuyama Y, Nakamura K (2007) Ecological aspects of hydrothermal vent animals in captivity at atmospheric pressure. Marine Ecology 28(1): 86-92

Miyake H, Lindsay DJ, Kitada M, Nemoto S, Miwa T, Itoh T. (2012) How to keep deep-sea animals. INTECH Open Access Publisher, Japan

Morris J, Thatje S, Ravaux J, Shillito B, Fernando D, Hauton C (2015) Acute combined pressure and temperature exposures on a shallow-water crustacean: novel insights into the stress response and high pressure neurological syndrome. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 181: 9-17

Nance JM (1981) Respiratory water flow and production of mucus in the cushion star, *Pteraster tesselatus* ives (Echinodermata: Asteroidea). Journal of Experimental Marine Biology and Ecology 50(1): 21-31

New P, Brown A, Oliphant A, Burchell P, Smith A, Thatje S (2014) The effects of temperature and pressure acclimation on the temperature and pressure tolerance of the shallow-water shrimp *Palaemonetes varians*. Marine Biology 161(3): 697-709

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. Journal of Experimental Biology 214(7): 1109-1117

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. Science 308(5730): 1912-1915

Pradillon F and Gaill F (2007) Pressure and life: Some biological strategies. Reviews in Environmental Science and Bio/Technology 6(1-3): 181-195

Pradillon F, Shillito B, Chervin J, Hamel G, Gaill F (2004) Pressure vessels for *in vivo* studies of deep-sea fauna. High Pressure Research 24(2): 237-246

Ravaux J, Cottin D, Chertemps T, Hamel G, Shillito B (2009) Hydrothermal shrimps display low expression of heat-inducible hsp70 gene in nature. Marine Ecology Progress Series 396: 153-156

Ravaux J, Gaill F, Le Bris N, Sarradin PM, Jollivet D, Shillito B (2003) Heat-shock response and temperature resistance in the deep-sea vent shrimp *Rimicaris exoculata*. Journal of Experimental Biology 206(14): 2345-2354

Robinson NJ, Thatje S, Osseforth C (2009) Heartbeat sensors under pressure: A new method for assessing hyperbaric physiology. High Pressure Research 29(3): 422-430

Rochette R, Hamel J, Himmelman JH (1994) Foraging strategy of the asteroid *Leptasterias polaris*: role of prey odors, current and feeding status. Marine Ecology Progress Series 106: 93-93

Schlieper C (1968) High pressure effects on marine invertebrates and fishes. Marine Biology 2(1): 5-12

Shillito B, Bris NL, Gaill F, Rees J, Zal F (2004) First access to live alvinellas. High Pressure Research 24(1): 169-172

Shillito B, Ravaux J, Sarrazin J, Zbinden M, Sarradin P, Barthelemy D (2015) Long-term maintenance and public exhibition of deep-sea Hydrothermal fauna: the AbyssBox project. Deep Sea Research Part II: Topical Studies in Oceanography 121: 137-145

Shillito B, Jollivet D, Sarradin P, Rodier P, Lallier F, Desbruyères D, Gaill F (2001) Temperature resistance of *Hesiolyra bergi*, a polychaetous annelid living on deep-sea vent smoker walls. Marine Ecology Progress Series 216: 141-149

Shillito B, Le Bris N, Hourdez S, Ravaux J, Cottin D, Caprais JC, Jollivet D, Gaill F (2006) Temperature resistance studies on the deep-sea vent shrimp *Mirocaris fortunata*. Journal of Experimental Biology 209(5): 945-955

Siebenaller J and Somero G (1989) Biochemical adaptation to the deep sea. CRC Critical Reviews of Aquatic Sciences 1: 1-25

Siikavuopio SI, Mortensen A, Dale T, Foss A (2007) Effects of carbon dioxide exposure on feed intake and gonad growth in green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 266(1): 97-101

Smithsonian Institution (1974) Department of Invertebrate Zoology, Research and Collections Information System, NMNH, Smithsonian Institution. See: http://www.mnh.si.edu/rc/db/collection_db_policy1.html. Accessed on 2017-07-01.

So J, Hamel J-F, Mercier A (2010) Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. Fisheries Management and Ecology 17(6): 473-484

Somero G (1992) Biochemical ecology of deep-sea animals. Experientia 48(6): 537-543

Stumpp M, Dupont S, Thorndyke M, Melzner F (2011) CO_2 induced seawater acidification impacts sea urchin larval development II: gene expression patterns in pluteus larvae. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 160(3): 320-330

Swezey RR and Somero GN (1985) Pressure effects on actin self-assembly: interspecific differences in the equilibrium and kinetics of the G to F transformation. Biochemistry 24(4): 852-860

Takahashi K, Kubo T, Arai Y, Kitajima I, Takigawa M, Imanishi J, Hirasawa Y (1998) Hydrostatic pressure induces expression of interleukin 6 and tumour necrosis factor alpha mRNAs in a chondrocyte-like cell line. Annals of the Rheumatic Diseases 57(4): 231-236

Taylor J, Lovera C, Whaling P, Buck K, Pane E, Barry J (2014) Physiological effects of environmental acidification in the deep-sea urchin *Strongylocentrotus fragilis*. Biogeosciences 11(5): 1413-1423

Thatje S and Robinson N (2011) Specific dynamic action affects the hydrostatic pressure tolerance of the shallow-water spider crab *Maja brachydactyla*. Naturwissenschaften 98(4): 299-313

Thatje S, Casburn L, Calcagno JA (2010) Behavioural and respiratory response of the shallow-water hermit crab *Pagurus cuanensis* to hydrostatic pressure and temperature. Journal of Experimental Marine Biology and Ecology 390(1): 22-30

Thistle D (2003) The deep-sea floor: An overview. Ecosystems of the World 34: 5-38

Tokuda G, Yamada A, Nakano K, Arita N, Yamasaki H (2006) Occurrence and recent long-distance dispersal of deep-sea hydrothermal vent shrimps. Biology Letters 2(2): 257-260

Tyler P and Dixon D (2000) Temperature/pressure tolerance of the first larval stage of *Mirocaris fortunata* from lucky strike hydrothermal vent field. Journal of the Marine Biological Association of the UK 80(4): 739-740

Tyler P and Young C (1998) Temperature and pressure tolerances in dispersal stages of the genus *Echinus* (Echinodermata: Echinoidea): prerequisites for deep-sea invasion and speciation. Deep Sea Research Part II: Topical Studies in Oceanography 45(1): 253-277

Tyler PA, Young CM, Clarke A (2000) Temperature and pressure tolerances of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri* (Echinodermata: Echinoidea): Potential for deep-sea invasion from high latitudes. Marine Ecology Progress Series 192: 173-180

VandenSpiegel D and Jangoux M (1987) Cuvierian tubules of the holothuroid *Holothuria forskali* (Echinodermata): a morphofunctional study. Marine Biology 96(2): 263-275

Verkaik K, Hamel J-F, Mercier A (2016a) Carry-over effects of ocean acidification in a cold-water lecithotrophic holothuroid. Marine Ecology Progress Series 557: 189-206

Verkaik K, Hamel J-F, Mercier A (2016b) Impact of ocean acidification on reproductive output in the deep-sea annelid *Ophryotrocha sp.* (Polychaeta: Dorvilleidae). Deep Sea Research Part II: Topical Studies in Oceanography

Villalobos FB, Tyler PA, Young CM (2006) Temperature and pressure tolerance of embryos and larvae of the Atlantic seastars *Asterias rubens* and *Marthasterias glacialis* (Echinodermata: Asteroidea): Potential for deep-sea invasion. Marine Ecology Progress Series 314: 109-117

Wilcock S, Wann K, Macdonald A (1978) The motor activity of *Crangon crangon* subjected to high hydrostatic pressure. Marine Biology 45(1): 1-7

WoRMS Editorial Board (2014) World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ. Accessed 2017-07-01

Yoshiki T, Yamanoha B, Kikuchi T, Shimizu A, Toda T (2008) Hydrostatic pressure-induced apoptosis on nauplii of *Calanus sinicus*. Marine Biology 156(2): 97-106

Young C, Tyler P, Fenaux L (1997) Potential for deep sea invasion by Mediterranean shallow water echinoids: pressure and temperature as stage-specific dispersal barriers. Marine Ecology Progress Series 154: 197-209

Young CM and Tyler PA (1993) Embryos of the deep-sea echinoid *Echinus affinis* require high pressure for development. Limnology and Oceanography 38(1): 178-181

Young CM, Vázquez E, Metaxas A, Tyler PA (1996) Embryology of vestimentiferan tube worms from deep-sea methane/sulphide seeps. Nature 381(6582): 514-516

3.8 Tables

Table 3-1 Bathymetric ranges (m) of focal species (rounded to the nearest hundred; e.g. 0.1 = 0 MPa = atmospheric pressure) with corresponding pressure tested. Note that for all three focal species the maximum depth of occurrence is based on only one record that met the search criteria described in the methods. The references used came from studies that conducted multi-species surveys by reputable institutions.

Species	Known depth range (m)	Pressure tested (MPa)
Strongylocentrotus droebachiensis	0-1200	Atmospheric (0) Medium (6) High (24)
Leptasterias polaris	0-1100	Atmospheric (0) Medium (5) High (22)
Cucumaria frondosa	0-1300	Atmospheric (0) Medium (6.5) High (26)

Table 3-2: Summary of experiments, including trial conditions and response variables measured.

Trial duration (h)	Species	Pressure condition (MPa)	pH [a]	Response variables	Time of response measurement[b]
24	*Strongylocentrotus droebachiensis*	Atmospheric (0) Medium (6) High (24)	Ambient Low	Feeding indices Righting movement Weight	During/Post-trial Post-trial Post-trial Pre-trial, Post-trial Post-trial
72		Atmospheric (0) High (25)	Ambient	Righting movement Survival (%)	Pre-trial, Post-trial, Post-recovery Post-recovery
24	*Leptasterias polaris*	Atmospheric (0) Medium (5) High (22)	Ambient Low	Feeding indices Righting movement Survival (%) Behaviour Weight	During/Post-trial Pre-trial, Post-trial, Post-recovery Post-recovery During, Post-trial Pre-trial, Post-trial
72		Atmospheric (0) High (25)	Ambient	Righting movement Survival (%)	Pre-trial, Post-trial, Post-recovery Post-recovery
216		Atmospheric (0) High (22)	Ambient	Feeding indices Righting movement Survival (%) Behavioural observations Weight	During/Post-recovery Pre-trial, Post-trial, Post-recovery Post-recovery During, Post-trial Pre-trial
24	*Cucumaria frondosa*	Atmospheric (0) Medium (6.5) High (26)	Ambient Low	Anchorage time Cloacal openings Predator escape response Time to feed Survival (%) Weight	Pre-trial, Post-trial, Post-recovery Pre-trial, Post-trial, Post-recovery Post-trial Post-trial Post-recovery Pre-trial
72		Atmospheric (0) High (25)	Ambient	Anchorage time Predator-escape Cloacal openings Survival (%)	Pre-trial, Post-trial, Post-recovery Post-trial Pre-trial, Post-trial, Post-recovery Post-recovery

[a] Low pH experiments tested at medium and atmospheric pressures.
[b] Time of measurement: Pre-trial (activity prior to pressure exposure), During (while being exposed to pressure), Post-trial (immediately after pressure exposure), Post-recovery (7-d after pressure exposure).

Table 3-3: Summary of results from 24-h, 72-h and 216-h experiments under ambient pH conditions, showing whether they are consistent with tolerance or acclimation to pressure by three different species. Combinations that were not tested are indicated with NT.

Species / metrics	**Tolerance**[a]		**Acclimation**[b]	
	Within natural depth range 24 h	**Beyond natural depth range 24 h**	**Beyond natural depth range**	
			72 h	**216 h**
S. droebachiensis				
• Feeding	Yes	No	NT	
• Righting to 90°	No	No	No	
• Righting to 180°	Yes	No	No	
• Recovery righting to 90°	Yes	NT	Yes	
• Recovery righting to 180°	Yes	NT	No	
• Weight	Yes	Yes	No	
• Coelomic fluid	Yes	Yes	NT	
L. polaris				
• Feeding	Yes	Yes	NT	NT
• Righting to 90°	Yes	No	No	No
• Righting to 180°	Yes	No	No	No
• Recovery righting to 90°	Yes	Yes	Yes	No
• Recovery righting to 180°	Yes	Yes	Yes	No
• Weight	No	Yes	No	NT
C. frondosa				
• Time to feed	No	No	Yes	
• Escape	Yes	Yes	Yes	
• Post-trial time to anchor	Yes	No	Yes	
• Post-trial cloacal respiration	Yes	No	No	
• Recovery time to anchor	Yes	Yes	Yes	
• Recovery cloacal respiration	Yes	Yes	Yes	

[a] Exposure to pressure treatments for 24 h under ambient pH conditions; individuals were considered tolerant if responses were not significantly different between experimental treatment and control treatment at atmospheric pressure.

[b] Exposure for 72 or 216 h under ambient pH conditions; individuals were considered to acclimate if responses were not significantly different from those obtained previously after 24 h.

Table 3-4: Summary of results from 24-h exposure to atmospheric and medium pressure (within natural depth range) under either acidified or ambient pH conditions, showing whether they are consistent with tolerance to pressure alone, acidification alone, and pressure-acidification combination.

Species / metrics	**Tolerance**[a]			
	Atmospheric pressure		**Medium pressure**	
	Ambient pH	**Acidified pH**	**Ambient pH**	**Acidified pH**
S. droebachiensis				
• Feeding	Yes	Yes	Yes	No
• Righting to 90°	Yes	Yes	Yes	Yes
• Righting to 180°	Yes	Yes	Yes	Yes
• Weight	Yes	Yes	Yes	Yes
• Coelomic fluid	Yes	Yes	Yes	Yes
L. polaris				
• Feeding	Yes	No	Yes	Yes
• Righting to 90°	Yes	Yes	Yes	Yes
• Righting to 180°	Yes	Yes	Yes	Yes
• Recovery righting to 90°	Yes	Yes	Yes	Yes
• Recovery righting to 180°	Yes	Yes	Yes	Yes
• Weight	Yes	Yes	No	Yes
C. frondosa				
• Time to feed	Yes	Yes	Yes	Yes
• Escape	Yes	Yes	No	Yes
• Post-trial time to anchor	Yes	No	Yes	Yes
• Post-trial cloacal respiration	Yes	Yes	Yes	Yes
• Recovery time to anchor	Yes	Yes	Yes	Yes
• Recovery cloacal respiration	Yes	Yes	Yes	Yes

[a] Exposure to pressure treatments for 24 h under ambient or acidified pH conditions; individuals were considered tolerant if the responses were not significantly different between experimental treatment and control treatment at atmospheric pressure under ambient pH.

Table 3-5: Survival rates (%; post-trial and post-recovery) associated with the 24-h, 72-h and 216-h pressure exposure trials under all pH conditions. Variable that were not tested are indicated with NT.

Trial duration (h)	Species	Pressure condition (MPa)	pH	Post-trial survival (%)[a]	Post-recovery survival (%)[b]
24	*Strongylocentrotus droebachiensis*	Atmospheric (0)	Ambient	100	NT
		Medium (6)		100	NT
		High (24)		50	NT
72		Atmospheric (0)		100	100
		High (25)		100	83.3
24	*Leptasterias polaris*	Atmospheric (0)	Ambient	100	100
		Medium (5)		100	100
		High (22)		92	83.3
72		Atmospheric (0)		100	100
		High (25)		100	100
216				100	86
				51.7	0
24	*Cucumaria frondosa*	Atmospheric (0)	Ambient	100	100
		Medium (6.5)		100	100
		High (26)		100	100
72		Atmospheric (0)		100	100
		High (25)		100	83.3
24	*Strongylocentrotus droebachiensis*	Atmospheric (0)	Acidified	100	NT
		Medium (6)		100	NT
	Leptasterias polaris	Atmospheric (0)		100	100
		Medium (5)		100	100
	Cucumaria frondosa	Atmospheric (0)		100	100
		Medium (6.5)		100	100

[a] Survival was measured post-trial (immediately after removal from experimental vessel).
[b] Survival was measured post-recovery (7 days after removal from experimental vessel).

Table 3-6: Proportion of sea stars *L. polaris* found in the various feeding positions after exposure to three pressure levels under either ambient or low pH. Pressure did not affect the final feeding positions of *L. polaris* after 24-h (H=2.47, df=2, p=0.291). In two-way comparisons, there was no significant effect of pressure (F1, 44 =1.02, p=0.751) or pH on the final positions (F1, 44 = 0.28, p=0.598), and no interaction between the factors (F1, 44 =2.54, p=0.118).

Pressure [a]	**pH**	**Proportion of individuals (%)**			
		Arms around mussel; stomach everted	**Arms around mussel; no stomach everted**	**Arms not around mussel; stomach everted**	**Arms not around mussel; no stomach everted**
Atmospheric	Ambient	58.3	0	16.7	25.0
Medium		41.7	8.3	0	50.0
High		8.3	25.0	33.3	33.3
Medium	Acidified	41.7	0	25.0	8.3
High		66.7	0	25.0	33.3

[a] See Table 3-1 for exact pressure values.

Table 3-7: Activity of *L. polaris* within the pressure vessel over a 216-h period at either atmospheric or high pressure (0 and 22 MPa; simultaneous trials). Scores included: feeding (arms wrapped around mussel, stomach everted on the prey), near mussels (no evident foraging or attempted opening of mussels), active (not visible from camera view because individuals were climbing on the sides of the pressure vessel), paralyzed (individual had not changed position for >8 hours). Proportion (%) of individuals displaying a given activity is shown in brackets.

Day	Activity under atmospheric pressure	Activity under high pressure
1	Active (100)	Active (66.7) Near mussel (33.3)
2	Active (66.7) Near mussel (33.3)	Active (33.3) Near mussel (66.7)
3	Active (66.7) Near mussel (33.3)	Active (66.7) Near mussel (33.3)
4	Active (100)	Active (66.7) Paralyzed (33.3)
5	Active (100)	Near mussel (66.7) Paralyzed (33.3)
6	Active (100)	Paralyzed (100)
7	Active (33.3) Near mussel (66.7)	Paralyzed (100)
8	Active (33.3) Near mussel (66.7)	Paralyzed (100)
9	Active (66.7) Near mussel (33.3)	Paralyzed (100)

3.9 Figures

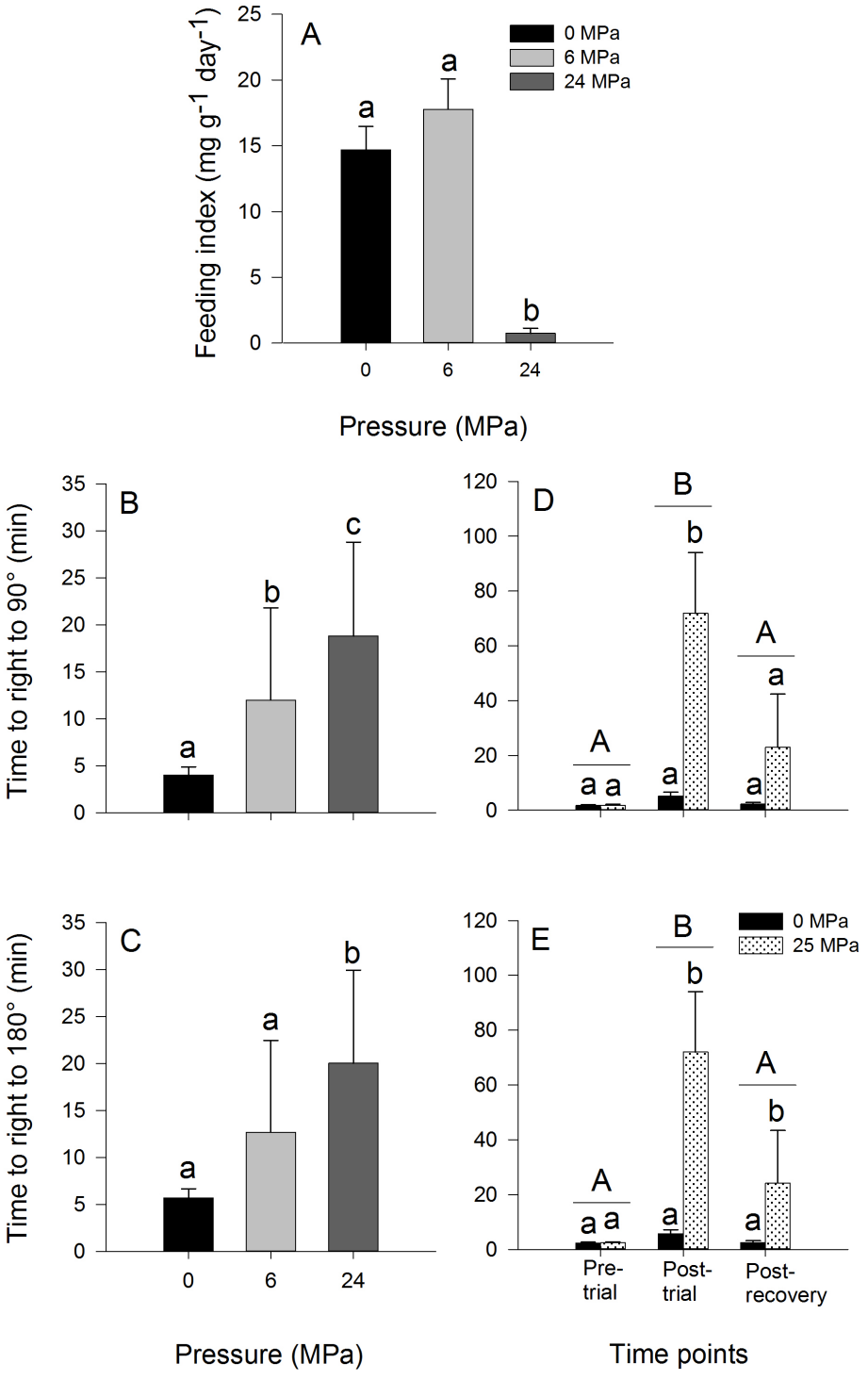
A
Feeding index (mg g-1 day-1)
0 MPa
6 MPa
24 MPa
Pressure (MPa)
B
Time to right to 90° (min)
C
Time to right to 180° (min)
Pressure (MPa)
D
E
0 MPa
25 MPa
Pre-trial
Post-trial
Post-recovery
Time points

Figure 3-1 (previous page): Response of *S. droebachiensis* to pressure. (A) Feeding index (mean ± SE, *n* = 12-24) during 24-h exposure to pressures within and beyond its natural bathymetric distribution. (B) Time to right to 90° (mean ± SE, *n* = 12-24) immediately after the 24-h trial. (C) Time to right to 180° (mean ± SE, *n* = 12-24) immediately after the 24-h trial. (D) Comparison of time to right to 90° (mean ± SE, n=6) pre-trial, post-trial or post-recovery following 72-h exposure. (E) Comparison of time to right to 180° (mean ± SE, n=6) pre-trial, post-trial or post-recovery following 72-h exposure. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b, c) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 (Appendix C) for full statistical results.

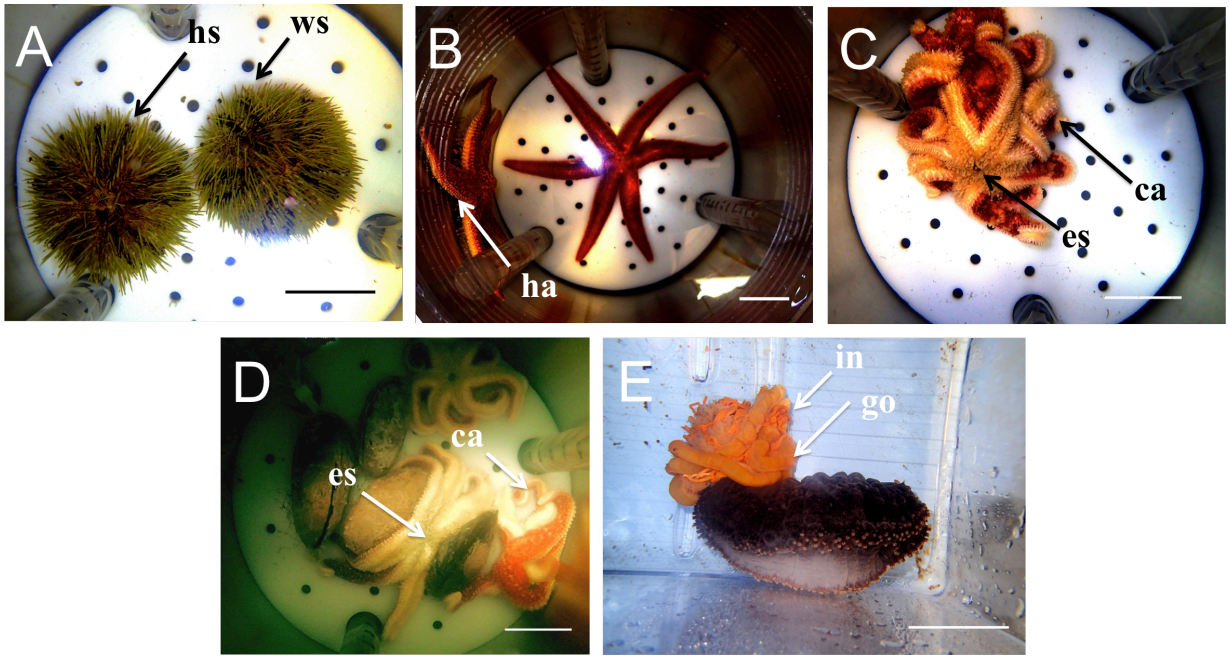

Figure 3-2: Post-trial condition of individuals immediately after pressure exposure for 72 and 216 h. (A) *S. droebachiensis*; individual on the left was exposed to 0 MPa and had healthy spines (hs); individual on the right was exposed to 25 MPa and had weak spines (ws); both individuals were tested in different pressure vessels. (B) *L. polaris*; after exposure to 0 MPa for 72 h, individuals were climbing and had healthy arms (ha) indicative of good health. (C) *L. polaris*; after exposure to 25 MPa for 72 h individuals all had convoluted arms (ca) and everted stomachs (es). (D) *L. polaris* after exposure to 22 MPa for 216 h individuals all had convoluted arms (ca) and everted stomachs (es) from stress. (E) *C. frondosa*; after exposure to 25 MPa this individual was found dead and eviscerated upon removal from the IPOCAMP; gonads (go) and intestines (in) are visible. Scale bars represent 4 cm.

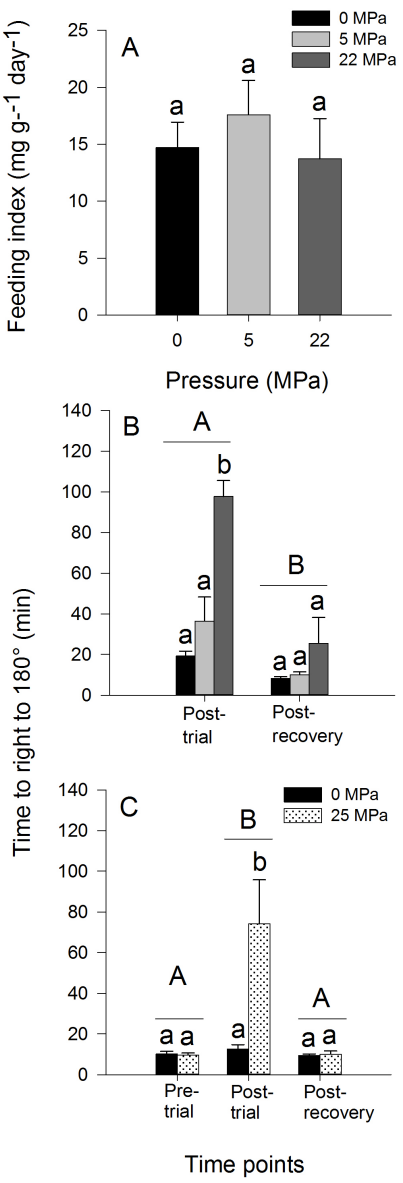

Figure 3-3: Feeding indices and righting of *L. polaris* post-trial and post-recovery following a 24-h exposure to pressures within and beyond its natural bathymetric distribution. (A) Feeding indices (mean ± SE, *n* = 12-24). (B) Time (mean ± SE, *n* = 12-24) required to right itself to 180°. (C) Time (mean ± SE, n=6) required to right itself to 180° pre-trial, post-trial or post-recovery following 72-h exposure to pressure. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results.

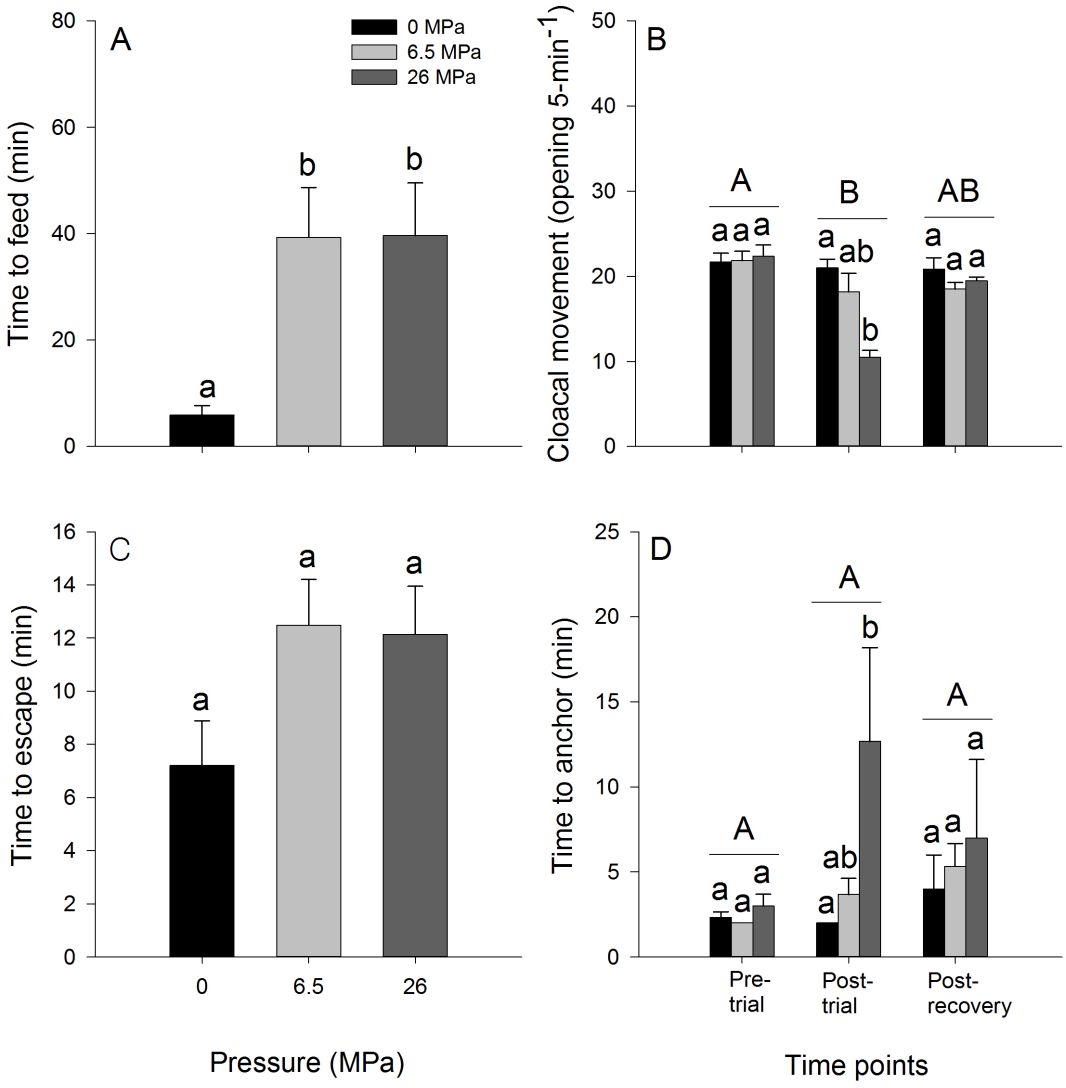

Figure 3-4: Time to feed, cloacal movement rate, time to escape response and time to anchor in *C. frondosa* measured either pre-trial, post-trial or post-recovery following 24-h exposure to pressures within and beyond its natural distribution (mean ± SE, $n = 6$). (A) Time to initiate feeding. (B) Cloacal movements. (C) Time to initiate escape from predator. (D) Time to anchor firmly to substrate. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results.

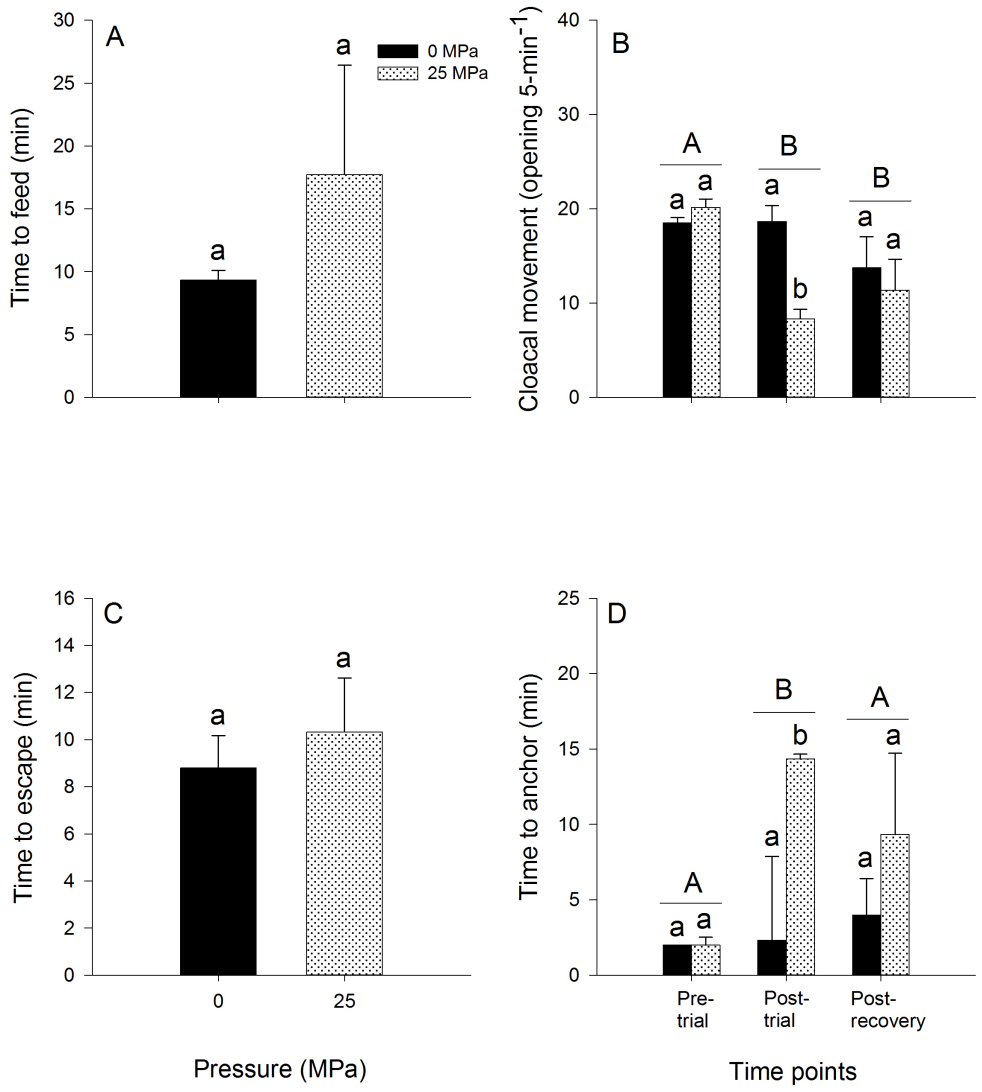

Figure 3-5: Time to feed, cloacal movement rate, time to escape response and time to anchor in *C. frondosa* measured either pre-trial, post-trial or post-recovery following 72-h exposure to pressures within and beyond its natural distribution (mean ± SE, $n = 6$). (A) Time to initiate feeding. (B) Cloacal movements. (C) Time to initiate escape from predator. (D) Time to anchor firmly to substrate. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results.

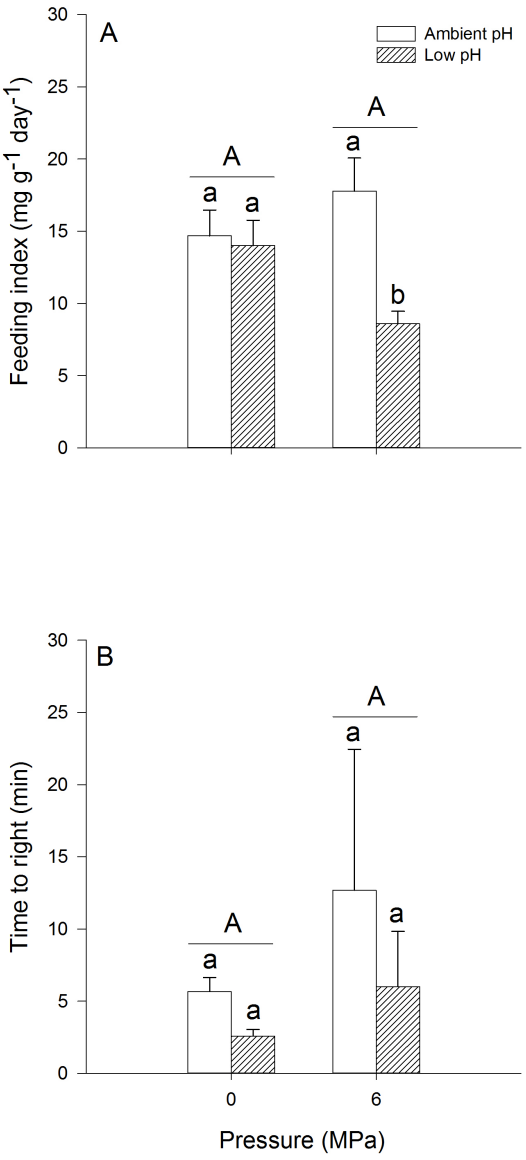

Figure 3-6: Feeding and righting of *S. droebachiensis* following 24-h exposure to ambient or low-pH conditions under pressures within and beyond its natural bathymetric distribution. (A) Feeding indices (mean ± SE, *n* = 12-24). (B) Time (mean ± SE, *n* = 12-24) required to right itself to 180°. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results.

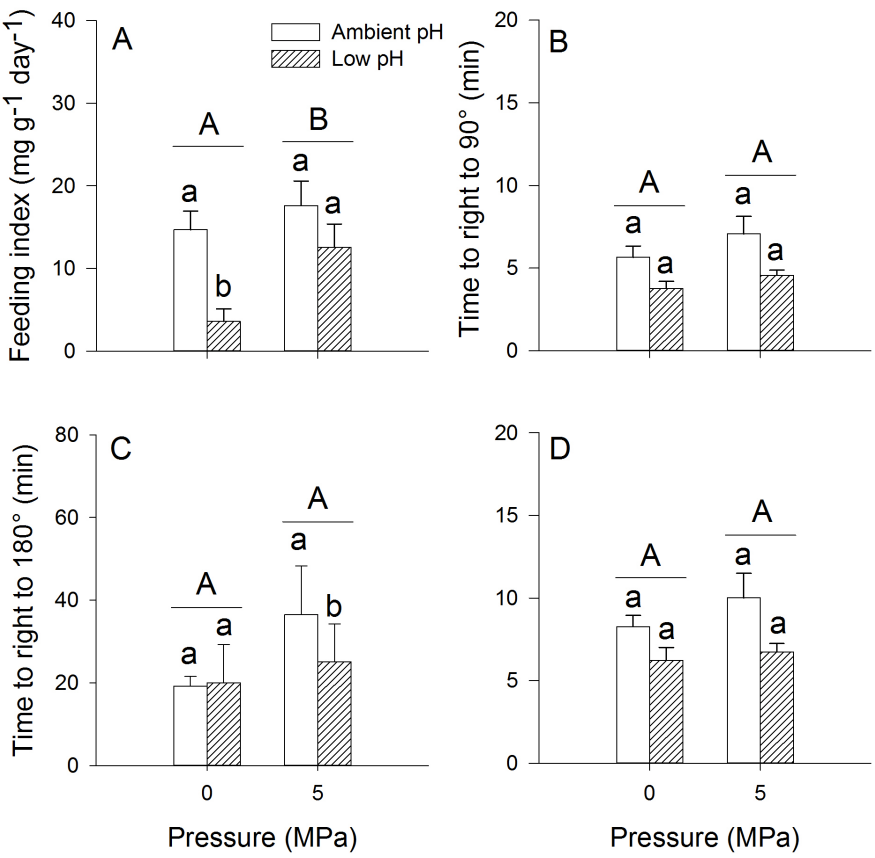

Figure 3-7: Feeding and righting in *L. polaris* following 24-h exposure to ambient and acidified conditions under pressures within its natural bathymetric distribution. (A) Feeding indices (mean ± SE, n = 12-24). Times to right: (B) post-trial to 90° (C) post-trial to 180°, (D) post-recovery to 180°. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results.

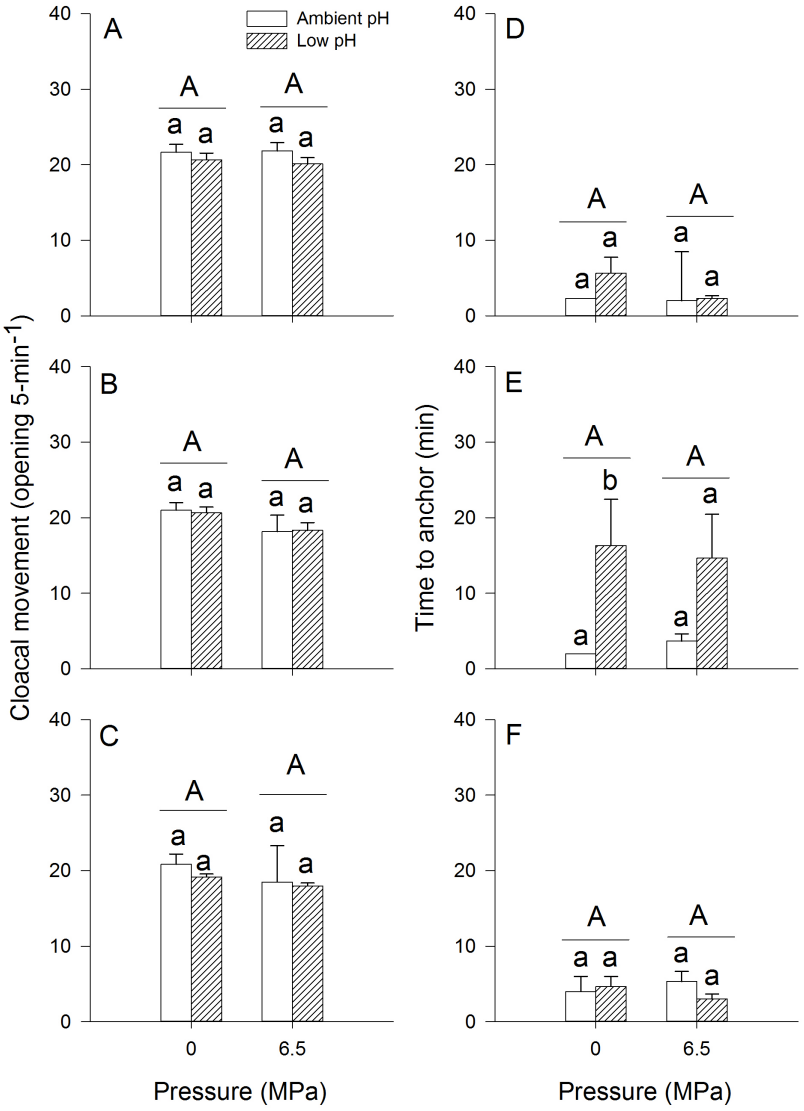

Figure 3-8: Cloacal movement and time to anchor (mean ± SE, n = 6) in *C. frondosa* measured pre-trial, post-trial and post-recovery following 24-h exposure to ambient and acidified conditions under pressures within its natural distribution. Cloacal movements (A) pre-trial, (B) post-trial, and (C) post-recovery. Time to anchor (D) pre-trial, (E) post-trial and (F) post-recovery. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results.

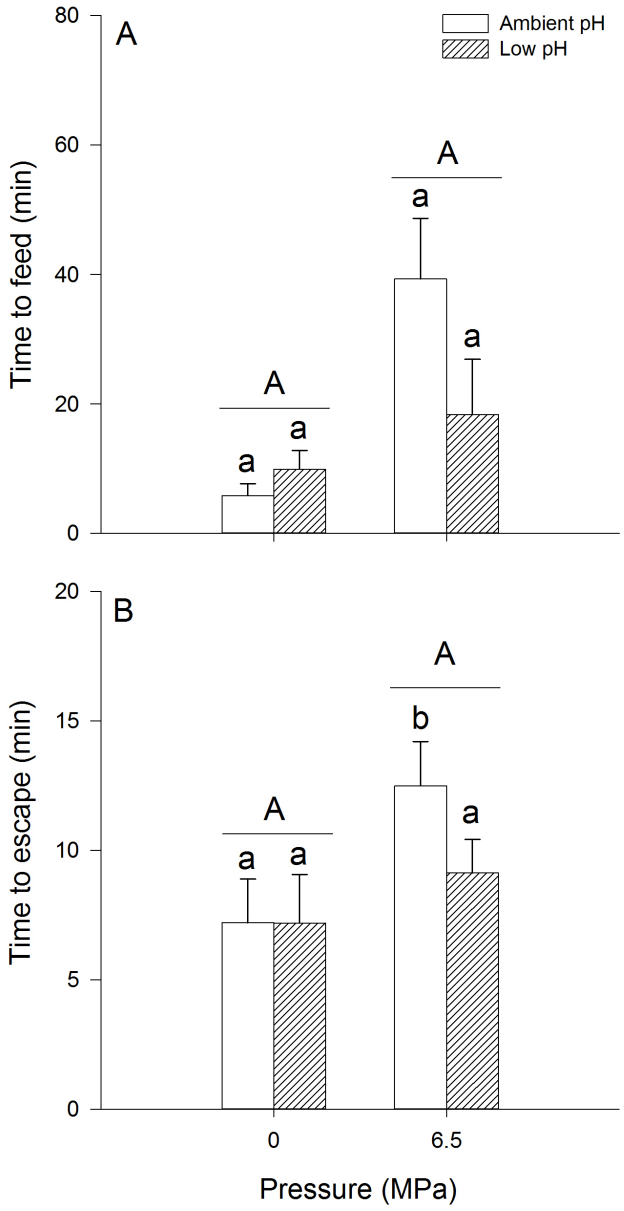

Figure 3-9: Time to initiate feeding and predator escape response (mean ± SE, *n* = 6) in *C. frondosa* following 24-h exposure to ambient and acidified conditions under pressures within its' natural distribution. (A) Time to feed. (B) Time to escape. Means with different letters are significantly different (ANOVA on ranks, $p > 0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments. See Tables C1-C3 for full statistical results.

Chapter 4: General Conclusions

4.1 Thesis summary

This thesis explored the effects of hydrostatic pressure, a critical parameter in the marine environment, and contributed to expand the current knowledge base by: (1) synthesizing and analyzing a large body of observational and experimental results on pressure tolerance (from 1961 to present date) to better understand how shallow-water and deep-sea species from different geographic locations cope with shifts in pressure conditions; and (2) examining the behavioural responses of three focal species of shallow-water echinoderms, including sea urchins (*Stronglyocentrotus droebachiensis*), sea stars (*Leptasterias polaris*) and sea cucumbers (*Cucumaria frondosa*), to various pressure and pH conditions.

In Chapter 2, data from some 134 studies were collated and synthesized to analyze how pressure tolerance differed between various taxa (>260 species) at different life stages (larvae and adults). Datasets were analyzed to determine if pressure tolerance was influenced by: (i) depth stratum of collection, (ii) geographic location of origin, and (iii) phylum. In terms of vertical migration, the results supported the relatively novel parsimony hypothesis, which suggests that species' pressure tolerance enables a bi-directional movement of animals from shallow waters to the deep sea, as well as the inverse. Also species from mid-depths could potentially migrate both ways (to greater and to shallower depths). Interestingly, the findings of the review also showed that deep-sea species generally survived better than shallow-water species when exposed to various non-native pressures. In addition, pressure tolerances were more limited for species originating from tropical locations relative to those from northern latitudes, likely due to confounding effects of thermal stress during ascent from cold depths to warmer surface

waters during collection. Lastly, phylum was a more significant driver of pressure tolerance for adults than for larvae (with the caveat of temporally constrained data on the latter).

In Chapter 3, the experimental results showed that shallow-water echinoderms from the Northwest Atlantic were generally not able to adapt to pressures that were atypical of their natural distribution, irrespective of exposure duration (24-216 h). With respect to motor functions, the time required to anchor or right in all three species increased after exposure to the highest pressures (beyond their known depth ranges). Similarly, during and after 24-h exposures to high-pressure, sea urchins reduced the amount of food they consumed at pressure, and sea cucumbers initiated a feeding response more slowly. By contrast, sea stars ate roughly the same amount of food under high-pressure than at atmospheric pressure. However, the follow-up experiment, testing whether sea stars could still feed under high-pressure conditions for a prolonged period of 216 h (9 days), yielded 100% mortality. Furthermore, complex interactions were found to exist between low pH and hydrostatic pressure (within the depth distributions of the focal species), as behavioural responses were essentially species-specific. For instance, sea urchins and sea stars fed less when low pH was combined to increased pressure, whereas sea cucumbers exhibited a faster feeding response after exposure to pressure and were seemingly not affected by pH. Lastly, motor activity was negatively impacted in sea stars and sea urchins under elevated pressures, while sea cucumbers were chiefly affected by pH.

Overall, the results of these two chapters exemplify that pressure is a complex abiotic parameter that has species-dependent effects at both the behavioural and

ecological scales. The review of the pressure literature has revealed that various traits related to the provenance of experimental species influence their pressure tolerance; these include geographic and bathymetric variables. Brown and Thatje (2014; 2015) had previously reviewed the pressure literature with an aim to assess the relationship between pressure and temperature and to model depth distributions with regards to future abiotic shifts associated with climate change. By contrast, the present study focused on evidence gathered from pressure tolerance and was able to highlight trends that merit further investigation. One significantly novel contribution of this review chapter was in demonstrating and highlighting that a fair number of deep-sea taxa have high barotolerance and are capable of surviving (and even reproducing) at atmospheric pressure. This includes the survival of freshly collected adults (e.g. Colaço et al. 2006; Kádár et al. 2006; Mercier and Hamel 2008; 2009; Miyake et al. 2007; 2012; Sun et al. 2009; 2010) as well as the ability of larvae obtained from parents collected in the deep sea to survive and sometimes to settle at atmospheric pressure (e.g. Epifanio et al. 1999; Hamasaki et al. 2010; Mercier and Hamel 2009; Mercier et al. 2011b). Taking this empirical evidence into account, the synthesis suggests that a shallow to deep sea invasion is unlikely, but that the parsimony hypothesis, and to some the degree the submergence hypothesis, have merit. Interestingly, the conclusion takes into consideration the results of the many studies that supported the deep-sea invasion (e.g. Aquino-Souza et al. 2008; Tyler and Young 1998; Tyler et al. 2000b; Villalobos et al. 2006; Young et al. 1997; Young and Tyler 1993; Young et al. 1996).

In addition to the synthesis of the literature, the experiments conducted in this study have also highlighted the generally limited ability of shallow-water echinoderms

belonging to three classes to cope with increases in pressure, particularly those that are atypical of their natural distributions for more than 24 hours.

4.2 Future directions

It is important to acknowledge that the relatively recent development of flow-through high-pressure chambers over the past few decades, combined with their high cost and rarity, make investigations of pressure tolerance essentially inaccessible to most investigators. Hence, the study of the response of marine organisms to hydrostatic pressure is still in its early stages. It is also essential for researchers to recognize the inherent limitations of simulating pressure in the laboratory and collecting animals from habitats defined by extreme pressure. There is a clear need for standardizing investigative methods, including gradual acclimation of experimental species to pressure without stressing them out (i.e. avoiding sudden pressurization). Similarly, there is also room for improving collection methods for deep-sea animals to minimize trauma from rapid depressurization.

As the field is developing, it is evident that certain species or taxa have been tested more frequently than others. Therefore, general assumptions about the universal ability for shallow-water or deep-sea animals to adapt to pressures atypical of their natural bathymetric distribution, based on the performance of a single species in a study, should not be encouraged (Oliphant et al. 2011). The present study strove to avoid such assumptions, which is why most of the contemporary literature was considered in order to ensure that trends regarding pressure tolerance were consistently observed across multiple studies. In addition to evaluating pressure tolerance in terms of survival length and survival proportion, future studies might also consider evaluating more complex

behaviours that are species-specific and may constitute good indicators of long-term survival. Previous studies have claimed that because early developmental stages of marine invertebrates could survive for a few hours under pressure, these species had the potential to disperse across depths and were potentially capable of colonizing deeper habitats; this despite the fact that settlement was not recorded and basic behaviours like feeding and mobility of adults were not evaluated (e.g. Macdonald 1997; Tyler and Young 1998; Young et al. 1997; Young et al. 1996). The present study showed that feeding and motor functions in adults exposed to non-native pressures (as proxies of an ability to survive and be successful at increased depths) were significantly impacted, albeit over short-term scales that were nevertheless comparable to or longer than those previously used for larval stages. It cannot be excluded, but remains difficult to prove, that slow incremental pressurization (over days or months) might produce different results. Because certain facilities, such as the Océanopolis aquarium in France, now have the capacity to keep tanks at pressure over long periods, slow-adaptation experiments may become possible in the not too distant future (Shillito et al. 2015).

Ideally, studies should integrate a multi-disciplinary approach when assessing pressure tolerance to gain a more holistic picture. For instance, Ravaux et al. (2009) were successful in evaluating the effects of pressure by examining survival, genome changes and behaviour in the shallow-water caridean shrimp *Palamonetes varians*. Future investigations should also aim to gather a more complete ontogenetic picture, whereby investigators test multiple life stages of the same species (i.e. early larval and later juvenile or adult stages). It is also recommended that future research maintain detailed

records of the ability for species at all stages to survive under experimental pressure conditions for longer periods of time to ground-truth barotolerance.

With the growing threat exerted by climate change, it is critical for future pressure studies to evaluate more combinations of pressure, temperature and acidification levels. It is crucial for us to gain a better understanding of how metazoans living at various depths will cope under new temperature and pH regimes. As climate change starts to drive increasingly serious modifications of oceanic conditions, there is an urgency to better understand whether certain species or phyla are capable of migrating vertically and perhaps even access new habitats beyond their current bathymetric range. Identifying potential bathymetric migrators could provide vital information on how marine ecosystems will change in the future.

4.3 References

Aquino-Souza R, Hawkins S, Tyler P (2008) Early development and larval survival of *Psammechinus miliaris* under deep-sea temperature and pressure conditions. Journal of the Marine Biological Association of the UK 88(3): 453-461

Brown A and Thatje S (2015) The effects of changing climate on faunal depth distributions determine winners and losers. Global Change Biology 21(1): 173-180

Brown A and Thatje S (2014) Explaining bathymetric diversity patterns in marine benthic invertebrates and demersal fishes: physiological contributions to adaptation of life at depth. Biological Reviews 89(2): 406-426

Colaço A, Martins I, Laranjo M, Pires L, Leal C, Prieto C, Costa V, Lopes H, Rosa D, Dando P (2006) Annual spawning of the hydrothermal vent mussel, *Bathymodiolus azoricus*, under controlled aquarium, conditions at atmospheric pressure. Journal of Experimental Marine Biology and Ecology 333(2): 166-171

Epifanio C, Perovich G, Dittel A, Cary S (1999) Development and behavior of megalopa larvae and juveniles of the hydrothermal vent crab *Bythograea thermydron*. Marine Ecology Progress Series 185: 147-154

Hamasaki K, Nakajima K, Tsuchida S, Kado R, Kitada S (2010) Number and duration of zoeal stages of the hydrothermal vent crab *Gandalfus yunohana* from laboratory reared specimens. Journal of Crustacean Biology 30(2): 236-240

Kádár E, Lobo-da-Cunha A, Santos RS, Dando P (2006) Spermatogenesis of *Bathymodiolus azoricus* in captivity matching reproductive behaviour at deep-sea hydrothermal vents. Journal of Experimental Marine Biology and Ecology 335(1): 19-26

Macdonald A (1997) Hydrostatic pressure as an environmental factor in life processes. Comparative Biochemistry and Physiology Part A: Physiology 116(4): 291-297

Mercier A and Hamel J-F (2009) Reproductive periodicity and host-specific settlement and growth of a deep-water symbiotic sea anemone. Canadian Journal of Zoology 87(11): 967-980

Mercier A and Hamel J-F (2008) Depth-related shift in life history strategies of a brooding and broadcasting deep-sea Asteroid. Marine Biology 156(2): 205-223

Mercier A, Sun Z, Hamel J-F (2011) Reproductive periodicity, spawning and development of the deep-sea scleractinian coral *Flabellum angulare*. Marine Biology 158(2): 371-380

Miyake H, Kitada M, Tsuchida S, Okuyama Y, Nakamura K (2007) Ecological aspects of hydrothermal vent animals in captivity at atmospheric pressure. Marine Ecology 28(1): 86-92

Miyake H, Lindsay DJ, Kitada M, Nemoto S, Miwa T, Itoh T. (2012) How to keep deep-sea animals. INTECH Open Access Publisher, Japan

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. Journal of Experimental Biology 214(7): 1109-1117

Ravaux J, Cottin D, Chertemps T, Hamel G, Shillito B (2009) Hydrothermal shrimps display low expression of heat-inducible hsp70 gene in nature. Marine Ecology Progress Series 396: 153-156

Shillito B, Ravaux J, Sarrazin J, Zbinden M, Sarradin P, Barthelemy D (2015) Long-term maintenance and public exhibition of deep-sea Hydrothermal fauna: the AbyssBox project. Deep Sea Research Part II: Topical Studies in Oceanography 121:137-145

Sun Z, Hamel J-F, Mercier A (2009) Planulation of deep-sea octocorals in the NW Atlantic. Coral Reefs 28(3): 781

Sun Z, Hamel J-F, Edinger E, Mercier A (2010) Reproductive biology of the deep-sea octocoral *Drifa glomerata* in the Northwest Atlantic. Marine Biology 157(4): 863-873

Tyler P and Young C (1998) Temperature and pressure tolerances in dispersal stages of the genus *Echinus* (Echinodermata: Echinoidea): prerequisites for deep-sea invasion and speciation. Deep Sea Research Part II: Topical Studies in Oceanography 45(1): 253-277

Tyler PA, Young CM, Clarke A (2000) Temperature and pressure tolerances of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri* (Echinodermata: Echinoidea): potential for deep-sea invasion from high latitudes. Marine Ecology Progress Series 192: 173-180

Villalobos FB, Tyler PA, Young CM (2006) Temperature and pressure tolerance of embryos and larvae of the Atlantic seastars *Asterias rubens* and *Marthasterias glacialis* (Echinodermata: Asteroidea): potential for deep-sea invasion. Marine Ecology Progress Series 314: 109-117

Young C, Tyler P, Fenaux L (1997) Potential for deep sea invasion by Mediterranean shallow water echinoids: pressure and temperature as stage-specific dispersal barriers. Marine Ecology Progress Series 154: 197-209

Young CM and Tyler PA (1993) Embryos of the deep-sea echinoid *Echinus affinis* require high pressure for development. Limnology and Oceanography 38(1): 178-181

Young CM, Vázquez E, Metaxas A, Tyler PA (1996) Embryology of vestimentiferan tube worms from deep-sea methane/sulphide seeps. Nature 381(6582): 514-516

APPENDICES

Appendix A: Datasets

Table A1. Shallow-water and deep-sea marine invertebrate species examined for tolerances to various hydrostatic pressures. Pressure was either experimentally applied using absolute (A) or incremental stepwise (I) methods, or corresponds to maintance in naturally fluctuating temperatures (F). The collection depths, ontogenic stage of animals and the experimental duration for exposure to pressure treatements was recorded. The effects of pressure were evaluated by examining the rate of survival (%) under experimental conditions and development (the maximum stage reaced with the percentage of individuals reaching that stage and total amount of time). The minimum adult survival time was recorded to denote how long deep-sea animals survive at ambient pressure (within and beyond the experimental time). The depth range was classified as Intertidal, Subtidal, Bathyal and Abyssal. Habitat types were also recorded. The geographic regions were classified as Antartica, Norwest Atlantic Ocean (NW Atl), Northeast Atlantic Ocean (NE Atl), Northeast Pacific Ocean (NE Pac), Northwest Pacific Ocean (NW Pac), Mediterranean Sea (Med), Tropical Indian Ocean (Trop Ind) Tropical East Pacific Ocean (Trop E Pac), Tropical West Pacific Ocean (Trop W Pac), Caribbean (Cari) and Gulf of Mexico (GOM). The corresponding climate zones used were: Polar, Temperate-cold, Temperate, Temperate-warm and Tropical.

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Annelida													
Shallow-water species													
Poeobius meseres	0	NE Pac		Intertidal			Larvae	0.1 (A)	5 (A)	8-13			Childress and Thuesen, 1993
Pomatoceros lamarcki	0-5	NE Atl	Temperate-warm	Intertidal			Larvae (Trochophore)	0.1 (A)	15 (A)	48	68.8	Trochophore; 68.8; (2)	Vevers et al., 2010
								10.1 (A)			69.1	Trochophore; 69.1; (2)	
								20.3 (A)			41.4	Trochophore; 41.4; (2)	
								30.4 (A)			19.3	Trochophore; 19.3; (2)	
								0.1 (A)		6	100		
								10.1 (A)			80		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Deep-sea species													
Alvinella pompejana	2500	NE Pac		Abyssal	Hydrothermal vent		Adult	0.1 (A)	4 (A)	4	0		Pradillon et al., 2005
							Larvae (Oocytes and spermatozoa)		2 (A)	72	100	Uncleaved; 100; (3)	
									10 (A)		83	16-cell stage and more; 11; (3)	
									14 (A)	63	95	16-cell stage and more; 77; (2.6)	
									20 (A)	48	0	Degrading embryos; 100; (2)	
									27 (A)	24		Degrading embryos; 100; (1)	
								26 (A)	2 (A)	576		Uncleaved; 100; (24)	
									10 (A)	72	100	16-cell stage and more; 55; (3)	
									20 (A)	48	0	Degrading embryos; 100; (2)	
Alvinella pompejana	2500	NE Pac		Abyssal	Hydrothermal vent		Adult	25 (A)	4 (A)	0	100		Ravaux et al., 2013
									4-20 (I)	3	68.4		
									4-20-42 (I)	5	91.6		
									4-20-42-55 (I)	7	0		
Alvinella pompejana	2500	NE Pac		Abyssal	Hydrothermal vent		Larvae (Embryo)	0.1 (A)	2 (A)	72	100	Uncleaved; 100; (3)	Pradillon et al., 2001
									10 (A)		90	Cleaved; 90; (3)	

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									14 (A)		80	Cleaved; 95; (3)	
									20 (A)		0	Uncleaved; 72; (3)	
								25.3 (A)	2 (A)	48	100	Uncleaved; 100; (2)	
									10 (A)			Cleaved; 50; (2)	
									20 (A)		0	Uncleaved; 100; (2)	
Alvinella pompejana	2600	NE Pac		Abyssal	Hydrothermal vent		Adult	26 (A)	15 (A)	20	36		Shillito et al., 2004
Hesiolyra bergi	2600	NE Pac		Abyssal	Hydrothermal vent		Adult	26 (A)	15 (A)	6	100		Shillito et al., 2001
										18			
									15-50 (I)		0		
									15-39 (I)		20		
										48	100		
Lamellibrachia luymesi	700	GOM		Bathyal	Hydrocarbon seep	15	Juvenile	0.1 (A)	6 (A)	600			Pflugfelder et al., 2009
Neopolynoe acanellae	466-1405	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		100		Hamel et al., 2015
Ophryotrocha sp.	500-1500	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-4 (F)		50	Juveniles; 80; to adult size (F1, F2, F3 generations)	Mercier et al., 2014
Paralvinella grasslei	2600	NE Pac		Abyssal	Hydrothermal vent		Adult	26 (A)	15 (A)	6			Cottin et al., 2008
										9	100		
									15-31.7 (I)	6	83		
									15 (A)	8.5	70		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									15-32.3 (I)				
									15 (A)	8	84		
									15-31.4 (I)				
									15 (A)	12	100		
Paralvinella grasslei	2600	NE Pac		Abyssal	Hydrothermal vent	0.75	Adult	0.1 (A)	15 (A)	6	100		Dixon et al., 2002
										18			
								26.3 (A)		6			
										18			
Paralvinella palmiformis	1800	NW Pac		Bathyal	Hydrothermal vent		Adult	17.9 (A)	10-60-10 (I)	10	0		Lee, 2003
Paralvinella palmiformis	1800	NW Pac		Bathyal	Hydrothermal vent	7	Adult	0.1 (A)	-	10	0		Lee, pers. comm.
Paralvinella sulfincola	1800	NW Pac		Bathyal	Hydrothermal vent		Adult	17.9 (A)	10-60-10 (I)	10	0		Lee, 2003
Paralvinella sulfincola	1800	NW Pac		Bathyal	Hydrothermal vent	7	Adult	0.1 (A)	-	10	0		Lee, pers. comm.
Paralvinella pandorae irlandei	2585	NW Pac		Abyssal	Hydrothermal vent		Adult	26 (A)	10 (A)	43	100		Boutet et al., 2009
									20 (A)				
Poeobius meseres	1000	NE Pac		Bathyal			Larvae	10.1 (A)	5 (A)	8-13			Childress and Thuesen, 1993
Riftia pachyptila	2500	NE Pac		Abyssal	Hydrothermal vent		Larvae	24.1 (A)	2 (A)	80	72*		Brooke and Young, 2009
									5 (A)		52*		
									10 (A)		0*		
								17.2 (A)	2 (A)		36*		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									5 (A)		0*		
									10 (A)				
								10.3 (A)	2 (A)				
									5 (A)				
									10 (A)				
								3.6 (A)	2 (A)				
									5 (A)				
									10 (A)				
Riftia pachyptila	2500	NE Pac		Abyssal	Hydrothermal vent		Larvae (Oocytes spermatozoa)	0.1 (A)	2 (A)	2.5		Fertilization; 90; (0.1)	Marsh et al., 2001
						34		25.3 (A)		816			
Riftia pachyptila	2500	NE Pac		Abyssal	Hydrothermal vent	0.5	Juvenile	0.1 (A)	8 (A)	1			Pflugfelder et al., 2009
										6			
								20.3 (A)		1			
										6			
Riftia pachyptila	2600	NE Pac		Abyssal	Hydrothermal vent		Adult	0.1 (A)	8 (A)	3	0**		Childress et al., 1984
						45		27.4 (A)		1080	100**		
								10 (A)					
Riftia pachyptila	2600	NE Pac		Abyssal	Hydrothermal vent	5	Adult	23.1 (A)	8 (A)	72	100		Gaill et al., 1997
										120	50		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Riftia pachyptila	2600	NE Pac		Abyssal			Adult	12.2 (A)	8 (A)	24	100		Childress et al., 1991
										72			
										120			
Arthopoda													
Shallow-water species													
Artemia franciscana	0				Lab culture		Larvae (Embryo)	0.1 (A)	25 (A)	3	80*****		Daiki et al., 2009
							Larvae (Anhydrobiotic embryo)	1200 (A)		0.33	86*****		
							Larvae (Hydrated embryo)				0*****		
Artemia franciscana	0				Lab culture		Larvae (First-stage nauplii)	0.1-60 (I)	24 (A)	3.33	100****		Seo et al., 2013
									4 (A)				
									24 (A)				
									4 (A)				
								20 (A)	8 (A)	1.7	91.6****		
								40 (A)		2.45	66****		
								60 (A)		3.17	0****		
Artemia franciscana	0				Lab culture		Larvae (First-stage nauplii)	0.1-60 (I)	24 (A)	3.33	100****		Seo et al., 2013

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									4 (A)				
									24 (A)				
									4 (A)				
								20 (A)	8 (A)	1.7	91.6****		
								40 (A)		2.45	66****		
								60 (A)		3.17	0****		
Balanus amphitrite	0				Lab culture		Larvae (Cyprid)	0.1 (A)	25 (A)	48	100		Kon-ya and Miki, 1994
								5 (A)					
								10 (A)					
								20 (A)					
								40 (A)			0		
Calanus finmarchius	0	NW Atl	Temperate-cold	Intertidal			Larvae	55.1 (A)	10 (A)	1	0		George and Marum, 1974
Calanus sinicus	0-199	NW Pac	Temperate	Intertidal, Subtidal, Bathyal		24	Larvae (Unhatched egg)	0.1 (A)	15 (A)	24	77********		Yoshiki et al., 2006
								1 (A)			60********		
								5.1 (A)			39********		
								10.1 (A)			17********		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Calanus sinicus	0-199	NW Pac	Temperate	Intertidal, Subtidal, Bathyal		24	Larvae (1 cell stage)	10.1 (A)	14 (A)	24	81********		Yoshiki et al., 2008
							Larvae (2 cell stage)				95********		
							Larvae (4 cell stage)				99********		
							Larvae (8 cell stage)				95********		
							Larvae (16 cell stage)				93********		
							Larvae (Blastula)				78********		
							Larvae (Limb-bud)				100*********		
							Larvae (1 cell stage)	10.1 (I)			91********		
							Larvae (2 cell stage)				90********		
							Larvae (4 cell stage)				88********		
							Larvae (8 cell stage)				100*********		
							Larvae (16 cell stage)				95********		
							Larvae (Blastula)				89********		
							Larvae (Limb-bud)				90********		
Crangon crangon	0-10	NE Atl	Temperate	Intertidal, Subtidal			Adult	23.3 (A)	25 (A)	1	50 ******		Schlieper, 1972
Crangon crangon	0-10	NE Atl	Temperate	Intertidal, Subtidal			Adult	0.1 (A)	8 (A)	8	100		Wilcock et al., 1978
								1 (A)					
								3 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								4.1 (A)					
								5.1 (A)					
								6.1 (A)					
								10.1 (A)			0		
								12.2 (A)					
Lithodes maja	60	NE Atl	Temperate-cold	Subtidal			Larvae (Zoea I)	0.1 (A)	6 (A)	4	100		Munro et al., 2015
								5 (A)					
								10 (A)					
								15 (A)					
								20 (A)					
								25 (A)					
								30 (A)					
							Larvae (Megalopa)	0.1 (A)					
								5 (A)					
								10 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								15 (A)					
								20 (A)					
								25 (A)					
								30 (A)					
							Larvae (Crab I Stage)	0.1 (A)					
								5 (A)					
								10 (A)					
Maja brachydactyla	4-12	NE Atl		Intertidal, Subtidal	Salt marsh		Juvenile	0.1-5.1-10.1-15.2 (I)	20 (A)	3.75	100		Robinson et al., 2009
								0.1 (A)					
Maja brachydactyla	4-12	NE Atl	Temperate	Intertidal, Subtidal	Salt marsh		Adult	0.1 (A)	20 (A)	90	100		Thatje and Robinson, 2011
								10.1 (A)					
								15.2 (A)					
Meganyctiphanes norvegica	0	NW Atl	Temperate-cold	Intertidal			Larvae	41.4 (A)	10 (A)	1	0		George and Marum, 1974
Pachygrapsus crassipes	0-15	Trop E Pac	Tropical	Intertidal, Subtidal			Adult	4.7 (A)	2 (A)	0.27	100		Menzies and Wilson, 1961
								5.4 (A)		0.32			

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								6 (A)		0.3			
								7.5 (A)		0.4	80		
								7.7 (A)		0.42	100		
								8.6 (A)		0.53			
								9 (A)		0.45	60		
								9.2 (A)		0.53	0		
								10.5 (A)		0.58			
								10.7 (A)		0.62			
								12.3 (A)		0.68			
								13.8 (A)		0.77			
								14.8 (A)		0.83			
								15.2 (A)		0.88			
								16.7 (A)		0.97			
								18.3 (A)		1.05			
								19.9 (A)		1.13			

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								21.5 (A)		1.25			
								10.4 (A)		4.43	33		
								22.3 (A)		5.78	0		
								35 (A)		5.95			
Pagurus cuanensis	0-10	NE Atl	Temperate	Intertidal, Subtidal	Salt marsh		Adult	2 (A)	5 (A)	1	100		Thatje et al., 2010
									10 (A)				
									15 (A)				
									20 (A)				
								5.1 (A)	5 (A)				
									10 (A)				
									15 (A)				
									20 (A)				
								10.1 (A)	5 (A)				
									10 (A)				
									15 (A)				

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									20 (A)				
								0.1-10.1-0.1 (I)	5 (A)	2.5			
									10 (A)				
									15 (A)				
									20 (A)				
Palaemonetes varians	0-10	NE Atl	Temperate	Intertidal, Subtidal	Salt marsh		Adult	0.1 (A)	10-28 (I)	10	100		Cottin et al., 2010
									10 (A)	7			
Palaemonetes varians	0-10	NE Atl	Temperate	Intertidal, Subtidal	Salt marsh	28	Adult	0.1 (A)	10 (A)	6	100		Cottin et al., 2012
								5 (A)					
								10 (A)					
								15 (A)					
								0.1 (A)	5 (A)	168			
								10 (A)					
								0.1 (A)	10 (A)				
								10 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								0.1 (A)	27 (A)		87		
								10 (A)			63		
									10 (A)	672	70		
Palaemonetes varians	0-10	NE Atl	Temperate	Intertidal, Subtidal	Salt marsh		Juvenile	0.1 (A)	5 (A)	2	100		Morris et al., 2015
									10 (A)				
									15 (A)				
									20 (A)				
								5 (A)	5 (A)				
									10 (A)				
									15 (A)				
									20 (A)				
								10 (A)	5 (A)				
									10 (A)				
									15 (A)				
									20 (A)				

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Palaemonetes varians	0-10	NE Atl	Temperate	Intertidal, Subtidal	Salt marsh		Adult	0.1-30 (I)	5 (A)	5	100***		Oliphant et al., 2011
									10 (A)				
									20 (A)		73.3***		
									30 (A)		56.7***		
								0.1 (A)	5 (A)	1.5	-		
									10 (A)	1			
									20 (A)	0.75			
									30 (A)	0.5			
								5 (A)	5 (A)	1.5			
									10 (A)	1			
									20 (A)	0.75			
									30 (A)	0.5			
								10 (A)	5 (A)	1.5			
									10 (A)	1			
									20 (A)	0.75			

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									30 (A)	0.5			
								15 (A)	5 (A)	1.5			
									10 (A)	1			
									20 (A)	0.75			
									30 (A)	0.5			
								20 (A)	5 (A)	1.5			
									10 (A)	1			
									20 (A)	0.75			
									30 (A)	0.5			
								25 (A)	5 (A)	1.5			
									10 (A)	1			
									20 (A)	0.75			
									30 (A)	0.5			
								30 (A)	5 (A)	1.5			
									10 (A)	1			

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									20 (A)	0.75			
									30 (A)	0.5			
Palaemonetes varians	0-10	NE Atl	Temperate	Intertidal, Subtidal			Adult	0.1 (A)	4 (A)	1.3	100		Smith et al., 2013
									8 (A)	1			
									16 (A)	0.83			
									21 (A)	0.67			
									25 (A)	0.5			
Parathemisto sp.	0	NW Atl	Temperate-cold	Intertidal			Larvae	65.5 (A)	10 (A)	1	0		George and Marum, 1974
Pontella sp.								34.5 (A)					
Sapphirina ovatolanceolata								41.4 (A)			40		
Vibilia sp.								38.6 (A)			0		
Parathemisto sp.								65.5 (A)					
								6.1 (A)			100		
Deep-sea species													
Acanthephyra eximia	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Abyssorchomene abyssorum	4050	Trop Ind		Abyssal			Adult	0.1 (A)	1.8 (A)	0	0		Treude et al., 2002
	4420												
Abyssorchomene distincta						21					100		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Agononida incerta	300	Trop W Pac		Bathyal		31	Adult	0.1 (A)	11 (A)	744			Konishi and Saito, 2000
							Larvae						
Alvinocaris sp.	1157	NW Pac		Bathyal		63	Adult	11.5-0.1 (I)	4.5 (A)	1512	100		Koyama et al., 2005b
						30		0.1 (A)		720			
						74	Larvae			1200	80		
										1776	0		
Amphipod sp. 1 (unknown species)	1100	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Amphipod sp. 2 (unknown species)	1100	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		100		Present study
Amphipod spp.	5900	NE Pac		Abyssal			Adult	60.1-0.1-60.1 (I)	2 (A)	2.9	100		Yayanos, 1981
Austinograea yunohana	450	NW Pac		Bathyal	Hydrothermal vent	365	Adult	0.1 (A)	10 (A)	8760	81.1		Miyake et al., 2007
									4 (A)		28.6		
									12 (A)		0	Unhatched 100	
									18.5 (A)		100	Hatched 100	
Aristeus antennatus	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Ashinkailepas seepiophilia	1300	NW Pac		Bathyal	Hydrothermal vent		Adult	0.1 (A)	4 (A)		100		Miyake et al., 2007
Barnacles (unknown species)	1400	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present Study

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Bythograea thermydron	2500	NE Pac		Abyssal	Hydrothermal vent	0.04	Adult	28-62-28 (I)	5 (A)	160	0		Airiess and Childress, 1994
						1			10 (A)				
						2		28-62-28 (I)	20 (A)		100		
Bythograea thermydron	2500	Trop E Pac		Abyssal	Hydrothermal vent	5	Adult	0.1 (A)	2 (A)	120	0		Mickel and Childress, 1982a
									7 (A)				
									10 (A)				
									12 (A)				
						21		12.2 (A)	2 (A)	504	100		
						548		24.1 (A)	5 (A)	13152			
Bythograea thermydron	2500	Trop E Pac		Abyssal	Hydrothermal vent	0.5	Adult	0.1 (A)	2 (A)	3-12	100		Mickel and Childress, 1982b
									8 (A)				
									12 (A)				
								13.8 (A)	2 (A)				
									8 (A)				
								27.6 (A)	2 (A)				
									8 (A)				

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									12 (A)				
									18 (A)				
									25 (A)				
Bythograea thermydron	2500	NE Pac		Abyssal	Hydrothermal vent	3.8	Adult	0.1 (A)	13 (A)	15	91		Martinez et al. 2001
										24	65		
Bythograea thermydron	2500	NE Pac		Abyssal	Hydrothermal vent	2	Adult	25 (A)	8 (A)	48			Toullec et al., 2007
								0.1 (A)					
										27			
										15			
										3			
										0			
Bythograea thermydron	2500-2600	NE Pac		Abyssal	Hydrothermal vent	201	Larvae (Megalopae)	0.1 (A)		4824	0	Stage 4 Juvenile; 1.4; (33)	Epifanio et al., 1999
						200	Juvenile			4800		Third Moult; 0.07; (199)	
Cancer macropthalmus	500	Trop W Pac			Reef	30-120	Adult	0.1 (A)	5 (A)	720, 2880			Wilson et al., 2013
Chaceon affinis	820-950	NE Atl		Bathyal		12	Adult	0.1 (A)	10 (A)	288			Mestre et al., 2015

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
									10-35 (I)	5	0		
								10 (A)		7			
Chaceon quinquedens	860	GOM		Bathyal		730	Adult	0.1 (A)	6 (A)	17520	100		Biesiot and Perry, 1995
						270	Larvae			6480	-		
	1043					730	Adult			17520	100		
						270	Larvae			6480	-		
Chorocaris chacei	1700	NE Atl		Bathyal	Hydrothermal vent	1	Adult	17 (A)	10 (A)	24	100**********		Shillito et al., 2006
Candacia ethiopica	800-1000	Cari		Bathyal			Larvae	27.5 (A)		1	0		George and Marum, 1974
Chionoecetes fenneri	300-700	GOM		Bathyal		547.5	Adult	0.1 (A)	5 (A)	13140	95		Henry et al., 1990
Chionoecetes tanneri	950-1050	NE Pac		Bathyal	Canyon	21	Adult	0.1 (A)	3.5 (A)	504	100		Pane and Barry, 2007
Chionoecetes quinquedens	300-700	GOM		Bathyal		180	Adult	0.1 (A)	5 (A)	4320	95		Henry et al., 1990
Chirostylidae	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	5 (A)	720, 2880			Wilson et al., 2013
Colossendeis sp.	1350-1450	NE Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		50		Present Study
Eurythenes grillus	4000-4325	NE Atl		Abyssal			Adult	0.1 (A)	5 (A)	6	0		Macdonald and Gilchrist, 1980
Eurythenes gryllus	3950	Trop Ind		Abyssal		9	Adult	0.1 (A)	1.8 (A)	0	100		Treude et al., 2002
						6							

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
											0		
Euphausia sp.	800-1000	Cari		Bathyal			Larvae	41.4 (A)		1	0		George and Marum, 1974
Gandalfus yunohana	445	NW Pac		Bathyal	Hydrothermal vent	66	Adult	0.1 (A)		1584			Hamasaki et al., 2010
						134			15 (A)	3216			
						60	Larvae (Zoea I)		17.9 (A)	300		Zoea I; 100; (12.5)	
									21.3 (A)	376.8		Zoea II; 10; (15.7)	
									24.2 (A)	816		Zoea V; 3.3; (34)	
									21 (A)	804		Zoea III; 6.7; (33.5)	
									24 (A)	1440		Zoea VI Metaphorsized; 3.3; (60)	
									27 (A)	1152		Zoea VI Metaphorsized 10 (48)	
									30 (A)	948		Zoea VI Metaphorsized 3.3 (39.5)	
Geryon longipes	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Gnathophausia gracilis	200	Trop E Pac		Bathyal		9.2	Adult	0.1-10.4 (I)	4.5 (A)	220	100		Quetin and Childress, 1980
Gnathophausia ingens						10.4		0.1-7.8-0.1 (I)	5.5 (A)	250			
						24		7.8 (A)		576	60		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Gnathophausia ingens	400-900	NE Pac		Bathyal		45	Adult	0.1 (A)	5.5 (A)	1080	100		Mickel and Childress, 1982c
										4-6			
								7.6 (A)					
								15.2 (A)					
								22.8 (A)					
								33.4 (A)					
						30		7.8 (A)		720			
								0.1 (A)		4-6			
								7.6 (A)					
								15.2 (A)					
								22.8 (A)			66.7		
								33.4 (A)			50		
Gnathophausia ingens	400-900	NE Pac		Bathyal		183	Adult	0.1 (A)		18300	100		Childress, personal observation
Gnathophausia ingens	200	Trop E Pac		Bathyal		365	Adult	0.1 (A)	6.5 (A)	8760			Childress, 1971
Goneplax rhomboides	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Gooseneck barnacle	1200	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Hermit crab sp. 1 (unknown species)	950					60-730				1440, 17520			
Heterocarpus ensifer	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	5 (A)	720, 2880			Wilson et al., 2013
Heterocarpus laevigatus	500												
Homeryon asper	1000												
Homola barbata	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Isopoda sp.	1150	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Liocarcinus depurator	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Lithodes longispina	1000	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	5 (A)	720, 2880			Wilson et al., 2013
Lycaea sp.	800-1000	Cari		Bathyal			Larvae	41.4 (A)		1	0		George and Marum, 1974
Macropipus tuberculatus	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Mirocaris fortunata	1700	NE Atl		Bathyal	Hydrothermal vent		Larvae (First zoeal stage)	0.1 (A)	10 (A)	20	100		Tyler and Dixon, 2000
								15.2 (A)					
								25.3 (A)					
								30.4 (A)			0		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								0.1 (A)	20 (A)				
								15.2 (A)			100		
								25.3 (A)					
								30.4 (A)			33.3		
Mirocaris fortunata	1700	NE Atl		Bathyal	Hydrothermal vent	343	Adult	0.1 (A)	7 (A)	1920	37		Shillito et al., 2015
Mirocaris fortunata	1700	NE Atl		Bathyal	Hydrothermal vent	91	Adult	0.1 (A)	24 (A)	2184	100		Matabos et al., 2015
								18 (A)	10-25 (A)				
Mirocaris fortunate	1617	NE Atl		Bathyal	Hydrothermal vent	450	Adult	0.1 (A)	8 (A)	10800			Smith et al., 2013
								0.1 (A)	4 (A)	1.33	100		
									8 (A)	1			
									16 (A)	0.83			
									21 (A)	0.67			
									25 (A)	0.5			
									10-25 (A)	5.5			
Mirocaris fortunata	850	NE Atl		Bathyal	Hydrothermal vent	9	Adult	0.1 (A)	21 (A)	216	70**********		Shillito et al., 2006
									16 (A)				
									10 (A)				
									25 (A)	144	80**********		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
	1700								10 (A)	1	100**********		
								17 (A)		7			
								0.1 (A) X		24	86**********		
								17 (A)		20.75	65**********		
	2300			Abyssal				0.1 (A)		36	50**********		
Monodaeus couchi	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100**********		Company and Sarda, 1998
Munida intermedia	200-1250												
Munida sp. 1	250	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	5 (A)	720, 2880			Wilson et al., 2013
Munida sp. 2	500												
Munida sp. 3	1000												
Munida striola	300	Trop W Pac		Bathyal		31	Adult	0.1 (A)	11 (A)	744			Konishi and Saito, 2000
Munida striola						-	Larvae			-			
Munida tenuimana	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Neocalanus cristatus	1000-1500	NW Pac		Bathyal			Larvae	0.1 (A)	4 (A)	24	51		Yoshiki et al., 2011
								1 (A)			37		
								5.1 (A)			69		
								10 (A)			49		
Neocalanus flemingeri	1000-1500	NW Pac		Bathyal			Larvae	0.1 (A)		24	50		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								1 (A)			37		
								5.1 (A)			69		
								10 (A)			49		
Neocalanus plumchrus	1000-1500	NW Pac		Bathyal			Larvae	0.1 (A)	2 (A)	24	79		
								1 (A)			79		
								5.1 (A)			65		
								10 (A)			78		
Neoverruca sp	1300	NW Pac		Bathyal	Hydrothermal vents			0.1 (A)	4 (A)		100		Miyake et al., 2007
Neoverruca sp.	1340	NW Pac		Bathyal	Hydrothermal vents	183	Larvae (N1)	0.1 (A)	4 (A)	72	100		Watanabe et al., 2004
							Larvae (N2)			384	100		
							Larvae (N3)			336			
							Larvae (N4)			384	97		
							Larvae (N5)			648			
							Larvae (N6)			2040	56		
Nephrops norvegicus	200-1250	NW Pac		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Nymphon hirtipes	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	5-8 (F)		100	Juvenile; 100; 390	Mercier et al., 2015
Opaepele spp.	1400	NW Pac		Bathyal	Hydrothermal vent	365	Adult	0.1 (A)	12 (A)	365	100		Miyake et al., 2007
	1500												
						365	Larvae	0.1 (A)	25 (A)	140	81**********		
							Adult	8.5 (A)	10-40 (I)	20	0**********		
						1097		0.1 (A)	7 (A)	1920	27		
						731					6		
Paguridae	250	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013
Palinurus mauritanicus	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Paralicella aff. alberti	4050	Trop Ind		Abyssal			Adult	0.1 (A)	1.8 (A)	0	0		Treude et al., 2002
Paralicella caperesca	1908			Bathyal	Seamount	26				624	100		
Paralicella caparesca	4000-4325	NE Atl		Abyssal			Adult	0.1 (A)	5 (A)	6	0		Macdonald and Gilchrist, 1980
Paralicella spp.	4050	Trop Ind		Abyssal			Adult	0.1 (A)	1.8 (A)	0	0		Treude et al., 2002
Paralicella spp.	4420												
Paramola japonica	250	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Parapenaeus longirostris	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Plesionika acanthonotus													
Plesionika edwardsi													
Plesionka giglolii													
Plesionka heterocarpus													
Plesionka martia													
Plesionika sp.	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	5 (A)	720, 2880			Wilson et al., 2013
Polycheles typhlops	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Polycheles typhlops	652-658	Med		Bathyal		7	Adult	0.1 (A)		168	0		Guerao and Abello, 1996
						5	Larvae			120			
Pontophilus norvegicus	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Pontella scicurifer	800-1000	Cari		Bathyal			Larvae	27.5 (A)		1	0		George and Marum, 1974
Processa canaliculata	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Rimicaris exoculata	2300	NE Atl		Abyssal	Hydrothermal vent	0.4	Adult	23 (A)	10 (A)	10	92		Cottin et al., 2010
									10-30 (I)		88		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Rimicaris exoculata	2300	NE Atl		Abyssal	Hydrothermal vent		Adult	23 (A)	15 (A)	4			Ravaux et al., 2003
										24	100		
						2				48			
									15-45 (I)	8	0		
										24	100		
Rimicaris exoculata	2320	NE Atl		Abyssal	Hydrothermal vent		Adult	23 (A)	15 (A)	4			Ponsard et al., 2013
										10			
										6			
										1			
Rimicaris exoculata	3650	NE Atl		Abyssal	Hydrothermal vent	3	Adult	30 (A)		8	100		Durand et al., 2010
										22			
										72			
Scyllarus aurora	250	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013
Segonzacia mesatlantica	1700	NE Atl		Bathyal	Hydrothermal vent	30	Adult	0.1 (A)	7 (A)	720	5.3		Shillito et al., 2015
											37.5		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Segonzacia mesatlantica	1700	NE Atl		Bathyal	Hydrothermal vent	30	Adult	0.1 (A)	24 (A)	2184	0		Matabos et al., 2015
						1095		18 (A)	10-25 (A)	26280			
Shinkaia crosnieri	1001	NW Pac		Bathyal	Hydrothermal vent	0.05	Adult	0.1 (A)	5 (A)	1.17	100		Watsuji et al., 2014
								12 (A)					
Shrimp sp. 1 (unknown species)	650-950	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		82		Present Study
Shrimp sp. 2 (unknown species)	500-700	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		50		Present Study
Shrimp sp. 3 (unknown species)	950-1250	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		50		Present Study
Shrimp sp. 4 (unknown species)	800-1400	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		50		Present Study
Solenorcera membranacea	200-1250	Med		Bathyal		1.5	Adult	0.1 (A)	13 (A)	12-36	100		Company and Sarda, 1998
Shinkaia crosnieri	1400	NW Pac		Bathyal	Hydrothermal vent	365	Adult	0.1 (A)	4 (A)	365	100		Miyake et al., 2007
	1500												
	1400						Larvae						
	1500												
Stephonyx biscayensis	1528, 1765	NE Atl		Bathyal	Canyon	60	Adult	0.1 (A)	1 (A)	0.17	100		Brown and Thatje, 2011
								5 (A)					
								10 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								15 (A)					
								20 (A)					
								25 (A)					
								30 (A)					
								0.1 (A)	3 (A)				
								5 (A)					
								10 (A)					
								15 (A)					
								20 (A)					
								25 (A)					
								30 (A)					
								0.1 (A)	5.5 (A)				
								5 (A)					
								10 (A)					
								15 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								20 (A)					
								25 (A)					
								30 (A)					
								0.1 (A)	10 (A)				
								5 (A)					
								10 (A)					
								15 (A)					
								20 (A)					
								25 (A)					
								30 (A)					
Stereomastis sculpta	1000	Trop W Pac		Bathyal	Reef	72	Adult	0.1 (A)		1728			Drazen, pers. comm.
Undinula vulgaris	800-1000	Cari		Bathyal			Larvae	20.7 (A)		1	50		George and Marum, 1974
Shallow-water and deep-sea species													
Gaussia princeps	0-1000	NE Pac	Temperate	Intertidal, Subtidal, Bathyal			Adult	0.1-18.3-0.1 (I)	10 (A)	0.67	100		Childress et al., 1976
									7 (A)				
									3.5 (A)				
Neocalanus cristatus	0-1300	NW Pac	Temperate-cold	Intertidal, Subtidal, Bathyal		115	Adult	0.1 (A)	2 (A)	2760	100		Saito and Tsuda, 2000

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
							Larvae (Egg)			-	-		
									4 (A)	105.6	81	CI (53)	
									6 (A)			CI (42)	
Neocalanus plumchrus	0-1300	NW Pac	Temperate-cold	Intertidal, Subtidal, Bathyal		6-35	Adult	0.1 (A)	2 (A)			CI (35)	
						60	Larvae (Egg)			100			
									4 (A)	105.6	89	NIII (60)	
Neocalanus yemingeri	0-1300	NW Pac	Temperate-cold	Intertidal, Subtidal, Bathyal		6-35	Adult		2 (A)				
						60	Larvae (Egg)			100.8	93		
									4 (A)				
Brachiopoda													
Deep-sea species													
Terebratulina sp.	1200-1300	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Chaetognatha													
Shallow-water and deep-sea species													
Eukrohnia fowleri	0-1000	NE Pac	Temperate	Intertidal, Subtidal, Bathyal		0.54	Larvae	0.1 (A)	5 (A)	8-13			Childress and Thuesen, 1993
Eukrohnia fowleri								10.1 (A)					
Pseudosagitta maxima								0.1 (A)					
Pseudosagitta maxima								10.1 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Solidosagitta zetesios								0.1 (A)					
Solidosagitta zetesios								10.1 (A)					
Chordata													
Shallow-water species													
Salpa fusiformis	0	NW Atl	Temperate-cold	Intertidal			Larvae	48.3 (A)	10 (A)	1	0		George and Marum, 1974
Urophycis sp.								27.6 (A)					
Deep-sea species													
Conger myriaster	1162				Lab culture	5	Adult	0.1 (A)	25 (A)	0.33	100		Koyama et al., 2005a
								20 (A)					
								40 (A)					
								70 (A)			95		
								80 (A)			70		
								100 (A)			55		
								130 (A)			0		
Eptatretus deani	1200	Trop W Pac		Bathyal	Reef	365	Adult	0.1 (A)		8760			Drazen, pers. comm.
Eptatretus stouti													
Megalodicopia hians	280-329	NE Pac		Bathyal	Canyon	220	Adult	0.1 (A)	4.4 (A)	5280	0		Havenhand et al., 2006
						180				4320			

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
						750				18000			
						210	Larvae			5040			
Simenchelys parasiticus	1162				Lab culture	5	Adult	0.1 (A)		120	100		Koyama et al., 2005a
								40 (A)	15 (A)	0.33			
								100 (A)					
								150 (A)					
								200 (A)			0		
Symphurus sp.	450	NW Pac		Bathyal	Hydrothermal vent	1	Larvae	0.1 (A)	26 (A)	7	100	Hatched; 100; (1)	Miyake et al., 2007
						3			20 (A)			Hatched; 100; (3)	
						14			12 (A)			Hatched; 100; (14)	
Symphurus sp.	450	NW Pac		Bathyal	Hydrothermal vent	365	Adult	0.1 (A)	4 (A)	8760			Miyake, pers. comm.
Cnidaria													
Shallow-water species													
Aegina citrea	0	NE Pac	Temperate-cold	Intertidal		0.54	Larvae	0.1 (A)	5 (A)	8-13			Childress and Thuesen, 1993
Crossota rufobrunnea													
Pelagia cyanella	0	Cari	Tropical	Intertidal			Larvae	62 (A)	10 (A)	1	25		George and Marum, 1974

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Porites astreoides	1-4	Cari	Tropical	Intertidal			Larvae (Planulae)	0.1-0.5-0.1 (I)	26 (A)	1.09	100		Stake and Sammarco, 2003
Deep-water species													
Aegina citrea	1000	NE Pac		Bathyal		0.54	Larvae	10.1 (A)	5 (A)	8-13			Childress and Thuesen, 1993
Actinostola callosa	650-1350	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		40		Present Study
Acanella arbuscula	700-1450			Bathyal		>730	Adult	0.1 (A)	1-8 (F)				Present study
Allantactis parasitica	725-1100	NW Atl		Bathyal		>730	Adult	0.1 (A)	-1-8 (F)		50	Juvenile; 5; 630 d	Mercier and Hamel 2009
Anthomastus ritteri	300	NE Pac		Bathyal	Canyon	496	Adult	0.1 (A)	6 (A)	11904			Cordes et al., 2001
						310				7440			
	450					496	Larvae			11904			
						310				7440			
Anthoptilum grandiflorum	650-1450	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Bolecera tuediae	750-1350	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		40		Present study
Crossota rufobrunnea	1000	NE Pac		Bathyal		0.54	Larvae	10.1 (A)	5 (A)	8-13			Childress and Thuesen, 1993
Desmophyllum dianthus	2100	NW Atl		Abyssal		60-730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Drifa sp.	360-1260	NW Atl		Bathyal		>730	Adult	0.1 (A)	0-8 (F)		60	Planula larvae; 100; unknown	Sun et al., 2009
Duva florida	535-2500	NW Atl		Bathyal, Abssyal		>730	Adult	0.1 (A)	1-3 (F)		100	Juvenile; 100; 100	Sun et al., 2011

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Duva fructicosa (Gersemia)	100-300	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-3 (F)		100	Juvenile; 100; 60	Sun et al., 2011
Drifa glomerata	350-1240	NW Atl		Bathyal		>730	Adult	0.1 (A)	0-9 (F)		70	Planula; 70; unknown	Sun et al., 2010
Flabellum alabastrum	600-1200	NW Atl		Bathyal		>730	Adult	0.1 (A)	-1.5-8 (F)		95 (growth of adults recorded for about 900 d)		Hamel et al., 2010
	2500			Abyssal		>730	Adult	0.1 (A)	-1.5-8 (F)				
Flabellum angulare	925-1430	NW Atl		Bathyal		>730	Adult	0.1 (A)	0-10 (F)		90	Planula; 90; unknown	Mercier et al., 2011
Hormatia digitata	500-1100	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Hormatia nodosa	500-950	NW Atl		Bathyal		60-730	Adult	0.1 (A)			100		Present Study
Keratoisis ornata	750-1500	NW Atl		Bathyal		1100	Adults	0.1 (A)			40		Present Study
Lophelia pertusa	218	Med		Bathyal		517	Adult	0.1 (A)	12 (A)	12408	100		Orejas et al., 2008
Madrepora oculata	214												
Paragorgia arborea	750-1250	NW Atl		Bathyal		60-730	Adult	0.1 (A)			100		Present Study
Pennatula grandis	466-1405	NW Atl		Bathyal		60-730	Adult	0.1 (A)			60		Hamel et al., 2010
Primnoa resedaeformis	800-1400	NW Atl		Bathyal		60-730	Adult	0.1 (A)			50		Present Study
Stephanauge nexilis	700-1350	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Umbellula sp.	1110-1505	NW Atl		Bathyal		60-730	Adult	0.1 (A)			80		Present Study
Urticina sp.	1100-1400	NW Atl		Bathyal		>730	Adult	0.1 (A)	-1-8 (F)		80	Juvenile; 80; 960	Mercier et al., 2017
Echinodermata													
Shallow-water species													

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Apostichopus japonicus	0-10	Trop W Pac	Temperate warm	Subtidal			Larvae (Early zygote)	45 (A)	21 (A)	0.12	21		Ding et al., 2007
								50 (A)			7		
								55 (A)			6		
								60 (A)			1		
								65 (A)			0		
							Larvae (Late zygote)	45 (A)			42		
								50 (A)			35		
								55 (A)			22		
								60 (A)			20		
								65 (A)			0		
Asterias rubens	16	NE Atl	Temperate-warm	Subtidal			Larvae (Zygote)	0.1 (A)	15 (A)	48		Late Blastula; 88; (2)	Villalobos et al., 2006
								5.1 (A)				Uncleaved; 15; (2)	
								10.1 (A)				Uncleaved; 20; (2)	
								15.2 (A)				Uncleaved; 23; (2)	

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								20.3 (A)				Abnormal; 100; (2)	
							Larvae (Early bipinnaria)	0.1 (A)			100		
								5.1 (A)			100		
								10.1 (A)			99		
								15.2 (A)			97		
								20.3 (A)			93		
							Larvae (Late bipinnaria)	0.1 (A)			95		
								5.1 (A)			96		
								10.1 (A)			95		
								15.2 (A)			93		
								20.3 (A)			91		
Cucumaria frondosa	10-15	NW Atl	Temperate-cold	Subtidal			Adult	0.1 (A)	6 (A)	24	100		Ammendolia et al. in preparation
								6.5 (A)					
								26 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								0.1 (A)		72			
								25 (A)			83.3		
Echinus esculentus	10-15	NE Atl	Temperate-cold	Subtidal			Larvae (Embryo)	0.1 (A)	7.5 (A)	24		Blastula; 62 ;(1)	Young and Tyler, 1998
								5.1 (A)				Blastula; 91; (1)	
								10.1 (A)				Blastula; 55; (1)	
								15.2 (A)				Abnormal; 100; (1)	
								20.3 (A)				Abnormal; 100; (1)	
	16							0.1 (A)	7 (A)			Blastula; 78; (1)	
								5.1 (A)				Blastula; 84; (1)	
								10.1 (A)				Blastula; 82; (1);	
								15.2 (A)				Blastula; 24; (1)	
								26.3 (A)				Abnormal; 100; (1)	
								0.1 (A)	11 (A)			Blastula; 81; (1)	
								5.1 (A)				Blastula; 92; (1)	

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								10.1 (A)				Blastula; 90; (1)	
								15.2 (A)				Blastula; 67; (1)	
Leptasterias polaris	10-15	NW Atl	Temperate-cold	Subtidal			Adult	0 (A)	6(A)	24	100		Ammendolia et al. in preparation
								5 (A)					
								22 (A)			83.3		
								0 (A)		72	100		
								25 (A)					
								0 (A)		216	28.5		
								22 (A)			0		
Marthasterias glacialis	10-15	NE Atl	Temperate-cold	Subtidal			Larvae (Zygote)	0.1 (A)				Early Gastrula; 99; (2)	Villalobos et al., 2006
								5.1 (A)				Early Gastrula; 97; (2)	
								10.1 (A)				Early Gastrula; 3; (2)	
								15.2 (A)				Early Gastrula; 31; (2)	
								20.3 (A)				Early Gastrula; 2; (2)	
							Larvae (Bipinnaria)	0.1 (A)			100		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								5.1 (A)			100		
								10.1 (A)			99		
								15.2 (A)			98		
								20.3 (A)			98		
Psammechinus miliaris	0-10	NE Atl	Temperate-warm	Subtidal			Larvae (Embryo)	0.1 (A)	5 (A)	12		2-cell stage; 6; (0.5)	Aquino-Souza et al., 2008
								5.1 (A)				Uncleaved; 89; (0.5)	
								10.1 (A)				Uncleaved; 100; (0.1)	
								15.2 (A)				Uncleaved; 70; (12)	
								20.3 (A)				Uncleaved; 47; (0.5)	
							Larvae (Gastrulae)	0.1 (A)		24	69		
								5.1 (A)			93		
								10.1 (A)			98		
								15.2 (A)			96		
								20.3 (A)			100		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
							Larvae (Early Prism)	0.1 (A)			96		
								5.1 (A)			89		
								10.1 (A)			90		
							Larvae (Late Prism)	15.2 (A)			90		
								20.3 (A)			92		
Sterechinus neumayeri	0-10	Antarctica	Polar	Subtidal		2	Larvae (Blastulae)	0.1 (A)	2.5 (A)	48		Blastula; 92; (2)	Tyler et al., 2000
								5.1 (A)				Blastula; 89; (2)	
								10.1 (A)				Blastula; 91; (2)	
								15.2 (A)				Blastula 83 ;(2);	
								20.3 (A)				Abnormal; 100; (2)	
							Larvae (Blastulae)	0.1 (A)		24	95		
								5.1 (A)			90		
								10.1 (A)			96		
								15.2 (A)			88		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								20.3 (A)			92		
								25.3 (A)			91		
							Larvae (Prism)	0.1 (A)			83		
								5.1 (A)			85		
								10.1 (A)			89		
								15.2 (A)			80		
								20.3 (A)			86		
								25.3 (A)			18		
							Larvae (4-arm plutei)	0.1 (A)			78		
								5.1 (A)			70		
								10.1 (A)			49		
								15.2 (A)			13		
								20.3 (A)			7		
								25.3 (A)			4		
Strongylocentrotus droebachiensis	10-15	NW Atl	Temperate-cold	Subtidal			Adult	0.1 (A)	6 (A)	24	100		Ammendolia et al. in preparation

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								6 (A)			100		
								24 (A)			50		
								0.1 (A)		72	100		
								25 (A)			83.3		
Deep-sea species													
Acanthocidaris hastigera	250	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720-2880			Wilson et al., 2013
Aspidodiadima hawaiiensis	500												
Aspidodiadima sp.	250												
Ceramaster granularis	650-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Crinoid sp.	970-1100	NW Atl		Bathyal		60-730	Adult	0.1 (A)			80		Present Study
Ctenodiscus crispatus	300-750	NW Atl		Bathyal		>730	Adult	0.1 (A)			5		Present Study
Echinus affinis	2000	Antarctica		Bathyal			Larvae	5.1 (A)	6 (A)	12		Uncleaved; 100; (0.5)	Young and Tyler, 1993
								10.1 (A)				Uncleaved; 97; (0.5)	
								15.2 (A)				Uncleaved; 45; (0.5)	
								20.3 (A)				8 cell stage; 65; (0.5)	
							Larvae (Oocyte and spermatozoa)	15.2 (A)		-		Uncleaved; 100	

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
							Larvae (oocytes, spermatozoa)	20.3 (A)		-		Uncleaved; 100	
Gorgonocephalus sp.	800-1250	NW Atl		Bathyal		>730	Adult	0.1 (A)			50		Present Study
Henricia lisa	600-1300	NW Atl		Bathyal		>730	Adult	0.1 (A)	-1-8 (F)		95	Juvenile; 100; 510	Mercier and Hamel, 2008
Henricia pauperrima	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013
Henricia robusta	1000												
Hippasteria phrygiana	450-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		80	Gastrula; 85; 32	Present Study
Histocidaris variabilis	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013
Hydroid colony	750-1350	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Leptycaster arcticus	950-1450					60-730	Adult	0.1 (A)			25		Present Study
Mediaster bairdi	700-1450					>730	Adult	0.1 (A)			35		Present Study
Mediaster ornatus	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013
Mesothuria lactea	1100-1375	NW Atl		Bathyal		>730	Adult	0.1 (A)			40		Present Study
Mesothuria sp.	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013
Micropyga sp. white	250												
Micropyga tuberculata													
Ophiura sarsi	550-1500	NW Atl		Bathyal		>730	Adult	0.1 (A)			5		Present Study

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Phormosoma placenta	730-802	Cari		Bathyal		4	Adult	0.1 (A)	9 (A)	96			Young and Cameron, 1987
Poraniomaorpha borealis	900-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Poraniomaorpha hispida	950-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Pteraster abyssorum	1050	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100	Juvenile; 2; 60	Present Study
Rathbunaster californicus	380-650	NE Pac		Bathyal	Canyon	300	Adult	0.1 (A)		7200			Lauerman, 1998
Solaster sp.	1250-1300	NW Atl		Bathyal		>730	Adult	0.1 (A)		17520	80		Present Study
Stereocidaris hawaiiensis	500	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720-2880			Wilson et al., 2013
Stylocidaris calacantha	250												
Strongylocentrotus pallidus	350-1250	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Stylocidans lineata	480-520	Cari		Bathyal	Reef	183	Adult		15 (A)	4392			Young et al., 1993
Stylocidaris rufa	250	Trop W Pac		Bathyal	Reef	30-120	Adult	0.1 (A)	10 (A)	720, 2880			Wilson et al., 2013
Tamaria scleroderma	250			Bathyal									
Tremaster mirabilis	700-1350	NW Atl		Bathyal		>730	Adult	0.1 (A)			100		Present Study
Zoreaster fulgens	900-1250	NW Atl		Bathyal		>730	Adult	0.1 (A)			30		Present Study

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Euglenozoa													
Deep-sea species													
Neobodo designis					Lab culture	360		50 (A)	2 (A)	360	10		Morgan-Smith et al., 2013
Foraminifera													
Deep-sea species													
Ammodiscus anguillae	220				Lab culture	56	Adult	0.1 (A)	12 (A)	1344			Bornmalm et al., 1997
Cibicidoides pachyderma													
Cibicides wuellerstorfi	1280	NW Atl		Bathyal	Mud volcano	120	Adult	12.7 (A)	0 (A)	2880			Wollenburg et al., 2015
Epistominella exigua	4300	NE Atl		Abyssal		36	Adult	45.6 (A)	2 (A)	864	100		Turley et al., 1993
Epistominella exigua										120	100		
Epistominella exigua	4549										100		
Gyroidinoides orbicularis	4300									864	100		
Gyroidinoides orbicularis										120	100		
Laticarinina pauperata	775	NE Atl		Bathyal		645	Adult	0.1 (A)	5 (A)	15480	33		Weinberg et al., 1990

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Tinogullmia riemann	4549	NE Atl		Abyssal		36	Adult	45.6 (A)	2 (A)	120	100		Turley et al., 1993
Tinogullmia sp											100		
Unidentified juvenile miliolid	4300			Abyssal			Juvenile				100		
Heterokontophyta													
Deep-sea species													
Cafeteria roenbergensis					Lab culture	8	Adult	50 (A)	2 (A)	192	1.6		Morgan-Smith et al., 2013
Mollusca													
Shallow-water species													
Buccinum undatum	0	NE Atl	Polar	Intertidal			Larvae (Veliger)	0.1 (A)	3 (A)	4			Smith and Thatje, 2012
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
								0.1(A)	6 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
	0	NE Atl	Polar	Intertidal			Juvenile (Hatching)	0.1 (A)	3 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
	0	NE Atl	Polar	Intertidal				20.3 (A)	6 (A)				
								30.4 (A)					
								40.5 (A)					
	5-10	NE Atl	Temperate-cold	Subtidal				0.1 (A)					
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
								0.1 (A)	10 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
								0.1 (A)	14 (A)				
								10.1 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
								0.1 (A)	18 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
	5-10	NE Atl	Temperate-cold	Subtidal				0.1 (A)	6 (A)				
								10.1 (A)					
	5-10	NE Atl	Temperate-cold	Subtidal				0.1 (A)	6 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
								0.1(A)	10 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								40.5 (A)					
								0.1 (A)	14 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
								0.1 (A)	18 (A)				
								10.1 (A)					
								20.3 (A)					
								30.4 (A)					
								40.5 (A)					
Buccinum undatum	10	NE Atl		Intertidal			Larvae (Egg)	0.1 (A)	10 (A)	480		Pediveliger; 56.7; (20)	Smith et al., 2015
								10.1 (A)		432		Pediveliger; 11.7; (18)	
								20.3 (A)	6 (A)	432		Pediveliger; 5; (18)	
								30.4 (A)		336		Pediveliger; 11.7; (18)	
Crepidula fornicata	0-10	NE Atl	Temperate	Subtidal			Larvae (Early veliger)	0.1 (A)	10 (A)	24	100		Mestre et al., 2013
								5 (A)			100		
								10 (A)			99		
								15 (A)			94		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								20 (A)			90		
								25 (A)			20		
								30 (A)			25		
								35 (A)			26		
								40 (A)			25		
							Larvae (Late veliger)	0.1 (A)			100		
								5 (A)			100		
								10 (A)			100		
								15 (A)			100		
								20 (A)			100		
								25 (A)			75		
								30 (A)			70		
								35 (A)			55		
								40 (A)			52		
Limacina sp.	0	NW Atl	Temperate-cold	Intertidal			Larvae	34.5 (A)	10 (A)	1	0		George and Marum, 1974
Mytilus edulis	10-15	NW Atl	Temperate-cold	Subtidal			Adult	0.1 (A)	6 (A)		100		Ammendolia et al. in preparation
								22 (A)			0		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Mytilus edulis	0-10	NE Atl	Temperate-warm	Subtidal			Larvae (Oocytes and spermatozoa)	0.1 (A)	10 (A)	4		Fertilized; 46; (0.17)	Mestre et al., 2009
								10.1 (A)				Fertilized; 82; (0.17)	
								20.3 (A)				Fertilized; 73; (0.17)	
								30.4 (A)				Fertilized; 78; (0.17)	
								40.5 (A)				Fertilized; 76; (0.17)	
								50.7 (A)				Fertilized; 52; (0.17)	
								0.1 (A)		24		Early blastula; 77; (1)	
								10.1 (A)				Early blastula; 9; (1)	
								20.3 (A)				Multi-cell; 13; (1)	
								30.4 (A)				Two-cell; 2; (1)	
							Larvae (Zygote)	0.1 (A)	5 (A)	50		Multi-cell; 63; (2.1)	
								10.1 (A)				Multi-cell; 64; (2.1)	
								20.3 (A)				Fertilized; 44; (2.1)	

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								30.4 (A)				Fertilized; 76; (2.1)	
								0.1 (A)	10 (A)			Early trochophore; 6; (2.1)	
								10.1 (A)				Gastrula; 59; (2.1)	
								20.3 (A)				Grastula; 1; (2.1)	
								30.4 (A)				Fertilized; 66; (2.1)	
Mytilus edulis diegensis	0-15	NE Pac	Temperate	Subtidal			Adult	10.4 (A)		4.4	100		Menzies and Wilson, 1961
								15.9 (A)		5	100		
								22.3 (A)		5.8	100		
								35 (A)		8	0		
Mytilus galloprovincialis	0-10	Med	Temperate	Subtidal		69	Adult	0.1 (A)	10 (A)	144	100		Galgani et al., 2005
								20.4 (A)			100		
								30.8 (A)			78.3		
								46.5 (A)		144	0		
								0.1 (A)		1656	86.6		
								4 (A)			79.4		
								24 (A)			67.9		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
								43 (A)			67.2		
								58 (A)			80.6		
								13 (A)			61.1		
								15.5 (A)			38		
Nucella lapillus	0	NW Atl	Temperate	Intertidal			Larvae (Excapsulated embryo)	0.1 (A)	12 (A)	12	7**********		Pechenik et al., 1984
								0.13 (A)			13**********		
							Larvae (Inact egg capsules)	0.1 (A)			0**********		
								0.13 (A)					
Deep-sea species													
Bathymodiolus azoricus	840	NE Atl		Bathyal	Hydrothermal vent	21	Adult	0.1 (A)	7.8 (A)	504			Dixon et al., 2004
Bathymodiolus azoricus	840	NE Atl		Bathyal	Hydrothermal vent	322	Adult	0.1 (A)	8 (A)	7728			Colaco et al., 2011
						365				8766	100		
Bathymodiolus azoricus	840	NE Atl		Bathyal	Hydrothermal vent	365	Adult	0.1 (A)	8 (A)	24	100		Martins et al., 2014
Bathymodiolus azoricus	840	NE Atl		Bathyal	Hydrothermal vent	248	Adult	0.1 (A)	7 (A)	5952			Pruski et al., 2003
Bathymodiolus azoricus	840	NE Atl		Bathyal	Hydrothermal vent	240	Adult	0.1 (A)	4 (A)	5760	100		Pruski et al., 2003 (Dando P., pers. comm.)

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Bathymodiolus azoricus	850	NE Atl		Bathyal	Hydrothermal vent	189	Adult	0.1 (A)	7.5 (A)	0	100		Barros et al., 2015
										12	100		
										24	100		
										36	100		
										48	100		
										168	100		
										504	100		
Bathymodiolus azoricus	850	NE Atl		Bathyal	Hydrothermal vent	180	Adult	0.1 (A)	9.5 (A)	4320	100		Bettencourt et al., 2008
Bathymodiolus azoricus	850	NE Atl		Bathyal	Hydrothermal vent	97	Adult	2-4-6-8-17 (I)	9 (A)	168	100		Bettencourt et al., 2010
								0.1 (A)		2328			
Bathymodiolus azoricus	850	NE Atl		Bathyal	Hydrothermal vent	2	Adult	0.1 (A)	9 (A)	48	100		Company et al., 2004
Bathymodiolus azoricus	850	NE Atl		Bathyal	Hydrothermal vent	45	Adult	0.1 (A)	8.5 (A)	1080	100		Kadar et al., 2005
Bathymodiolus azoricus	850	NE Atl		Bathyal	Hydrothermal vent	365	Adult	0.1 (A)		8760	50		Kadar et al., 2006
Bathymodiolus azoricus	860	NE Atl		Bathyal	Hydrothermal vent	8	Adult	8.5 (A)	9 (A)	24	100		Serafilm et al., 2006

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
										48			
										144			
Bathymodiolus azoricus	1700	NE Atl		Bathyal	Hydrothermal vent	10	Adult	0.1 (A)	6 (A)	240			Dixon et al., 2004
Bathymodiolus azoricus	1700	NE Atl		Bathyal	Hydrothermal vent		Adult	0.1 (A)	9.5 (A)	12	100		Kadar et al., 2008
								2 (A)			100		
						120	Adult	0.1 (A)	7 (A)	2880	100		
								8.5 (A)		240	100		
								17.5 (A)			100		
								23 (A)			100		
Bathymodiolus azoricus	1700	NE Atl		Bathyal	Hydrothermal vent	10	Adult	0.1 (A)	7 (A)	240			Pruski et al., 2003
						240			4 (A)	72	0		
						240					0		
						-			7 (A)	0	0		
Bathymodiolus childressi	650	GOM		Bathyal	Cold Seep	150	Adult	0.1 (A)	7.5 (A)	3600	100		Arellano and Young, 2009

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
						183				4392	100		
						15				360			
						75				1800			
						60				1440			
						90				2160			
						14	Larvae			336			
						365			-	8760			Fisher, pers. comm. Arellano and Young, 2009
Bathymodiolus childressi	750	GOM		Bathyal	Cold Seep	3	Adult	0.1 (A)	8 (A)	6	100		Berger and Young, 2006
									12 (A)				
									16 (A)				
									20 (A)				
Bathynerita childressi	540-650	GOM		Bathyal	Cold Seep		Adult		7 (A)				Arellano et al., 2011
							Larvae		7 (A)	24	64		
									15 (A)		37		
									20 (A)		30		
									25 (A)		17		

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
							Larvae (Blastulae)		7 (A)		58		
							Larvae (Trochophore)						
Bathynerita naticoide	650	GOM		Bathyal	Cold Seep		Adult	0.1 (A)	7 (A)				Arellano et al., 2014
							Larvae		15 (A)	72	100		
									25 (A)		100		
									29 (A)		100		
									32 (A)		84		
									35 (A)		0		
Bathymodiolus thermophilus	2400	Trop E Pac		Abyssal	Hydrothermal vent		Adult	12.4 (A)	6 (A)	24	12.5		Page et al., 1991
Bathymodiolus thermophilus	2585	NE Pac		Abyssal	Hydrothermal vent		Adult	26 (A)	10 (A)	43	100		Boutet et al., 2009
									20 (A)	43	100		
Bivalve sp.	700-1350	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		50		Present Study
Buccinum sp.	550-950	NW Atl		Bathyal		>730		0.1 (A)	1-8 (F)		100		
Buccinum scalariforme	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	5-6 (F)		90	Juvenile; 5 (natural canibalism); 900	Montgomery et al., in press
Buccinum cyaneum	650	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present study

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Neptunea despecta	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present study
Neptunea lyrata	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present study
Neptunea decemcostata	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present study
Aporrhais occidentalis	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present study
Colus pubescens	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present study
Colus stimpsoni	700-1450	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Present study
Depressigyra globulus	1800	NW Pac		Bathyal	Hydrothermal vent		Adult	17.93 (A)	10-60-10 (I)	10			Lee, 2003
Depressigyra globulus	1800	NW Pac		Bathyal	Hydrothermal vent	14	Adult	0.1 (A)	4 (A)	336			Lee, pers. comm.
Frigidoalvania brychia	775	NE Atl		Bathyal	Hydrothermal vent	772	Adult	0.1 (A)	5 (A)	18528	50		Weinberg et al., 1990
Ifremeria nautilei	1700-2900	Trop W Pac		Bathyal, Abyssal	Hydrothermal vent		Larvae	0.1 (A)	23 (A)	360	100	Shelled veliger; 100; (15)	Reynolds et al., 2010
									4 (A)		0		
Lepetodrilus fucensis	1800	NW Pac		Bathyal	Hydrothermal vent		Adult	17.93 (A)	10-60-10 (I)	10			Lee, 2003
Lepetodrilus fucensis	1800	NW Pac		Bathyal	Hydrothermal vent	14	Adult	0.1 (A)	4 (A)	336			Lee personal commications
Nucula granulosa	775	NE Atl		Bathyal		772	Adult	0.1 (A)	5 (A)	18528	45.9		Weinberg et al., 1990

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Nucula subovata													
Thyasira ferruginea													
Thyasira minutus													
Thyasira obsoleta													
Thyasira minutus													
Tritonia sp. 1	975	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Tritonia sp. 2	850	NW Atl		Bathyal		60-730	Adult	0.1 (A)	1-8 (F)		100		Present Study
Porifera													
Deep-sea species													
Polymastia sp. 1	1000	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Robertson et al. (submitted)
Polymastia sp. 2	1000	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Robertson et al. (submitted)
Radiella hemisphaerica	1000	NW Atl		Bathyal		>730	Adult	0.1 (A)	1-8 (F)		100		Robertson et al. (submitted)
Sipuncula													
Deep-sea species													
Phascolosoma turnerae	520	Cari		Bathyal			Adult	0.1 (A)	14 (A)	17520			Rice et al., 2012
							Larvae			1440			
Vestimentifera													

Species	Collection depth (m)	Geographic region	Climate zone	Depth range	Habitat type	Minimum survival time (d)	Initial life history stage tested	Pressure (MPa) and methods	Temperature (°C) and methods	Experimental duration (h)	Survival rate (%)	Max stage reached; % individuals reaching that stage; max age reached (d)	Reference
Deep-sea species													
Escarpia sp.	600	GOM		Bathyal	Cold seep	21	Larvae	0.1 (A)	9 (A)	504	100		Young et al., 1996
								5 (A)			100		
								10 (A)			100		
Lamellibrachia sp.	600	GOM		Bathyal	Cold seep	21	Larvae	0.1 (A)	9 (A)	504	100		
								5 (A)			100		

Footnotes

Citation	Definition of survival
Brooke and Young, 2009	*% Normal development
Childress et al., 1984	**The maximum holding time for an individual specimen was 45 days
Oliphant et al., 2011	***Survival recorded 3 days after termination of experiment
Seo et al., 2013	****Survival was recorded 30 minutes after decompression experiment
Daiki et al., 2009	*****Survival was recorded 24 hours after exposure
Schlieper, 1972	******Survival was recorded immediately after exposure to pressure
Yoshiki et al., 2006	*******Hatching success (%)
Yoshiki et al., 2008	********Hatching success (%)
Shillito et al., 2006	*********Survival was recored once 50% of tank mortalities occurred
Pechenik et al., 1984	**********Survival was recorded 3 days after decompression

Table A2. Data used in FAMD to assess the association between phylum, geographic location and survival time (rank and duration) for adults from chemosynthetic environments.

Phylum	Geographic regions	Survival time rank	Survival time (d)
Arthopoda	NW Pac	3	365
Arthopoda	NW Pac	3	66
Arthopoda	NW Pac	3	134
Arthopoda	NE Atl	1	9
Arthopoda	NE Atl	1	6
Arthopoda	NE Atl	4	1097
Arthopoda	NE Atl	4	731
Arthopoda	NE Atl	3	343
Arthopoda	NE Atl	3	91
Arthopoda	NE Atl	3	450
Arthopoda	NE Atl	1	9
Arthopoda	NE Atl	1	9
Arthopoda	NE Atl	1	9
Arthopoda	NW Pac	4	1095
Arthopoda	NW Pac	3	365
Arthopoda	NW Pac	3	365
Arthopoda	NE Atl	2	30
Arthopoda	NW Pac	3	365
Arthopoda	NW Pac	3	365
Mollusca	NE Atl	2	21
Mollusca	NE Atl	3	365
Mollusca	NE Atl	3	365
Mollusca	NE Atl	3	248
Mollusca	NE Atl	3	240
Mollusca	NE Atl	2	10
Mollusca	NE Atl	3	189
Mollusca	NE Atl	3	189
Mollusca	NE Atl	3	189
Mollusca	NE Atl	3	189
Mollusca	NE Atl	3	189
Mollusca	NE Atl	3	189
Mollusca	NE Atl	3	180
Mollusca	NE Atl	3	97
Mollusca	NE Atl	3	365
Mollusca	NE Atl	3	120
Mollusca	NE Atl	3	120
Mollusca	GOM	3	150
Mollusca	GOM	3	183
Mollusca	GOM	2	15
Mollusca	GOM	3	75

Phylum	Geographic regions	Survival time rank	Survival time (d)
Mollusca	GOM	2	60
Mollusca	GOM	3	90
Mollusca	GOM	3	365

Table A3. Data used in FAMD to assess the association between phylum, geographic location and survival time (rank and duration) for adults from non-chemosynthetic environments.

Phylum	Geographic location	Survival time rank	Survival time (d)
Arthopoda	NW Atl	4	730
Arthopoda	NW Atl	4	730
Arthopoda	NW Atl	4	730
Arthopoda	Trop W Pac	3	120
Arthopoda	GOM	4	730
Arthopoda	GOM	4	730
Arthopoda	GOM	3	548
Arthopoda	GOM	3	548
Arthopoda	Trop W Pac	3	120
Arthopoda	NW Atl	4	730
Arthopoda	NE Pac	2	45
Arthopoda	NE Pac	3	183
Arthopoda	NW Atl	4	730
Arthopoda	NW Atl	4	730
Arthopoda	Trop W Pac	3	120
Arthopoda	Trop W Pac	3	120
Arthopoda	Trop W Pac	3	120
Arthopoda	NW Atl	4	730
Arthopoda	Trop W Pac	3	120
Arthopoda	Trop W Pac	3	120
Arthopoda	Trop W Pac	3	120
Arthopoda	Trop W Pac	3	120
Arthopoda	NW Atl	4	730
Arthopoda	Trop W Pac	3	120
Arthopoda	Trop W Pac	3	120
Arthopoda	Trop W Pac	3	120
Arthopoda	Med	1	7
Arthopoda	Trop W Pac	3	120
Arthopoda	NW Atl	4	730
Arthopoda	NW Atl	4	730
Arthopoda	NW Atl	4	730
Arthopoda	NW Atl	4	730
Arthopoda	NE Atl	3	60
Arthopoda	NE Atl	3	60
Arthopoda	Trop W Pac	3	72
Chordata	Trop W Pac	3	365
Chordata	Trop W Pac	3	365
Chordata	NE Pac	4	730
Cnidaria	NW Atl	4	730

Phylum	Geographic location	Survival time rank	Survival time (d)
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NE Pac	3	496
Cnidaria	NE Pac	3	496
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	Med	3	517
Cnidaria	Med	3	517
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Cnidaria	NW Atl	4	730
Echinodermata	Trop W Pac	3	120
Echinodermata	Trop W Pac	3	120
Echinodermata	Trop W Pac	3	120
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	Trop W Pac	3	120
Echinodermata	Trop W Pac	3	120
Echinodermata	NW Atl	4	730
Echinodermata	Trop W Pac	3	120
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	Trop W Pac	3	120
Echinodermata	NW Atl	4	730
Echinodermata	Trop W Pac	3	120
Echinodermata	Trop W Pac	3	120

Phylum	Geographic location	Survival time rank	Survival time (d)
Echinodermata	Trop W Pac	3	120
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Echinodermata	NE Pac	3	300
Echinodermata	NW Atl	4	730
Echinodermata	Trop W Pac	3	120
Echinodermata	Trop W Pac	3	120
Echinodermata	NW Atl	4	730
Echinodermata	Trop W Pac	3	120
Echinodermata	Trop W Pac	3	120
Echinodermata	NW Atl	4	730
Echinodermata	NW Atl	4	730
Mollusca	NW Atl	4	730
Mollusca	NW Atl	4	730
Mollusca	NW Atl	4	730
Mollusca	NE Atl	4	772
Mollusca	NW Atl	4	730
Mollusca	NW Atl	4	730
Porifera	NW Atl	4	730
Porifera	NW Atl	4	730
Porifera	NW Atl	4	730
Porifera	NW Atl	4	730

Table A4. Data used in FAMD to assess the association between phylum, geographic location, depth range and survival time (rank and duration) for adults from non-chemosynthetic environments.

Phylum	Geographic regions	Depth range	Survival time rank	Survival time (d)
Arthopoda	NW Atl	Bathyal	4	730
Arthopoda	NW Atl	Bathyal	4	730
Arthopoda	NW Atl	Bathyal	4	730
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	GOM	Bathyal	4	730
Arthopoda	GOM	Bathyal	4	730
Arthopoda	GOM	Bathyal	3	548
Arthopoda	GOM	Bathyal	3	548
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	NW Atl	Bathyal	4	730
Arthopoda	NE Pac	Bathyal	2	730
Arthopoda	NE Pac	Bathyal	3	730
Arthopoda	NW Atl	Bathyal	4	120
Arthopoda	NW Atl	Bathyal	4	120
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	Trop W Pac	Bathyal	3	730
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	NW Atl	Bathyal	4	120
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	Trop W Pac	Bathyal	3	730
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	NW Atl	Bathyal	4	120
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	Trop W Pac	Bathyal	3	7
Arthopoda	Trop W Pac	Bathyal	3	120
Arthopoda	Med	Bathyal	1	730
Arthopoda	Trop W Pac	Bathyal	3	730
Arthopoda	NW Atl	Bathyal	4	730
Arthopoda	NW Atl	Bathyal	4	730
Arthopoda	NW Atl	Bathyal	4	60
Arthopoda	NW Atl	Bathyal	4	60
Arthopoda	NE Atl	Bathyal	3	72
Arthopoda	NE Atl	Bathyal	3	365
Arthopoda	Trop W Pac	Bathyal	3	365
Chordata	Trop W Pac	Bathyal	3	730
Chordata	Trop W Pac	Bathyal	3	730
Chordata	NE Pac	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730

Phylum	Geographic regions	Depth range	Survival time rank	Survival time (d)
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	496
Cnidaria	NW Atl	Bathyal	4	496
Cnidaria	NE Pac	Bathyal	3	730
Cnidaria	NE Pac	Bathyal	3	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Abyssal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Abyssal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Abyssal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	517
Cnidaria	NW Atl	Bathyal	4	517
Cnidaria	Med	Bathyal	3	730
Cnidaria	Med	Bathyal	3	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	730
Cnidaria	NW Atl	Bathyal	4	120
Cnidaria	NW Atl	Bathyal	4	120
Echinodermata	Trop W Pac	Bathyal	3	120
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NW Atl	Bathyal	4	120
Echinodermata	NW Atl	Bathyal	4	120
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	Trop W Pac	Bathyal	3	120
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NW Atl	Bathyal	4	120
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	Trop W Pac	Bathyal	3	120

Phylum	Geographic regions	Depth range	Survival time rank	Survival time (d)
Echinodermata	NW Atl	Bathyal	4	120
Echinodermata	Trop W Pac	Bathyal	3	120
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NW Atl	Bathyal	4	300
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NE Pac	Bathyal	3	120
Echinodermata	NW Atl	Bathyal	4	120
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	Trop W Pac	Bathyal	3	120
Echinodermata	NW Atl	Bathyal	4	120
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	Trop W Pac	Bathyal	3	730
Echinodermata	NW Atl	Bathyal	4	730
Echinodermata	NW Atl	Bathyal	4	730
Mollusca	NW Atl	Bathyal	4	730
Mollusca	NW Atl	Bathyal	4	772
Mollusca	NW Atl	Bathyal	4	730
Mollusca	NE Atl	Bathyal	4	730
Mollusca	NW Atl	Bathyal	4	730
Mollusca	NW Atl	Bathyal	4	730
Porifera	NW Atl	Bathyal	4	730
Porifera	NW Atl	Bathyal	4	730
Porifera	NW Atl	Bathyal	4	730
Porifera	NW Atl	Bathyal	4	730

Table A5. Data used in FAMD to assess the association between phylum, pressure, experimental duration and percent survival (rank and percent) for adults from chemosynthetic environments.

Phylum	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Annelida	0.1	4	1	0
Annelida	25	0	10	100
Annelida	26	20	4	36
Annelida	26	6	10	100
Annelida	26	18	10	100
Annelida	26	48	10	100
Annelida	26	9	10	100
Annelida	26	8.5	8	70
Annelida	26	8	9	84
Annelida	26	12	10	100
Annelida	0.1	6	10	100
Annelida	0.1	18	10	100
Annelida	26.3	6	10	100
Annelida	26.3	18	10	100
Annelida	26	43	10	100
Annelida	0.1	3	1	0
Annelida	27.4	1080	10	100
Annelida	10	1080	10	100
Annelida	23.1	72	10	100
Annelida	23.1	120	6	50
Annelida	12.2	24	10	100
Annelida	12.2	72	10	100
Annelida	12.2	120	10	100
Arthopoda	0.1	8760	9	81.1
Arthopoda	0.1	120	1	0
Arthopoda	12.2	504	10	100
Arthopoda	24.1	13152	10	100
Arthopoda	0.1	15	10	91
Arthopoda	0.1	24	7	65
Arthopoda	0.1	24	10	100
Arthopoda	0.1	216	8	70
Arthopoda	0.1	144	9	80
Arthopoda	18	26328	1	2.6
Arthopoda	18	17544	1	2.4
Arthopoda	18	8232	4	32.6
Arthopoda	0.1	216	8	70
Arthopoda	0.1	216	8	70
Arthopoda	0.1	216	8	70
Arthopoda	0.1	144	9	80

Phylum	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Arthopoda	0.1	1	10	100
Arthopoda	17	7	10	100
Arthopoda	0.1	24	9	86
Arthopoda	17	20.75	7	65
Arthopoda	0.1	36	6	50
Arthopoda	23	10	10	92
Arthopoda	23	24	10	100
Arthopoda	23	48	10	100
Arthopoda	30	8	10	100
Arthopoda	30	22	10	100
Arthopoda	30	72	10	100
Arthopoda	18	17568	1	0
Arthopoda	18	18264	1	5.3
Arthopoda	18	9054	4	37.5
Arthopoda	0.1	1.17	10	100
Arthopoda	12	1.17	10	100
Mollusca	0.1	8766	10	100
Mollusca	0.1	24	10	100
Mollusca	0.1	4320	10	100
Mollusca	0.1	48	10	100
Mollusca	0.1	1080	10	100
Mollusca	0.1	8760	6	50
Mollusca	8.5	24	10	100
Mollusca	8.5	48	10	100
Mollusca	8.5	144	10	100
Mollusca	0.1	12	10	100
Mollusca	2	12	10	100
Mollusca	0.1	2880	10	100
Mollusca	8.5	240	10	100
Mollusca	17.5	240	10	100
Mollusca	23	240	10	100
Mollusca	12.4	24	2	12.5
Mollusca	26	43	10	100

Table A6. Data used in FAMD to assess the association between phylum, geographic location, depth range and percent survival (rank and percent) for adults from chemosynthetic environments.

Phylum	Geographic region	Depth range	Survival time rank	Survival time (d)
Annelida	NE Pac	Abyssal	1	0
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	4	36
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	8	70
Annelida	NE Pac	Abyssal	9	84
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	1	0
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	6	50
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	100
Arthopoda	NW Pac	Bathyal	9	81.1
Arthopoda	Trop E Pac	Abyssal	1	0
Arthopoda	Trop E Pac	Abyssal	10	100
Arthopoda	Trop E Pac	Abyssal	10	100
Arthopoda	NE Pac	Abyssal	10	91
Arthopoda	NE Pac	Abyssal	7	65
Arthopoda	NE Atl	Bathyal	10	100
Arthopoda	NE Atl	Bathyal	8	70
Arthopoda	NE Atl	Bathyal	9	80
Arthopoda	NE Atl	Bathyal	1	2.6
Arthopoda	NE Atl	Bathyal	1	2.4
Arthopoda	NE Atl	Bathyal	4	32.6
Arthopoda	NE Atl	Bathyal	8	70
Arthopoda	NE Atl	Bathyal	8	70
Arthopoda	NE Atl	Bathyal	8	70
Arthopoda	NE Atl	Bathyal	9	80

Phylum	Geographic region	Depth range	Survival time rank	Survival time (d)
Arthopoda	NE Atl	Bathyal	10	100
Arthopoda	NE Atl	Bathyal	10	100
Arthopoda	NE Atl	Bathyal	9	86
Arthopoda	NE Atl	Bathyal	7	65
Arthopoda	NE Atl	Abyssal	6	50
Arthopoda	NE Atl	Abyssal	10	92
Arthopoda	NE Atl	Abyssal	10	100
Arthopoda	NE Atl	Abyssal	10	100
Arthopoda	NE Atl	Abyssal	10	100
Arthopoda	NE Atl	Abyssal	10	100
Arthopoda	NE Atl	Abyssal	10	100
Arthopoda	NE Atl	Bathyal	1	0
Arthopoda	NE Atl	Bathyal	1	5.3
Arthopoda	NE Atl	Bathyal	4	37.5
Arthopoda	NW Pac	Bathyal	10	100
Arthopoda	NW Pac	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	6	50
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	NE Atl	Bathyal	10	100
Mollusca	Trop E Pac	Abyssal	2	12.5
Mollusca	NE Pac	Abyssal	10	100

Table A7. Data used in FAMD to assess the association between phylum, depth, pressure, experimental duration and percent survival (rank and percent) for adults from non-chemosynthetic environments.

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Arthopoda	Shallow	23.3	1	6	50
Arthopoda	Shallow	0.1	8	10	100
Arthopoda	Shallow	1	8	10	100
Arthopoda	Shallow	3	8	10	100
Arthopoda	Shallow	4.1	8	10	100
Arthopoda	Shallow	5.1	8	10	100
Arthopoda	Shallow	6.1	8	10	100
Arthopoda	Shallow	10.1	8	1	0
Arthopoda	Shallow	12.2	8	1	0
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	10.1	24	10	100
Arthopoda	Shallow	15.2	24	10	100
Arthopoda	Shallow	4.7	0.27	10	100
Arthopoda	Shallow	5.4	0.32	10	100
Arthopoda	Shallow	6	0.3	10	100
Arthopoda	Shallow	7.5	0.4	9	80
Arthopoda	Shallow	7.7	0.42	10	100
Arthopoda	Shallow	8.6	0.53	10	100
Arthopoda	Shallow	9	0.45	7	60
Arthopoda	Shallow	9.2	0.53	1	0
Arthopoda	Shallow	10.5	0.58	1	0
Arthopoda	Shallow	10.7	0.62	1	0
Arthopoda	Shallow	12.3	0.68	1	0
Arthopoda	Shallow	13.8	0.77	1	0
Arthopoda	Shallow	14.8	0.83	1	0
Arthopoda	Shallow	15.2	0.88	1	0
Arthopoda	Shallow	16.7	0.97	1	0
Arthopoda	Shallow	18.3	1.05	1	0
Arthopoda	Shallow	19.9	1.13	1	0
Arthopoda	Shallow	21.5	1.25	1	0
Arthopoda	Shallow	10.4	4.43	4	33
Arthopoda	Shallow	22.3	5.78	1	0
Arthopoda	Shallow	35	5.95	1	0
Arthopoda	Shallow	2	1	10	100
Arthopoda	Shallow	5.1	1	10	100
Arthopoda	Shallow	10.1	1	10	100
Arthopoda	Shallow	0.1	7	10	100
Arthopoda	Shallow	0.1	6	10	100
Arthopoda	Shallow	5	6	10	100

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Arthopoda	Shallow	10	6	10	100
Arthopoda	Shallow	15	6	10	100
Arthopoda	Shallow	0.1	168	10	100
Arthopoda	Shallow	10	168	10	100
Arthopoda	Shallow	0.1	672	8	70
Arthopoda	Deep	0.1	504	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	504	10	100
Arthopoda	Deep	0.1	6	1	0
Arthopoda	Deep	0.1	216	10	100
Arthopoda	Deep	0.1	144	10	100
Arthopoda	Deep	7.8	576	7	60
Arthopoda	Deep	0.1	117	10	100
Arthopoda	Deep	0.1	10	10	100
Arthopoda	Deep	0.1	1080	10	100
Arthopoda	Deep	0.1	720	10	100
Arthopoda	Deep	7.6	720	10	100
Arthopoda	Deep	0.1	18300	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	624	10	100
Arthopoda	Deep	0.1	6	1	0
Arthopoda	Deep	0.1	168	1	0
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	0.1	0.17	10	100
Arthopoda	Deep	5	0.17	10	100
Arthopoda	Deep	10	0.17	10	100
Arthopoda	Deep	15	0.17	10	100
Arthopoda	Deep	20	0.17	10	100
Arthopoda	Deep	25	0.17	10	100
Arthopoda	Deep	30	0.17	10	100
Arthopoda	Deep	0.1	0.17	10	100
Arthopoda	Deep	5	0.17	10	100
Arthopoda	Deep	10	0.17	10	100
Arthopoda	Deep	15	0.17	10	100
Arthopoda	Deep	20	0.17	10	100
Arthopoda	Deep	25	0.17	10	100

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Arthopoda	Deep	30	0.17	10	100
Cnidaria	Deep	0.1	17520	5	40
Cnidaria	Deep	0.1	17520	6	50
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	17520	5	40
Cnidaria	Deep	0.1	17520	7	60
Cnidaria	Deep	0.1	17520	8	70
Cnidaria	Deep	0.1	17520	10	95
Cnidaria	Deep	0.1	17520	10	95
Cnidaria	Deep	0.1	17520	9	90
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	12408	10	100
Cnidaria	Deep	0.1	12408	10	100
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	17520	7	60
Cnidaria	Deep	0.1	17520	6	50
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	17520	9	80
Cnidaria	Deep	0.1	17520	9	80
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Deep	0.1	17520	9	80
Echinodermata	Deep	0.1	17520	1	5
Echinodermata	Shallow	0.1	24	10	100
Echinodermata	Shallow	6.5	24	10	100
Echinodermata	Shallow	26	24	10	100
Echinodermata	Shallow	0.1	72	10	100
Echinodermata	Shallow	25	72	9	83.3
Echinodermata	Deep	0.1	17520	6	50
Echinodermata	Deep	0.1	17520	10	95
Echinodermata	Deep	0.1	17520	9	80
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Shallow	0.1	24	10	100
Echinodermata	Shallow	5	24	10	100
Echinodermata	Shallow	22	24	9	83.3
Echinodermata	Shallow	0.1	72	10	100
Echinodermata	Shallow	25	72	10	100
Echinodermata	Shallow	0.1	216	3	28.5
Echinodermata	Shallow	22	216	1	0
Echinodermata	Deep	0.1	17520	3	25
Echinodermata	Deep	0.1	17520	4	35

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Echinodermata	Deep	0.1	17520	1	0
Echinodermata	Deep	0.1	17520	1	5
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Deep	0.1	17520	9	80
Echinodermata	Shallow	0.1	24	10	100
Echinodermata	Shallow	6	24	10	100
Echinodermata	Shallow	26	24	6	50
Echinodermata	Shallow	0.1	72	10	100
Echinodermata	Shallow	25	72	9	83.3
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Deep	0.1	17520	1	5
Foraminifera	Deep	45.6	864	10	100
Foraminifera	Deep	45.6	864	10	100
Foraminifera	Deep	45.6	864	10	100
Foraminifera	Deep	0.1	15480	4	33
Foraminifera	Deep	45.6	120	10	100
Foraminifera	Deep	45.6	864	10	100
Mollusca	Shallow	0.1	216	10	100
Mollusca	Shallow	22	216	1	0
Mollusca	Shallow	10.4	4.4	10	100
Mollusca	Shallow	15.9	5	10	100
Mollusca	Shallow	22.3	5.8	10	100
Mollusca	Shallow	35	8	1	0
Mollusca	Shallow	0.1	144	10	100
Mollusca	Shallow	20.4	144	10	100
Mollusca	Shallow	30.8	144	8	78.3
Mollusca	Shallow	46.5	144	1	0
Mollusca	Shallow	0.1	1656	9	86.6
Mollusca	Shallow	4	1656	8	79.4
Mollusca	Shallow	24	1656	7	67.9
Mollusca	Shallow	43	1656	7	67.2
Mollusca	Shallow	58	1656	9	80.6
Mollusca	Shallow	13	1656	7	61.1
Mollusca	Shallow	15.5	1656	4	38
Mollusca	Deep	0.1	17520	6	50
Mollusca	Deep	0.1	17520	10	100
Mollusca	Deep	0.1	17520	10	90
Mollusca	Deep	0.1	18528	6	50
Mollusca	Deep	0.1	17520	10	100

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Mollusca	Deep	0.1	17520	10	100
Porifera	Deep	0.1	17520	10	100
Porifera	Deep	0.1	17520	10	100
Porifera	Deep	0.1	17520	10	100
Porifera	Deep	0.1	17520	10	100

Table A8. Data used in FAMD to assess the association between phylum, depth, geographic location, depth range and percent survival (rank and percent) for adults from non-chemosynthetic environments.

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Arthopoda	Shallow	NE Atl	Subtidal	6	50
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	1	0
Arthopoda	Shallow	NE Atl	Subtidal	1	0
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	Trop E Pac	Subtidal	10	100
Arthopoda	Shallow	Trop E Pac	Subtidal	10	100
Arthopoda	Shallow	Trop E Pac	Subtidal	10	100
Arthopoda	Shallow	Trop E Pac	Subtidal	9	80
Arthopoda	Shallow	Trop E Pac	Subtidal	10	100
Arthopoda	Shallow	Trop E Pac	Subtidal	10	100
Arthopoda	Shallow	Trop E Pac	Subtidal	7	60
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	4	33
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	Trop E Pac	Subtidal	1	0
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	8	70
Arthopoda	Deep	Trop Ind	Abyssal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NE Pac	Bathyal	10	100
Arthopoda	Deep	NE Atl	Abyssal	1	0
Arthopoda	Deep	Trop Ind	Abyssal	10	100
Arthopoda	Deep	Trop Ind	Abyssal	10	100
Arthopoda	Deep	Trop E Pac	Bathyal	7	60
Arthopoda	Deep	Trop E Pac	Bathyal	10	100
Arthopoda	Deep	Trop E Pac	Bathyal	10	100
Arthopoda	Deep	NE Pac	Bathyal	10	100
Arthopoda	Deep	NE Pac	Bathyal	10	100
Arthopoda	Deep	NE Pac	Bathyal	10	100
Arthopoda	Deep	NE Pac	Bathyal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	Trop Ind	Bathyal	10	100
Arthopoda	Deep	NE Atl	Abyssal	1	0
Arthopoda	Deep	Med	Bathyal	1	0
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NW Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100
Arthopoda	Deep	NE Atl	Bathyal	10	100

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Arthopoda	Deep	NE Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	5	40
Cnidaria	Deep	NW Atl	Bathyal	6	50
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	5	40
Cnidaria	Deep	NW Atl	Bathyal	7	60
Cnidaria	Deep	NW Atl	Bathyal	8	70
Cnidaria	Deep	NW Atl	Bathyal	10	95
Cnidaria	Deep	NW Atl	Abyssal	10	95
Cnidaria	Deep	NW Atl	Bathyal	9	90
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	Med	Bathyal	10	100
Cnidaria	Deep	Med	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	7	60
Cnidaria	Deep	NW Atl	Bathyal	6	50
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	9	80
Cnidaria	Deep	NW Atl	Bathyal	9	80
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Deep	NW Atl	Bathyal	9	80
Echinodermata	Deep	NW Atl	Bathyal	1	5
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	9	83.3
Echinodermata	Deep	NW Atl	Bathyal	6	50
Echinodermata	Deep	NW Atl	Bathyal	10	95
Echinodermata	Deep	NW Atl	Bathyal	9	80
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	9	83.3
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	3	28.5
Echinodermata	Shallow	NW Atl	Subtidal	1	0
Echinodermata	Deep	NW Atl	Bathyal	3	25
Echinodermata	Deep	NW Atl	Bathyal	4	35

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Echinodermata	Deep	NW Atl	Bathyal	1	0
Echinodermata	Deep	NW Atl	Bathyal	1	5
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Deep	NW Atl	Bathyal	9	80
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	6	50
Echinodermata	Shallow	NW Atl	Subtidal	10	100
Echinodermata	Shallow	NW Atl	Subtidal	9	83.3
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Deep	NW Atl	Bathyal	1	5
Foraminifera	Deep	NE Atl	Abyssal	10	100
Foraminifera	Deep	NE Atl	Abyssal	10	100
Foraminifera	Deep	NE Atl	Abyssal	10	100
Foraminifera	Deep	NE Atl	Bathyal	4	33
Foraminifera	Deep	NE Atl	Abyssal	10	100
Foraminifera	Deep	NE Atl	Abyssal	10	100
Mollusca	Shallow	NW Atl	Subtidal	10	100
Mollusca	Shallow	NW Atl	Subtidal	1	0
Mollusca	Shallow	NE Pac	Subtidal	10	100
Mollusca	Shallow	NE Pac	Subtidal	10	100
Mollusca	Shallow	NE Pac	Subtidal	10	100
Mollusca	Shallow	NE Pac	Subtidal	1	0
Mollusca	Shallow	Med	Subtidal	10	100
Mollusca	Shallow	Med	Subtidal	10	100
Mollusca	Shallow	Med	Subtidal	8	78.3
Mollusca	Shallow	Med	Subtidal	0	0
Mollusca	Shallow	Med	Subtidal	9	86.6
Mollusca	Shallow	Med	Subtidal	8	79.4
Mollusca	Shallow	Med	Subtidal	7	67.9
Mollusca	Shallow	Med	Subtidal	7	67.2
Mollusca	Shallow	Med	Subtidal	9	80.6
Mollusca	Shallow	Med	Subtidal	7	61.1
Mollusca	Shallow	Med	Subtidal	4	38
Mollusca	Deep	NW Atl	Bathyal	6	50
Mollusca	Deep	NW Atl	Bathyal	10	100
Mollusca	Deep	NW Atl	Bathyal	10	90
Mollusca	Deep	NE Atl	Bathyal	6	50
Mollusca	Deep	NW Atl	Bathyal	10	100

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Mollusca	Deep	NW Atl	Bathyal	10	100
Porifera	Deep	NW Atl	Bathyal	10	100
Porifera	Deep	NW Atl	Bathyal	10	100
Porifera	Deep	NW Atl	Bathyal	10	100
Porifera	Deep	NW Atl	Bathyal	10	100

Table A9. Data used in FAMD to assess the association between phylum, pressure, experimental duration and percent survival (rank and percent) for larvae from chemosynthetic environments.

Phylum	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Annelida	0.1	72	10	100
Annelida	0.1	72	10	90
Annelida	25.3	48	10	100
Annelida	24.1	80	8	72
Annelida	17.2	80	4	36
Annelida	10.3	80	1	0
Annelida	3.6	80	1	0
Arthopoda	0.1	20	10	100
Arthopoda	15.2	20	10	100
Arthopoda	25.3	20	10	100
Arthopoda	30.4	20	1	0
Arthopoda	0.1	72	10	100
Arthopoda	0.1	384	10	100
Arthopoda	0.1	336	10	100
Arthopoda	0.1	384	10	97
Arthopoda	0.1	648	10	97
Arthopoda	0.1	2040	6	56
Arthopoda	0.1	140	9	81
Mollusca	0.1	24	7	64
Mollusca	0.1	24	6	58
Mollusca	0.1	24	6	58
Mollusca	0.1	72	10	100

Table A10. Data used in FAMD to assess the association between phylum, depth, geographic location, depth range and percent survival (rank and percent) for larvae from chemosynthetic environments.

Phylum	Geographic regions	Depth range	Survival rank	Survival (%)
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	10	90
Annelida	NE Pac	Abyssal	10	100
Annelida	NE Pac	Abyssal	8	72
Annelida	NE Pac	Abyssal	4	36
Annelida	NE Pac	Abyssal	1	0
Annelida	NE Pac	Abyssal	1	0
Arthopoda	NE Atl	Bathyal	10	100
Arthopoda	NE Atl	Bathyal	10	100
Arthopoda	NE Atl	Bathyal	10	100
Arthopoda	NE Atl	Bathyal	1	0
Arthopoda	NW Pac	Bathyal	10	100
Arthopoda	NW Pac	Bathyal	10	100
Arthopoda	NW Pac	Bathyal	10	100
Arthopoda	NW Pac	Bathyal	10	97
Arthopoda	NW Pac	Bathyal	10	97
Arthopoda	NW Pac	Bathyal	6	56
Arthopoda	NW Pac	Bathyal	9	81
Mollusca	GOM	Bathyal	7	64
Mollusca	GOM	Bathyal	6	58
Mollusca	GOM	Bathyal	6	58
Mollusca	GOM	Bathyal	10	100

Table A11. Data used in FAMD to assess the association between phylum, pressure, experimental duration and percent survival (rank and percent) for larvae from non-chemosynthetic environments.

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Annelida	Shallow	0.1	48	7	68.8
Annelida	Shallow	10.1	48	8	69.1
Annelida	Shallow	20.3	48	2	41.4
Annelida	Shallow	30.4	48	2	19.3
Annelida	Shallow	0.1	6	10	100
Annelida	Shallow	10.1	6	9	80
Annelida	Deep	0.1	8760	6	50
Arthopoda	Deep	0.1	720	9	80
Arthopoda	Shallow	0.1	4	10	100
Arthopoda	Shallow	5	4	10	100
Arthopoda	Shallow	10	4	10	100
Arthopoda	Shallow	15	4	10	100
Arthopoda	Shallow	20	4	10	100
Arthopoda	Shallow	25	4	10	100
Arthopoda	Shallow	10	4	10	100
Arthopoda	Shallow	55.1	1	1	0
Arthopoda	Shallow	0.1	24	8	77
Arthopoda	Shallow	1	24	7	60
Arthopoda	Shallow	5.1	24	4	39
Arthopoda	Shallow	10.1	24	2	17
Arthopoda	Shallow	10.1	24	9	81
Arthopoda	Shallow	10.1	24	10	95
Arthopoda	Shallow	10.1	24	10	99
Arthopoda	Shallow	10.1	24	10	95
Arthopoda	Shallow	10.1	24	10	93
Arthopoda	Shallow	10.1	24	8	78
Arthopoda	Shallow	10.1	24	10	100
Arthopoda	Shallow	10.1	24	10	91
Arthopoda	Shallow	10.1	24	10	90
Arthopoda	Shallow	10.1	24	9	88
Arthopoda	Shallow	10.1	24	10	100
Arthopoda	Shallow	10.1	24	10	95
Arthopoda	Shallow	10.1	24	9	89
Arthopoda	Shallow	10.1	24	10	90
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	100

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Arthopoda	Shallow	0.1	24	10	98
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	98
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Shallow	0.1	24	10	99
Arthopoda	Shallow	0.1	24	10	100
Arthopoda	Deep	27.5	1	1	0
Arthopoda	Deep	41.4	1	1	0
Arthopoda	Shallow	41.4	1	1	0
Arthopoda	Deep	0.1	24	6	51
Arthopoda	Deep	1	24	4	37
Arthopoda	Deep	5.1	24	7	69
Arthopoda	Deep	10	24	5	49
Arthopoda	Deep	0.1	24	6	50
Arthopoda	Deep	1	24	4	37
Arthopoda	Deep	5.1	24	7	69
Arthopoda	Deep	10	24	5	49
Arthopoda	Deep	0.1	24	8	79
Arthopoda	Deep	1	24	8	79
Arthopoda	Deep	5.1	24	7	65
Arthopoda	Deep	10	24	8	78
Arthopoda	Deep	0.1	17520	10	100
Arthopoda	Deep	41.4	1	1	0
Arthopoda	Shallow	65.5	1	1	0
Arthopoda	Shallow	6.1	1	10	100
Arthopoda	Deep	27.5	1	1	0
Arthopoda	Shallow	34.5	1	1	0
Arthopoda	Shallow	41.4	1	5	40
Arthopoda	Deep	20.7	1	6	50
Arthopoda	Shallow	38.6	1	1	0
Cnidaria	Deep	0.1	17520	5	40
Cnidaria	Deep	0.1	17520	6	50
Cnidaria	Deep	0.1	17520	7	60
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	17520	10	100
Cnidaria	Deep	0.1	17520	8	70
Cnidaria	Deep	0.1	17520	10	95

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Cnidaria	Deep	0.1	17520	10	90
Cnidaria	Shallow	62	1	3	25
Cnidaria	Shallow	0.5	1.09	10	100
Cnidaria	Deep	0.1	17520	9	80
Echinodermata	Shallow	45	0.12	3	21
Echinodermata	Shallow	50	0.12	1	7
Echinodermata	Shallow	55	0.12	1	6
Echinodermata	Shallow	60	0.12	1	1
Echinodermata	Shallow	65	0.12	1	0
Echinodermata	Shallow	45	0.12	5	42
Echinodermata	Shallow	50	0.12	4	35
Echinodermata	Shallow	55	0.12	3	22
Echinodermata	Shallow	60	0.12	3	20
Echinodermata	Shallow	65	0.12	1	0
Echinodermata	Shallow	0.1	48	10	100
Echinodermata	Shallow	5.1	48	10	100
Echinodermata	Shallow	10.1	48	10	99
Echinodermata	Shallow	15.2	48	10	97
Echinodermata	Shallow	20.3	48	10	93
Echinodermata	Shallow	0.1	48	10	95
Echinodermata	Shallow	5.1	48	10	96
Echinodermata	Shallow	10.1	48	10	95
Echinodermata	Shallow	15.2	48	10	93
Echinodermata	Shallow	20.3	48	10	91
Echinodermata	Deep	0.1	17520	10	95
Echinodermata	Deep	0.1	17520	10	95
Echinodermata	Shallow	0.1	24	10	100
Echinodermata	Shallow	5.1	24	10	100
Echinodermata	Shallow	10.1	24	10	99
Echinodermata	Shallow	15.2	24	10	98
Echinodermata	Shallow	20.3	24	10	98
Echinodermata	Shallow	0.1	24	7	69
Echinodermata	Shallow	5.1	24	10	93
Echinodermata	Shallow	10.1	24	10	98
Echinodermata	Shallow	15.2	24	10	96
Echinodermata	Shallow	20.3	24	10	100
Echinodermata	Shallow	0.1	24	10	96
Echinodermata	Shallow	5.1	24	9	89
Echinodermata	Shallow	10.1	24	10	90
Echinodermata	Shallow	15.2	24	10	90
Echinodermata	Shallow	20.3	24	10	92

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Echinodermata	Deep	0.1	17520	10	100
Echinodermata	Shallow	0.1	24	10	95
Echinodermata	Shallow	5.1	24	10	90
Echinodermata	Shallow	10.1	24	10	96
Echinodermata	Shallow	15.2	24	9	88
Echinodermata	Shallow	20.3	24	10	92
Echinodermata	Shallow	25.3	24	10	91
Echinodermata	Shallow	0.1	24	9	83
Echinodermata	Shallow	5.1	24	9	85
Echinodermata	Shallow	10.1	24	9	89
Echinodermata	Shallow	15.2	24	9	80
Echinodermata	Shallow	20.3	24	9	86
Echinodermata	Shallow	25.3	24	2	18
Echinodermata	Shallow	0.1	24	8	78
Echinodermata	Shallow	5.1	24	8	70
Echinodermata	Shallow	10.1	24	5	49
Echinodermata	Shallow	15.2	24	2	13
Echinodermata	Shallow	20.3	24	1	7
Echinodermata	Shallow	25.3	24	1	4
Mollusca	Deep	0.1	17520	10	90
Mollusca	Shallow	0.1	24	10	100
Mollusca	Shallow	5	24	10	100
Mollusca	Shallow	10	24	10	99
Mollusca	Shallow	15	24	10	94
Mollusca	Shallow	20	24	10	90
Mollusca	Shallow	25	24	3	20
Mollusca	Shallow	30	24	3	25
Mollusca	Shallow	35	24	3	26
Mollusca	Shallow	40	24	3	25
Mollusca	Shallow	0.1	24	10	100
Mollusca	Shallow	5	24	10	100
Mollusca	Shallow	10	24	10	100
Mollusca	Shallow	15	24	10	100
Mollusca	Shallow	20	24	10	100
Mollusca	Shallow	25	24	8	75
Mollusca	Shallow	30	24	8	70
Mollusca	Shallow	35	24	6	55
Mollusca	Shallow	40	24	6	52
Mollusca	Shallow	0.1	12	1	7
Mollusca	Shallow	0.13	12	2	13
Mollusca	Shallow	0.1	12	1	0

Phylum	Deep or Shallow	Pressure (MPa)	Experimental duration (h)	Survival rank	Survival (%)
Mollusca	Shallow	0.13	12	1	0
Mollusca	Shallow	34.5	1	1	0

Table A12. Data used in FAMD to assess the association between phylum, depth, geographic location, depth range and percent survival (rank and percent) for larvae from chemosynthetic environments.

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Annelida	Shallow	NE Atl	Intertidal	7	68.8
Annelida	Shallow	NE Atl	Intertidal	8	69.1
Annelida	Shallow	NE Atl	Intertidal	2	41.4
Annelida	Shallow	NE Atl	Intertidal	2	19.3
Annelida	Shallow	NE Atl	Intertidal	10	100
Annelida	Shallow	NE Atl	Intertidal	9	80
Annelida	Deep	NW Atl	Bathyal	6	50
Arthopoda	Deep	NW Pac	Bathyal	9	80
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NE Atl	Subtidal	10	100
Arthopoda	Shallow	NW Atl	Intertidal	1	0
Arthopoda	Shallow	NW Pac	Subtidal	8	77
Arthopoda	Shallow	NW Pac	Subtidal	7	60
Arthopoda	Shallow	NW Pac	Subtidal	4	39
Arthopoda	Shallow	NW Pac	Subtidal	2	17
Arthopoda	Shallow	NW Pac	Subtidal	9	81
Arthopoda	Shallow	NW Pac	Subtidal	10	95
Arthopoda	Shallow	NW Pac	Subtidal	10	99
Arthopoda	Shallow	NW Pac	Subtidal	10	95
Arthopoda	Shallow	NW Pac	Subtidal	10	93
Arthopoda	Shallow	NW Pac	Subtidal	8	78
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	91
Arthopoda	Shallow	NW Pac	Subtidal	10	90
Arthopoda	Shallow	NW Pac	Subtidal	9	88
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	95
Arthopoda	Shallow	NW Pac	Subtidal	9	89
Arthopoda	Shallow	NW Pac	Subtidal	10	90
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	98

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	98
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Shallow	NW Pac	Subtidal	10	99
Arthopoda	Shallow	NW Pac	Subtidal	10	100
Arthopoda	Deep	Cari	Bathyal	1	0
Arthopoda	Deep	Cari	Bathyal	1	0
Arthopoda	Shallow	NW Atl	Intertidal	1	0
Arthopoda	Deep	NW Pac	Bathyal	6	51
Arthopoda	Deep	NW Pac	Bathyal	4	37
Arthopoda	Deep	NW Pac	Bathyal	7	69
Arthopoda	Deep	NW Pac	Bathyal	5	49
Arthopoda	Deep	NW Pac	Bathyal	6	50
Arthopoda	Deep	NW Pac	Bathyal	4	37
Arthopoda	Deep	NW Pac	Bathyal	7	69
Arthopoda	Deep	NW Pac	Bathyal	5	49
Arthopoda	Deep	NW Pac	Bathyal	8	79
Arthopoda	Deep	NW Pac	Bathyal	8	79
Arthopoda	Deep	NW Pac	Bathyal	7	65
Arthopoda	Deep	NW Pac	Bathyal	8	78
Arthopoda	Deep	NW Pac	Bathyal	10	100
Arthopoda	Deep	Cari	Bathyal	1	0
Arthopoda	Shallow	NW Atl	Intertidal	1	0
Arthopoda	Shallow	NW Atl	Intertidal	10	100
Arthopoda	Deep	Cari	Bathyal	1	0
Arthopoda	Shallow	NW Atl	Intertidal	1	0
Arthopoda	Shallow	NW Atl	Intertidal	5	40
Arthopoda	Deep	Cari	Bathyal	6	50
Arthopoda	Shallow	NW Atl	Intertidal	1	0
Cnidaria	Deep	NW Atl	Bathyal	5	40
Cnidaria	Deep	NW Atl	Bathyal	6	50
Cnidaria	Deep	NW Atl	Bathyal	7	60
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	10	100
Cnidaria	Deep	NW Atl	Bathyal	8	70
Cnidaria	Deep	NW Atl	Bathyal	10	95
Cnidaria	Deep	NW Atl	Bathyal	10	90
Cnidaria	Shallow	Cari	Intertidal	3	25

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Cnidaria	Shallow	Cari	Intertidal	10	100
Cnidaria	Deep	NW Atl	Bathyal	9	80
Echinodermata	Shallow	Trop W Pac	Subtidal	3	21
Echinodermata	Shallow	Trop W Pac	Subtidal	1	7
Echinodermata	Shallow	Trop W Pac	Subtidal	1	6
Echinodermata	Shallow	Trop W Pac	Subtidal	1	1
Echinodermata	Shallow	Trop W Pac	Subtidal	1	0
Echinodermata	Shallow	Trop W Pac	Subtidal	5	42
Echinodermata	Shallow	Trop W Pac	Subtidal	4	35
Echinodermata	Shallow	Trop W Pac	Subtidal	3	22
Echinodermata	Shallow	Trop W Pac	Subtidal	3	20
Echinodermata	Shallow	Trop W Pac	Subtidal	1	0
Echinodermata	Shallow	NE Atl	Subtidal	10	100
Echinodermata	Shallow	NE Atl	Subtidal	10	100
Echinodermata	Shallow	NE Atl	Subtidal	10	99
Echinodermata	Shallow	NE Atl	Subtidal	10	97
Echinodermata	Shallow	NE Atl	Subtidal	10	93
Echinodermata	Shallow	NE Atl	Subtidal	10	95
Echinodermata	Shallow	NE Atl	Subtidal	10	96
Echinodermata	Shallow	NE Atl	Subtidal	10	95
Echinodermata	Shallow	NE Atl	Subtidal	10	93
Echinodermata	Shallow	NE Atl	Subtidal	10	91
Echinodermata	Deep	NW Atl	Bathyal	10	95
Echinodermata	Deep	NW Atl	Bathyal	10	95
Echinodermata	Shallow	NE Atl	Subtidal	10	100
Echinodermata	Shallow	NE Atl	Subtidal	10	100
Echinodermata	Shallow	NE Atl	Subtidal	10	99
Echinodermata	Shallow	NE Atl	Subtidal	10	98
Echinodermata	Shallow	NE Atl	Subtidal	10	98
Echinodermata	Shallow	NE Atl	Subtidal	7	69
Echinodermata	Shallow	NE Atl	Subtidal	10	93
Echinodermata	Shallow	NE Atl	Subtidal	10	98
Echinodermata	Shallow	NE Atl	Subtidal	10	96
Echinodermata	Shallow	NE Atl	Subtidal	10	100
Echinodermata	Shallow	NE Atl	Subtidal	10	96
Echinodermata	Shallow	NE Atl	Subtidal	9	89
Echinodermata	Shallow	NE Atl	Subtidal	10	90
Echinodermata	Shallow	NE Atl	Subtidal	10	90
Echinodermata	Shallow	NE Atl	Subtidal	10	92
Echinodermata	Deep	NW Atl	Bathyal	10	100
Echinodermata	Shallow	Antarctica	Subtidal	10	95
Echinodermata	Shallow	Antarctica	Subtidal	10	90

Phylum	Deep or Shallow	Geographic regions	Depth range	Survival rank	Survival (%)
Echinodermata	Shallow	Antarctica	Subtidal	10	96
Echinodermata	Shallow	Antarctica	Subtidal	9	88
Echinodermata	Shallow	Antarctica	Subtidal	10	92
Echinodermata	Shallow	Antarctica	Subtidal	10	91
Echinodermata	Shallow	Antarctica	Subtidal	9	83
Echinodermata	Shallow	Antarctica	Subtidal	9	85
Echinodermata	Shallow	Antarctica	Subtidal	9	89
Echinodermata	Shallow	Antarctica	Subtidal	9	80
Echinodermata	Shallow	Antarctica	Subtidal	9	86
Echinodermata	Shallow	Antarctica	Subtidal	2	18
Echinodermata	Shallow	Antarctica	Subtidal	8	78
Echinodermata	Shallow	Antarctica	Subtidal	8	70
Echinodermata	Shallow	Antarctica	Subtidal	5	49
Echinodermata	Shallow	Antarctica	Subtidal	2	13
Echinodermata	Shallow	Antarctica	Subtidal	1	7
Echinodermata	Shallow	Antarctica	Subtidal	1	4
Mollusca	Deep	NW Atl	Bathyal	10	90
Mollusca	Shallow	NE Atl	Subtidal	10	100
Mollusca	Shallow	NE Atl	Subtidal	10	100
Mollusca	Shallow	NE Atl	Subtidal	10	99
Mollusca	Shallow	NE Atl	Subtidal	10	94
Mollusca	Shallow	NE Atl	Subtidal	10	90
Mollusca	Shallow	NE Atl	Subtidal	3	20
Mollusca	Shallow	NE Atl	Subtidal	3	25
Mollusca	Shallow	NE Atl	Subtidal	3	26
Mollusca	Shallow	NE Atl	Subtidal	3	25
Mollusca	Shallow	NE Atl	Subtidal	10	100
Mollusca	Shallow	NE Atl	Subtidal	10	100
Mollusca	Shallow	NE Atl	Subtidal	10	100
Mollusca	Shallow	NE Atl	Subtidal	10	100
Mollusca	Shallow	NE Atl	Subtidal	10	100
Mollusca	Shallow	NE Atl	Subtidal	8	75
Mollusca	Shallow	NE Atl	Subtidal	8	70
Mollusca	Shallow	NE Atl	Subtidal	6	55
Mollusca	Shallow	NE Atl	Subtidal	6	52
Mollusca	Shallow	NW Atl	Intertidal	1	7
Mollusca	Shallow	NW Atl	Intertidal	2	13
Mollusca	Shallow	NW Atl	Intertidal	1	0
Mollusca	Shallow	NW Atl	Intertidal	1	0
Mollusca	Shallow	NW Atl	Subtidal	1	0

Table A13. Pearson correlation data that were used to test the relationship between percent survival with pressure and experimental duration, respectively, for chemosynthetic adults.

Pressure (MPa)	Experimental duration (h)	Survival (%)
0.1	4	0
25	0	100
26	20	36
26	6	100
26	18	100
26	48	100
26	9	100
26	8.5	70
26	8	84
26	12	100
0.1	6	100
0.1	18	100
26.3	6	100
26.3	18	100
26	43	100
0.1	3	0
27.4	1080	100
10	1080	100
23.1	72	100
23.1	120	50
12.2	24	100
12.2	72	100
12.2	120	100
0.1	8760	81.1
0.1	120	0
12.2	504	100
24.1	13152	100
0.1	15	91
0.1	24	65
0.1	24	100
0.1	216	70
0.1	144	80
18	26328	2.6
18	17544	2.4
18	8232	32.6
0.1	216	70
0.1	216	70
0.1	216	70

Pressure (MPa)	Experimental duration (h)	Survival (%)
0.1	144	80
0.1	1	100
17	7	100
0.1	24	86
17	20.75	65
0.1	36	50
23	10	92
23	24	100
23	48	100
30	8	100
30	22	100
30	72	100
18	17568	0
18	18264	5.3
18	9054	37.5
0.1	1.17	100
12	1.17	100
0.1	8766	100
0.1	24	100
0.1	4320	100
0.1	48	100
0.1	1080	100
0.1	8760	50
8.5	24	100
8.5	48	100
8.5	144	100
0.1	12	100
2	12	100
0.1	2880	100
8.5	240	100
17.5	240	100
23	240	100
12.4	24	12.5
26	43	100

Table A14. Pearson correlation data that were used to test the relationship between percent survival with pressure and experimental duration, respectively, for chemosynthetic adults.

Pressure (MPa)	Experimental duration (h)	Survival (%)
23.3	1	50
0.1	8	100
1	8	100
3	8	100
4.1	8	100
5.1	8	100
6.1	8	100
10.1	8	0
12.2	8	0
0.1	24	100
10.1	24	100
15.2	24	100
4.7	0.27	100
5.4	0.32	100
6	0.3	100
7.5	0.4	80
7.7	0.42	100
8.6	0.53	100
9	0.45	60
9.2	0.53	0
10.5	0.58	0
10.7	0.62	0
12.3	0.68	0
13.8	0.77	0
14.8	0.83	0
15.2	0.88	0
16.7	0.97	0
18.3	1.05	0
19.9	1.13	0
21.5	1.25	0
10.4	4.43	33
22.3	5.78	0
35	5.95	0
2	1	100
5.1	1	100
10.1	1	100
0.1	7	100
0.1	6	100

Pressure (MPa)	Experimental duration (h)	Survival (%)
5	6	100
10	6	100
15	6	100
0.1	168	100
10	168	100
0.1	672	70
0.1	504	100
0.1	17520	100
0.1	17520	100
0.1	504	100
0.1	6	0
0.1	216	100
0.1	144	100
7.8	576	60
0.1	117	100
0.1	10	100
0.1	1080	100
0.1	720	100
7.6	720	100
0.1	18300	100
0.1	17520	100
0.1	17520	100
0.1	17520	100
0.1	624	100
0.1	6	0
0.1	168	0
0.1	17520	100
0.1	17520	100
0.1	17520	100
0.1	17520	100
0.1	0.17	100
5	0.17	100
10	0.17	100
15	0.17	100
20	0.17	100
25	0.17	100
30	0.17	100
0.1	0.17	100
5	0.17	100
10	0.17	100
15	0.17	100
20	0.17	100

Pressure (MPa)	Experimental duration (h)	Survival (%)
25	0.17	100
30	0.17	100
0.1	17520	40
0.1	17520	50
0.1	17520	100
0.1	17520	100
0.1	17520	40
0.1	17520	60
0.1	17520	70
0.1	17520	95
0.1	17520	95
0.1	17520	90
0.1	17520	100
0.1	17520	100
0.1	12408	100
0.1	12408	100
0.1	17520	100
0.1	17520	60
0.1	17520	50
0.1	17520	100
0.1	17520	80
0.1	17520	80
0.1	17520	100
0.1	17520	80
0.1	17520	5
0.1	24	100
6.5	24	100
26	24	100
0.1	72	100
25	72	83.3
0.1	17520	50
0.1	17520	95
0.1	17520	80
0.1	17520	100
0.1	24	100
5	24	100
22	24	83.3
0.1	72	100
25	72	100
0.1	216	28.5
22	216	0
0.1	17520	25

Pressure (MPa)	Experimental duration (h)	Survival (%)
0.1	17520	35
0.1	17520	0
0.1	17520	5
0.1	17520	100
0.1	17520	100
0.1	17520	100
0.1	17520	80
0.1	24	100
6	24	100
26	24	50
0.1	72	100
25	72	83.3
0.1	17520	100
0.1	17520	100
0.1	17520	5
45.6	864	100
45.6	864	100
45.6	864	100
0.1	15480	33
45.6	120	100
45.6	864	100
0.1	216	100
22	216	0
10.4	4.4	100
15.9	5	100
22.3	5.8	100
35	8	0
0.1	144	100
20.4	144	100
30.8	144	78.3
46.5	144	0
0.1	1656	86.6
4	1656	79.4
24	1656	67.9
43	1656	67.2
58	1656	80.6
13	1656	61.1
15.5	1656	38
0.1	17520	50
0.1	17520	100
0.1	17520	90
0.1	18528	50

Pressure (MPa)	Experimental duration (h)	Survival (%)
0.1	17520	100
0.1	17520	100
0.1	17520	100
0.1	17520	100
0.1	17520	100
0.1	17520	100

Table A15. Pearson correlation data that were used to test the relationship between percent survival with pressure and experimental duration, respectively, for chemosynthetic larvae.

Pressure (MPa)	Experimental duration (h)	Survival (%)
0.1	72	100
0.1	72	90
25.3	48	100
24.1	80	72
17.2	80	36
10.3	80	0
3.6	80	0
0.1	20	100
15.2	20	100
25.3	20	100
30.4	20	0
0.1	72	100
0.1	384	100
0.1	336	100
0.1	384	97
0.1	648	97
0.1	2040	56
0.1	140	81
0.1	24	64
0.1	24	58
0.1	24	58
0.1	72	100

Table A16. Pearson correlation data that were used to test the relationship between percent survival with pressure and experimental duration, respectively, for non-chemosynthetic larvae.

Pressure (MPa)	Experimental duration (h)	Survival (%)
0.1	48	68.8
10.1	48	69.1
20.3	48	41.4
30.4	48	19.3
0.1	6	100
10.1	6	80
0.1	8760	50
0.1	720	80
0.1	4	100
5	4	100
10	4	100
15	4	100
20	4	100
25	4	100
10	4	100
55.1	1	0
0.1	24	77
1	24	60
5.1	24	39
10.1	24	17
10.1	24	81
10.1	24	95
10.1	24	99
10.1	24	95
10.1	24	93
10.1	24	78
10.1	24	100
10.1	24	91
10.1	24	90
10.1	24	88
10.1	24	100
10.1	24	95
10.1	24	89
10.1	24	90
0.1	24	100
0.1	24	100
0.1	24	100
0.1	24	100

0.1	24	98
0.1	24	100
0.1	24	98
0.1	24	100
0.1	24	100
0.1	24	100
0.1	24	100
0.1	24	100
0.1	24	99
0.1	24	100
27.5	1	0
41.4	1	0
41.4	1	0
0.1	24	51
1	24	37
5.1	24	69
10	24	49
0.1	24	50
1	24	37
5.1	24	69
10	24	49
0.1	24	79
1	24	79
5.1	24	65
10	24	78
0.1	17520	100
41.4	1	0
65.5	1	0
6.1	1	100
27.5	1	0
34.5	1	0
41.4	1	40
20.7	1	50
38.6	1	0
0.1	17520	40
0.1	17520	50
0.1	17520	60
0.1	17520	100
0.1	17520	100
0.1	17520	70
0.1	17520	95
0.1	17520	90
62	1	25

0.5	1.09	100
0.1	17520	80
45	0.12	21
50	0.12	7
55	0.12	6
60	0.12	1
65	0.12	0
45	0.12	42
50	0.12	35
55	0.12	22
60	0.12	20
65	0.12	0
0.1	48	100
5.1	48	100
10.1	48	99
15.2	48	97
20.3	48	93
0.1	48	95
5.1	48	96
10.1	48	95
15.2	48	93
20.3	48	91
0.1	17520	95
0.1	17520	95
0.1	24	100
5.1	24	100
10.1	24	99
15.2	24	98
20.3	24	98
0.1	24	69
5.1	24	93
10.1	24	98
15.2	24	96
20.3	24	100
0.1	24	96
5.1	24	89
10.1	24	90
15.2	24	90
20.3	24	92
0.1	17520	100
0.1	24	95
5.1	24	90
10.1	24	96

15.2	24	88
20.3	24	92
25.3	24	91
0.1	24	83
5.1	24	85
10.1	24	89
15.2	24	80
20.3	24	86
25.3	24	18
0.1	24	78
5.1	24	70
10.1	24	49
15.2	24	13
20.3	24	7
25.3	24	4
0.1	17520	90
0.1	24	100
5	24	100
10	24	99
15	24	94
20	24	90
25	24	20
30	24	25
35	24	26
40	24	25
0.1	24	100
5	24	100
10	24	100
15	24	100
20	24	100
25	24	75
30	24	70
35	24	55
40	24	52
0.1	12	7
0.13	12	13
0.1	12	0
0.13	12	0
34.5	1	0

References

Airriess CN and Childress JJ (1994) Homeoviscous properties implicated by the interactive effects of pressure and temperature on the hydrothermal vent crab *Bythograea thermydron*. Biology Bulletin 187(2): 208-214

Aquino-Souza R, Hawkins S, Tyler P (2008) Early development and larval survival of *Psammechinus miliaris* under deep-sea temperature and pressure conditions. Journal of the Marine Biological Association of the UK 88(03): 453-461

Arellano SM and Young CM (2011) Temperature and salinity tolerances of embryos and larvae of the deep-sea mytilid mussel "*Bathymodiolus*" *childressi*. Marine Biology 158(11): 2481-2493

Arellano SM and Young CM (2009) Spawning, development, and the duration of larval life in a deep-sea cold-seep mussel. Biology Bulletin 216(2): 149-162

Arellano SM, Van Gaest AL, Johnson SB, Vrijenhoek RC, Young CM (2014) Larvae from deep-sea methane seeps disperse in surface waters. Proceedings of the Royal Society of London B: Biological Sciences 281(1786): 20133276

Baillon S, Hamel J-F, Wareham VE, Mercier A (2014) Seasonality in reproduction of the deep-water pennatulacean coral *Anthoptilum grandiflorum*. Marine Biology 161(1): 29-43

Barros I, Divya B, Martins I, Vandeperre F, Santos RS, Bettencourt R (2015) Post-capture immune gene expression studies in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* acclimatized to atmospheric pressure. Fish and Shellfish Immunology 42(1): 159-170

Berger MS and Young CM (2006) Physiological response of the cold-seep mussel *Bathymodiolus childressi* to acutely elevated temperature. Marine Biology 149(6): 1397-1402

Bettencourt R, Dando P, Rosa D, Riou V, Colaço A, Sarrazin J, Sarradin P, Santos RS (2008) Changes of gill and hemocyte-related bio-indicators during long term maintenance of the vent mussel *Bathymodiolus azoricus* held in aquaria at atmospheric pressure. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 150(1): 1-7

Bettencourt R, Costa V, Laranjo M, Rosa D, Pires L, Colaco A, Sarradin PM, Lopes H, Sarrazin MJ, Santos RS (2010) Out of the deep-sea into a land-based aquarium environment: Investigating innate immunity in the hydrothermal vent mussel *Bathymodiolus azoricus*. Cahiers De Biologie Marine 51(4): 341-350

Biesiot PM and Perry H (1995) Biochemical composition of the deep-sea red crab *Chaceon quinquedens* (Geryonidae): organic reserves of developing embryos and adults. Marine Biology 124(3): 407-416

Bornmalm L, Corliss BH, Tedesco K (1997) Laboratory observations of rates and patterns of movement of continental margin benthic foraminifera. Marine Micropaleontology 29(3): 175-184

Boutet I, Jollivet D, Shillito B, Moraga D, Tanguy A (2009) Molecular identification of differentially regulated genes in the hydrothermal-vent species *Bathymodiolus thermophilus* and *Paralvinella pandorae* in response to temperature. BMC Genomics 10(1): 1

Brooke SD and Young CM (2009) Where do the embryos of *Riftia pachyptila* develop? Pressure tolerances, temperature tolerances, and buoyancy during prolonged embryonic dispersal. Deep Sea Research Part II: Topical Studies in Oceanography 56(19): 1599-1606

Brown A and Thatje S (2011) Respiratory response of the deep-sea amphipod *Stephonyx biscayensis* indicates bathymetric range limitation by temperature and hydrostatic pressure. PLoS One 6(12): e28562

Childress JJ (1971) Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. Biological Bulletin 141(1): 109-121

Childress J (1976) Effects of pressure, temperature and oxygen on the oxygen consumption rate of the midwater copepod *Gaussia princeps*. Marine Biology 39(1): 19-24

Childress J and Thuesen E (1993) Effects of hydrostatic pressure on metabolic rates of six species of deep-sea gelatinous zooplankton. Limnology and Oceanography 38(3): 665-670

Childress J, Arp A, Fisher Jr C (1984) Metabolic and blood characteristics of the hydrothermal vent tube-worm *Riftia pachyptila*. Marine Biology 83(2): 109-124

Childress J, Fisher C, Favuzzi J, Kochevar R, Sanders N, Alayse A (1991) Sulfide-driven autotrophic balance in the bacterial symbiont-containing hydrothermal vent tubeworm, *Riftia pachyptila jones*. Biology Bulletin 180(1): 135-153

Colaço A, Bettencourt R, Costa V, Lino S, Lopes H, Martins I, Pires L, Prieto C, Santos RS (2011) LabHorta: a controlled aquarium system for monitoring physiological characteristics of the hydrothermal vent mussel *Bathymodiolus azoricus*. ICES Journal of Marine Science: Journal Du Conseil 68(2): 349-356

Company J and Sardà F (1998) Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. Deep-Sea Research Part I 45(11): 1861-1880

Company R, Serafim A, Bebianno M, Cosson R, Shillito B, Fiala-Medioni A (2004) Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. Marine Environmental Research 58(2): 377-381

Cordes E, Nybakken J, VanDykhuizen G (2001) Reproduction and growth of *Anthomastus ritteri* (Octocorallia: Alcyonacea) from Monterey Bay, California, USA. Marine Biology 138(3): 491-501

Cottin D, Shillito B, Chertemps T, Tanguy A, Léger N, Ravaux J (2010a) Identification of differentially expressed genes in the hydrothermal vent shrimp *Rimicaris exoculata* exposed to heat stress. Marine Genomics 3(2): 71-78

Cottin D, Shillito B, Chertemps T, Thatje S, Léger N, Ravaux J (2010b) Comparison of heat-shock responses between the hydrothermal vent shrimp *Rimicaris exoculata* and the related coastal shrimp *Palaemonetes varians*. Journal Experimental Marine Biology and Ecology 393(1): 9-16

Cottin D, Brown A, Oliphant A, Mestre NC, Ravaux J, Shillito B, Thatje S (2012) Sustained hydrostatic pressure tolerance of the shallow water shrimp *Palaemonetes varians* at different temperatures: insights into the colonisation of the deep sea. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 162(4): 357-363

Cottin D, Ravaux J, Leger N, Halary S, Toullec JY, Sarradin PM, Gaill F, Shillito B (2008) Thermal biology of the deep-sea vent annelid *Paralvinella grasslei*: *in vivo* studies. Journal of Experimental Biology 211(14): 2196-2204

Ding J, Chang Y, Wang Z, Song J (2007) Polyploidy induction by hydrostatic pressure shock and embryo development of sea cucumber *Apostichopus japonicus*. Chinese Journal of Oceanography and Limnology 25: 184-190

Dixon DR, Pruski AM, Dixon LR (2004) The effects of hydrostatic pressure change on DNA integrity in the hydrothermal-vent mussel *Bathymodiolus azoricus*: implications for future deep-sea mutagenicity studies. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 552(1): 235-246

Dixon DR, Dixon LR, Shillito B, Gwynn JP (2002) Background and induced levels of DNA damage in Pacific deep-sea vent polychaetes: the case for avoidance. Cahiers De Biologie Marine 43(3/4): 333-336

Durand L, Zbinden M, Cueff-Gauchard V, Duperron S, Roussel EG, Shillito B, Cambon-Bonavita MA (2010) Microbial diversity associated with the hydrothermal shrimp *Rimicaris exoculata* gut and occurrence of a resident microbial community. FEMS Microbiological Ecology 71(2): 291-303

Epifanio C, Perovich G, Dittel A, Cary S (1999) Development and behavior of megalopa larvae and juveniles of the hydrothermal vent crab *Bythograea thermydron*. Marine Ecology Progress Series 185: 147-154

Gaill F, Shillito B, Ménard F, Goffinet G, Childress JJ (1997) Rate and process of tube production by the deep-sea hydrothermal vent tubeworm *Riftia pachyptila*. Marine Ecology Progress Series 148: 135-143

Galgani F, Chiffoleau J, Gall PL, Pichot Y, Andral B, Martin C (2005) Deep-sea caging of the mussel *Mytilus galloprovincialis*: Potential application in ecotoxicological studies. Chemical Ecology 21(2): 133-141

George RY and Marum JP (1974) The effects of hydrostatic pressure on living aquatic organisms III. behavior and tolerance of euplanktonic organisms to increased hydrostatic pressure. Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie 59(2): 175-186

Guerao G and Abelló P (1996) Description of the first larval stage of *Polycheles typhlops* (Decapoda: Eryonidea: Polychelidae). Journal Natural History 30(8): 1179-1184

Hamasaki K, Nakajima K, Tsuchida S, Kado R, Kitada S (2010) Number and duration of zoeal stages of the hydrothermal vent crab *Gandalfus yunohana* from laboratory reared specimens. Journal of Crustacean Biology 30(2): 236-240

Hamel J-F, Montgomery EM, Barnich R, Mercier A (2015) Range extension of the deep-sea polychaete worm *Neopolynoe acanellae* in Canada. Marine Biodiversity Records 8

Hamel J-F, Sun Z, Mercier A (2010) Influence of size and seasonal factors on the growth of the deep-sea coral *Flabellum alabastrum* in mesocosm. Coral Reefs 29(2): 521-525

Havenhand JN, Matsumoto GI, Seidel E (2006) *Megalodicopia hians* in the Monterey Submarine Canyon: distribution, larval development, and culture. Deep Sea Research Part I: Oceanographic Research Papers 53(2): 215-222

Henry RP, Perry HM, Trigg CB, Handley HL, Krarup A (1990) Physiology of two species of deep-water crabs, *Chaceon fenneri* and *C. quinquedens*: gill morphology, and hemolymph ionic and nitrogen concentrations. Journal of Crustacean Biology 375-381

Horikawa DD, Iwata K, Kawai K, Koseki S, Okuda T, Yamamoto K (2009) High hydrostatic pressure tolerance of four different anhydrobiotic animal species. Zoological Science 26(3): 238-242

Kadar E, Tschuschke IG, Checa A (2008) Post-capture hyperbaric simulations to study the mechanism of shell regeneration of the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* (Bivalvia: Mytilidae). Journal of Experimental Marine Biology and Ecology 364(2): 80-90

Kádár E, Bettencourt R, Costa V, Santos RS, Lobo-da-Cunha A, Dando P (2005) Experimentally induced endosymbiont loss and re-acquirement in the hydrothermal vent bivalve *Bathymodiolus azoricus*. Journal of Experimental Marine Biology and Ecology 318(1): 99-110

Konishi K and Saito T (2000) Larvae of the deep-sea squat lobsters, *Agononida incerta* (Henderson, 1888) and *Munida striola macpherson* and baba, 1993 with notes on larval morphology of the family (Crustacea: Anomura: Galatheidae). Zoological Science 17(7): 1021-1029

Kon-ya K and Miki W (1994) Effects of environmental factors on larval settlement of the barnacle *Balanus amphitrite* reared in the laboratory. Fisheries Science 60(5): 563-565

Koyama S, Kobayashi H, Inoue A, Miwa T, Aizawa M (2005a) Effects of the piezo-tolerance of cultured deep-sea eel cells on survival rates, cell proliferation, and cytoskeletal structures. Extremophiles 9(6): 449-460

Koyama S, Nagahama T, Ootsu N, Takayama T, Horii M, Konishi S, Miwa T, Ishikawa Y, Aizawa M (2005b) Survival of deep-sea shrimp (*Alvinocaris sp.*) during decompression and larval hatching at atmospheric pressure. Marine Biotechnology 7(4): 272-278

Lauerman LM (1998) Diet and feeding behavior of the deep-water sea star *Rathbunaster californicus* (Fisher) in the Monterey Submarine Canyon. Bulletin of Marine Sciences 63(3): 523-530

Lee RW (2003) Thermal tolerances of deep-sea hydrothermal vent animals from the Northeast Pacific. Biology Bulletin 205(2): 98-101

Macdonald A and Gilchrist I (1980) Effects of hydraulic decompression and compression on deep sea amphipods. Comparative Biochemistry and Physiology Part A: Physiology 67(1): 149-153

Marsh AG, Mullineaux LS, Young CM, Manahan DT (2001) Larval dispersal potential of the tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents. Nature 411(6833): 77-80

Martinez AS, Toullec JY, Shillito B, Charmantier-Daures M, Charmantier G (2001) Hydromineral regulation in the hydrothermal vent crab *Bythograea thermydron*. Biological Bulletin 201(2): 167-174

Martins E, Figueras A, Novoa B, Santos RS, Moreira R, Bettencourt R (2014) Comparative study of immune responses in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* and the shallow-water mussel *Mytilus galloprovincialis* challenged with vibrio bacteria. Fish Shellfish Immunology 40(2): 485-499

Matabos M, Cuvelier D, Brouard J, Shillito B, Ravaux J, Zbinden M, Barthelemy D, Sarradin P, Sarrazin J (2015) Behavioural study of two hydrothermal crustacean decapods: *Mirocaris fortunata* and *Segonzacia mesatlantica*, from the Lucky Strike vent field (Mid-Atlantic Ridge). Deep Sea Research Part II: Topical Studies in Oceanography 121: 146-158

Menzies RJ and Wilson JB (1961) Preliminary field experiments on the relative importance of pressure and temperature on the penetration of marine invertebrates into the deep sea. Oikos 12(2): 302-309

Mercier A and Hamel J-F (2009) Reproductive periodicity and host-specific settlement and growth of a deep-water symbiotic sea anemone. Canadian Journal of Zoology 87(11): 967-980

Mercier A and Hamel J-F (2008) Depth-related shift in life history strategies of a brooding and broadcasting deep-sea asteroid. Marine Biology 156(2): 205-223

Mercier A, Baillon S, Hamel J-F (2015) Life history and feeding biology of the deep-sea pycnogonid *Nymphon hirtipes*. Deep Sea Research Part I: Oceanographic Research Papers 106: 1-8

Mercier A, Baillon S, Hamel J-F (2014) Life history and seasonal breeding of the deep-sea annelid *Ophryotrocha sp.* (Polychaeta: Dorvelleidae). Deep Sea Research Part I: Oceanographic Research Papers 91: 27-35

Mercier A, Sun Z, Hamel J-F (2011) Reproductive periodicity, spawning and development of the deep-sea scleractinian coral *Flabellum angulare*. Marine Biology 158(2): 371-380

Mestre NC, Cottin D, Bettencourt R, Colaço A, Correia SP, Shillito B, Thatje S, Ravaux J (2015) Is the deep-sea crab *Chaceon affinis* able to induce a thermal stress response? Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 181: 54-61

Mestre NC, Thatje S, Tyler PA (2009) The ocean is not deep enough: Pressure tolerances during early ontogeny of the blue mussel *Mytilus edulis*. Proceedings of the Royal Society of London B: Biological Sciences 276(1657): 717-726

Mickel TJ and Childress J (1982a) Effects of temperature, pressure, and oxygen concentration on the oxygen consumption rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). Physiological Zoology 1982: 199-207

Mickel TJ and Childress JJ (1982b) Effects of pressure and temperature on the EKG and heart rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). Biological Bulletin 162(1): 70-82

Mickel TJ and Childress J (1982c) Effects of pressure and pressure acclimation on activity and oxygen consumption in the bathypelagic mysid *Gnathophausia ingens.* Deep Sea Research Part A.Oceanographic Research Papers 29(11): 1293-1301

Miyake H, Kitada M, Tsuchida S, Okuyama Y, Nakamura K (2007) Ecological aspects of hydrothermal vent animals in captivity at atmospheric pressure. Marine Ecology 28(1): 86-92

Morgan-Smith D, Garrison CE, Bochdansky AB (2013) Mortality and survival of cultured surface-ocean flagellates under simulated deep-sea conditions. Journal of Experimental Marine Biology and Ecology 445: 13-20

Morris J, Thatje S, Ravaux J, Shillito B, Fernando D, Hauton C (2015) Acute combined pressure and temperature exposures on a shallow-water crustacean: novel insights into the stress response and high pressure neurological syndrome. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 181: 9-17

Munro C, Morris JP, Brown A, Hauton C, Thatje S (2015) The role of ontogeny in physiological tolerance: Decreasing hydrostatic pressure tolerance with development in the northern stone crab *Lithodes maja*. Proceedings of the Royal Society of London B: Biological Sciences 282(1809): 20150577

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. Journal of Experimental Biology 214(7): 1109-1117

Orejas C, Gori A, Gili JM (2008) Growth rates of live *Lophelia pertusa* and *Madrepora oculata* from the Mediterranean Sea maintained in aquaria. Coral Reefs 27(2): 255-255

Page H, Fiala-Medioni A, Fisher C, Childress J (1991) Experimental evidence for filter-feeding by the hydrothermal vent mussel, *Bathymodiolus thermophilus*. Deep Sea Research Part A. Oceanographic Research Papers 38(12): 1455-1461

Pane EF and Barry JP (2007) Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. Marine Ecology Progress Series 334: 1-9

Pechenik J, Chang S, Lord A (1984) Encapsulated development of the marine prosobranch gastropod *Nucella lapillus*. Marine Biology 78(2): 223-229

Pflugfelder B, Cary SC, Bright M (2009) Dynamics of cell proliferation and apoptosis reflect different life strategies in hydrothermal vent and cold seep vestimentiferan tubeworms. Cell Tissue Research 337(1): 149-165

Ponsard J, Cambon-Bonavita M, Zbinden M, Lepoint G, Joassin A, Corbari L, Shillito B, Durand L, Cueff-Gauchard V, Compère P (2013) Inorganic carbon fixation by chemosynthetic ectosymbionts and nutritional transfers to the hydrothermal vent host-shrimp *Rimicaris exoculata*. The ISME Journal 7(1): 96-109

Pradillon F, Shillito B, Young CM, Gaill F (2001) Deep-sea ecology: Developmental arrest in vent worm embryos. Nature 413(6857): 698-699

Pradillon F, Le Bris N, Shillito B, Young CM, Gaill F (2005) Influence of environmental conditions on early development of the hydrothermal vent polychaete *Alvinella pompejana*. Journal of Experimental Biology 208(8): 1551-1561

Pruski AM and Dixon DR (2003) Toxic vents and DNA damage: First evidence from a naturally contaminated deep-sea environment. Aquatic Toxicology 64(1): 1-13

Quetin LB and Childress JJ (1980) Observations on the swimming activity of two bathypelagic mysid species maintained at high hydrostatic pressures. Deep Sea Research Part A. Oceanographic Research Papers 27(5): 383-391

Ravaux J, Hamel G, Zbinden M, Tasiemski AA, Boutet I, Léger N, Tanguy A, Jollivet D, Shillito B (2013) Thermal limit for metazoan life in question: *in vivo* heat tolerance of the pompeii worm. PLoS One 8(5): e64074

Ravaux J, Gaill F, Le Bris N, Sarradin PM, Jollivet D, Shillito B (2003) Heat-shock response and temperature resistance in the deep-sea vent shrimp *Rimicaris exoculata*. Journal of Experimental Biology 206(14): 2345-2354

Reynolds KC, Watanabe H, Strong EE, Sasaki T, Uematsu K, Miyake H, Kojima S, Suzuki Y, Fujikura K, Kim S, Young CM (2010) New molluscan larval form: brooding and development in a hydrothermal vent gastropod, *Ifremeria nautilei* (Provannidae). Biological Bulletin 219(1): 7-11

Rice ME, Reichardt HF, Piraino J, Young CM (2012) Reproduction, development, growth, and the length of larval life of *Phascolosoma turnerae*, a wood-dwelling deep-sea sipunculan. Invertebrate Biology 131(3): 204-215

Robinson NJ, Thatje S, Osseforth C (2009) Heartbeat sensors under pressure: a new method for assessing hyperbaric physiology. High Pressure Research 29(3): 422-430

Saito H and Tsuda A (2000) Egg production and early development of the subarctic copepods *Neocalanus cristatus*, *N. plumchrus* and *N. flemingeri*. Deep Sea Research Part I: Oceanographic Research Papers 47(11): 2141-2158

Schlieper C (1968) High pressure effects on marine invertebrates and fishes. Marine Biology 2(1): 5-12

Seo M, Koyama S, Toyofuku T, Kojima S, Watanabe H (2013) Determination of extremely high pressure tolerance of brine shrimp larvae by using a new pressure chamber system. Zoological Science 30: 919-923

Serafim A, Cosson R, Camus L, Shillito B, Fiala-Médioni A, Bebianno MJ (2006) The effect of cadmium on antioxidant responses and the susceptibility to oxidative stress in the hydrothermal vent mussel *Bathymodiolus azoricus*. Marine Biology 148(4): 817-825

Shillito B, Bris NL, Gaill F, Rees J, Zal F (2004) First access to live alvinellas. High Pressure Research 24(1): 169-172

Shillito B, Ravaux J, Sarrazin J, Zbinden M, Sarradin P, Barthelemy D (2015) Long-term maintenance and public exhibition of deep-sea Hydrothermal fauna: the AbyssBox project. Deep Sea Research Part II: Topical Studies in Oceanography 121:137-145

Shillito B, Jollivet D, Sarradin P, Rodier P, Lallier F, Desbruyères D, Gaill F (2001) Temperature resistance of *Hesiolyra bergi*, a polychaetous annelid living on deep-sea vent smoker walls. Marine Ecology Progress Series 216: 141-149

Shillito B, Le Bris N, Hourdez S, Ravaux J, Cottin D, Caprais JC, Jollivet D, Gaill F (2006) Temperature resistance studies on the deep-sea vent shrimp *Mirocaris fortunata*. Journal of Experimental Biology 209(5): 945-955

Smith F, Brown A, Mestre NC, Reed AJ, Thatje S (2013) Thermal adaptations in deep-sea hydrothermal vent and shallow-water shrimp. Deep Sea Research Part II: Topical Studies in Oceanography 92: 234-239

Smith KE and Thatje S (2012) The secret to successful deep-sea invasion: does low temperature hold the key? PLoS One 7(12): e51219

Smith KE, Brown A, Thatje S (2015) The metabolic cost of developing under hydrostatic pressure: experimental evidence supports macroecological pattern. Marine Ecology Progress Series 524: 71-82

Stake JL and Sammarco PW (2003) Effects of pressure on swimming behavior in planula larvae of the coral *Porites astreoides* (Cnidaria, Scleractinia). Journal of Experimental Marine Biology and Ecology 288(2): 181-201

Sun Z, Hamel J-F, Mercier A (2009) Planulation of deep-sea octocorals in the NW Atlantic. Coral Reefs 28(3): 781

Sun Z, Hamel J-F, Edinger E, Mercier A (2010) Reproductive biology of the deep-sea octocoral *Drifa glomerata* in the Northwest Atlantic. Marine Biology 157(4): 863-873

Thatje S and Robinson N (2011) Specific dynamic action affects the hydrostatic pressure tolerance of the shallow-water spider crab *Maja brachydactyla*. Naturwissenschaften 98(4): 299-313

Thatje S, Casburn L, Calcagno JA (2010) Behavioural and respiratory response of the shallow-water hermit crab *Pagurus cuanensis* to hydrostatic pressure and temperature. Journal of Experimental Marine Biology and Ecology 390(1): 22-30

Toullec J, Vinh J, Le Caer J, Shillito B, Soyez D (2002) Structure and phylogeny of the crustacean hyperglycemic hormone and its precursor from a hydrothermal vent crustacean: the crab *Bythograea thermydron*. Peptides 23(1): 31-42

Treude T, Janßen F, Queisser W, Witte U (2002) Metabolism and decompression tolerance of scavenging lysianassoid deep-sea amphipods. Deep Sea Research Part I: Oceanographic Research Papers 49(7): 1281-1289

Turley C, Gooday A, Green J (1993) Maintenance of abyssal benthic foraminifera under high pressure and low temperature: some preliminary results. Deep Sea Research Part I: Oceanographic Research Papers 40(4): 643-652

Tyler P and Dixon D (2000) Temperature/pressure tolerance of the first larval stage of *Mirocaris fortunata* from Lucky Strike hydrothermal vent field. Journal of the Marine Biological Association of the UK 80(04): 739-740

Tyler P and Young C (1998) Temperature and pressure tolerances in dispersal stages of the genus *Echinus* (Echinodermata: Echinoidea): prerequisites for deep-sea invasion and speciation. Deep Sea Research Part II: Topical Studies in Oceanography 45(1): 253-277

Vevers WF, Dixon DR, Dixon LR (2010) The role of hydrostatic pressure on developmental stages of *Pomatoceros lamarcki* (Polychaeta: Serpulidae) exposed to water accommodated fractions of crude oil and positive genotoxins at simulated depths of 1000–3000 m. Environmental Pollution 158(5): 1702-1709

Villalobos FB, Tyler PA, Young CM (2006) Temperature and pressure tolerance of embryos and larvae of the Atlantic seastars *Asterias rubens* and *Marthasterias glacialis* (Echinodermata: Asteroidea): potential for deep-sea invasion. Marine Ecology Progress Series 314: 109-117

Watanabe H, Kado R, Tsuchida S, Miyake H, Kyo M, Kojima S (2004) Larval development and intermoult period of the hydrothermal vent barnacle *Neoverruca sp.* Journal of the Marine Biological Association of the UK 84(4): 743-745

Watsuji T, Yamamoto A, Takaki Y, Ueda K, Kawagucci S, Takai K (2014) Diversity and methane oxidation of active epibiotic methanotrophs on live *Shinkaia crosnieri*. The ISME Journal 8(5): 1020-1031

Weinberg JR (1990) High rates of long-term survival of deep-sea infauna in the laboratory. Deep Sea Research Part A. Oceanographic Research Papers 37(8): 1375-1379

Wilcock S, Wann K, Macdonald A (1978) The motor activity of *Crangon crangon* subjected to high hydrostatic pressure. Marine Biology 45(1): 1-7

Wilson S, Yeh J, Korsmeyer KE, Drazen JC (2013) Metabolism of shallow and deep-sea benthic crustaceans and echinoderms in Hawaii. Marine Biology 160(9): 2363-2373

Wollenburg JE, Raitzsch M, Tiedemann R (2015) Novel high-pressure culture experiments on deep-sea benthic foraminifera—Evidence for methane seepage-related $\delta^{13}C$ of *Cibicides wuellerstorfi*. Marine Micropaleontology 117: 47-64

Yayanos AA (1981) Reversible inactivation of deep-sea amphipods (*Paralicella capresca*) by a decompression from 601 bars to atmospheric pressure. Comparative Biochemistry and Physiology Part A: Physiology 69(3): 563-565

Yoshiki T, Ono T, Shimizu A, Toda T (2011) Effect of hydrostatic pressure on eggs of neocalanus copepods during spawning in the deep-layer. Marine Ecology Progress Series 430: 63-70

Yoshiki T, Toda T, Yoshida T, Shimizu A (2006) A new hydrostatic pressure apparatus for studies of marine zooplankton. Journal of Plankton Research 28(6): 563-570

Yoshiki T, Yamanoha B, Kikuchi T, Shimizu A, Toda T (2008) Hydrostatic pressure-induced apoptosis on nauplii of *Calanus sinicus*. Marine Biology 156(2): 97-106

Young C, Tyler P, Emson R, Gage J (1993) Perception and selection of macrophyte detrital falls by the bathyal echinoid *Stylocidaris lineata*. Deep Sea Research Part I: Oceanographic Research Papers 40(7): 1475-1486

Young CM and Tyler PA (1993) Embryos of the deep-sea echinoid *Echinus affinis* require high pressure for development. Limnology and Oceanography 38(1): 178-181

Young CM and Cameron JL (1987) Laboratory and in situ flotation rates of lecithotrophic eggs from the bathyal echinoid *Phormosoma placenta*. Deep Sea Research Part A. Oceanographic Research Papers 34(9): 1629-1639

Young CM, Vázquez E, Metaxas A, Tyler PA (1996) Embryology of vestimentiferan tube worms from deep-sea methane/sulphide seeps. Nature 381(6582): 514-516

Appendix B: Supplementary experiment for *Strongylocentrotus droebachiensis*

Extracellular coelomic pH in *Strongylocentrotus droebachiensis*

Methods

A syringe was used to extract 2.0 ml of perivisceral coelomic fluid from the sea urchins after the combined pH/pressure trials (~150 min after removal from the vessel). Immediately after extraction, the pH of the fluid was measured with an AccumetR pH probe (Fisher Scientific, USA). The pH experiment was only done for *S. droebachiensis* since previous studies had shown that perivisceral fluid of different urchin species was sensitive to acidified conditions (Kurihara et al. 2013; Spicer et al. 2011). Although great care was taken to prevent unnecessary internal damage the invasive sampling may still have affected the health status of the individuals. Thus, to avoid any bias, *S. droebachiensis* was not monitored for post-recovery health responses after the pH/pressure trials.

Results

Pressure did not affect the pH of the coelomic fluid in *S. droebachiensis* under ambient pH conditions and under different pressures ($F_{2, 45}$ =1.97, p=0.151). Under ambient pH conditions the coelomic pH of animals following exposure to atmospheric and medium pressure was 7.58 and 7.4 units, respectively. While under low pH conditions the coelomic pH of animals following exposure to atmospheric and medium pressure was 7.51 and 7.57 units, respectively. A significant interaction between the effects of pH and pressure on the pH of the coelomic fluid was detected ($F_{1, 56}$ =4.81, p=0.032), although independent tests failed to identify pairwise differences. The pH of the coelomic fluid was not significantly affected by pressure ($F_{1, 56}$ =0.10, p=0.749) or by

the pH of seawater ($F_{1, 56}$=0.10, p=0.758). There was also a significant interaction between pH and pressure on the pH of the coelomic fluid ($F_{1, 56}$=4.81, p=0.032), although independent tests failed to identify pairwise differences. The pH of the coelomic fluid was not significantly affected by pressure ($F_{1, 56}$=0.10, p=0.749) or by the pH of seawater ($F_{1, 56}$=0.10, p=0.758).

Appendix C: Statistical results

Table C1. Complete statistical results for *Strongylocentrotus droebachiensis* health responses for the following experimental conditions: ambient pH/24 h, ambient pH/0-4 h (dissolved oxygen experiments), ambient pH/72 h and acidified pH/24 h. Asterisks indicate significance ($p<0.05$).

Time (h)	pH	Experiment	Pressure (atm, med, high)[a]	Time relative to trial (pre, post and recovery)[b]	Results
24	Ambient	Feeding Indices	AtmxMedxHigh	Post	H=26.56, df=2, p<0.001
			Atm×Med		Atm<Med
			Atm×High		Atm>High*
			Med×High		Med>High*
		Weight	AtmxMedxHigh		H=0.848, df=2, p=0.655
		pH coelomic fluid	AtmxMedxHigh		$F_{2,45}$ =1.97, p=0.151
		Righting to 90°	AtmxMedxHigh		H=6.26, df=2, p=0.044
			Atm×Med		Atm<Med*
			Atm×High		Atm<High*
			Med×High		Med<High*
		Righting to 180°	AtmxMedxHigh		H=7.80, df=2, p=0.020
			Atm×Med		Atm<Med
			Atm×High		Atm<High
			Med×High		Med<High*
72		Righting to 90°	AtmxHigh	PrexPostxRecovery	Interaction between pressure and time* $F_{2, 35}$ =4.71, p=0.017
				Post	Atm<High* t=-3.86, df=10 p=0.003
				Pre	Atm=High t=0.20, df=10, p=0.848
				Recovery	Atm=High U=7.00, df=10, p=0.093
			Atm	PrexPost	Pre<Post* U=5.00, df=10, p=0.041
				PostxRecovery	Post=Recovery t=1.82, df=10, p=0.099
				PrexRecovery	Pre=Recovery t=0.70, df=10, p=0.500
			High	PrexPost	Pre<Recovery* U=2.00, df=10, p=0.009
				PostxPre	Pre<Post* t=5.72, df=10, p=<0.001
				PostxRecovery	Post>Recovery* t=2.42, df=10, p=0.036
		Righting to 180°	AtmxHigh	PrexPostxRecovery	Interaction between pressure and time* $F_{2, 35}$ =4.71, p=0.017
				Post	Atm<High* t=-3.75, df=10 p=0.04
				Recovery	Atm<High* U=4.00, df=10, p=0.026
				Pre	Atm=High t=-0.162, df=10, p=0.875
			Atm	PrexPost	Pre<Post* t=-2.45, df=10, p=0.034
				PrexRecovery	Pre=Recovery t=0.01, df=10, p=0.094
				PostxRecovery	Post=Recovery t=-2.10, df=10, p=0.062
			High	PrexPost	Pre<Post*

					t=-5.31, df=10, p=<0.001
				PrexRecovery	Pre<Recovery* U=3.00, df=10, p=0.015
				PostxRecovery	Post=recovery t=2.21, df=10, p=0.052
0, 2, 4[c]		Dissolved oxygen	0, 5, 10, 25 MPa	0x2x4 h	No interaction between pressure and time $F_{6, 24}$=0.10, p=0.975 Effect of pressure level $F_{3, 24}$=1.46, p=0.249
				0x2x4 h	Effect of time level $F_{2, 24}$=8.41, p=0.002 0>4 h* 0=4 h 2=4 h
24	Ambient ×Acidified	Feeding Indices	AtmxMed	Post	Interaction between pH and pressure level* $F_{1, 56}$ = 4.25, p=0.044
	Acidified				Atm>Med* t=2.19, df=22, p=0.039
	Ambient				Atm=Med t=-1.03, df=34, p=0.312
	Ambient ×Acidified		Med		Ambient>Acidified* t=-3.18, df=22, p=0.004
			Atm		Ambient=Acidified U=219.0, df=34, p=0.933
	Ambient ×Acidified	Weight	Atm×Med		Atm=Med $F_{2, 56}$=1.06, p=0.353 Ambient=Acidified $F_{1, 56}$=0.02, p=0.880
	Ambient ×Acidified	Righting to 90°			Interaction between pH and pressure level* $F_{1, 59}$ = 0.29, p=0.592 No effect of pressure $F_{1, 59}$= 1.69, p=0.199
	Acidified				Atm<Med
	Ambient				Atm<Med
	Ambient ×Acidified				No effect of pH $F_{1, 59}$ = 1.04, p=0.312
			Atm		Ambient>Acidified
			Med		Ambient>Acidified
	Ambient ×Acidified	pH of the coelomic fluid	Atm×Med		Interaction between pH and pressure level* $F_{1, 56}$=4.81, p=0.032 pH level $F_{1, 56}$=0.10, p=0.758 Pressure level $F_{1, 56}$=0.10, p=0.749

[a] Pressure atm (atmospheric), med (medium) and high refer to 0, 6 and 24 MPa, respectively
[b] Time of measurement: Pre (activity prior to pressure exposure <2 h), During (while being exposed to pressure) Post (immediately after pressure exposure), Recovery (7-d after pressure exposure)
[c] Dissolved oxygen experiments tested 0, 2 and 4 h of pressure duration

Table C2. Complete statistical results for *Leptasterias polaris* health responses for the following experimental conditions: ambient pH/24 h, ambient pH/72 h, ambient pH/216 and acidified pH/24 h. Asterisks indicate significance ($p<0.05$).

Time (h)	pH	Experiment	Pressure (atm, med, high)[a]	Time relative to trial (pre, post and recovery)[b]	Results
24	Ambient	Feeding Indices	AtmxMedxHigh	Post	$F_{2, 45}$ =0.40, p=0.669
		Feeding positions			H=2.47, df=2, p=0.291
		Weight			H=10.89, df=2, p=0.004*
			Atm×Med		Atm<High*
			Atm×High		Med<High*
			Med×High		Med=High
		Righting to 90°	AtmxMedxHigh		H=10.28, df=2, p=0.006*
			Atm×Med		Atm<High*
			Atm×High		Med<High*
			Med×High		Atm=Med
		Righting to 180°	AtmxMedxHigh		H=22.98, df=2, p=<0.001*
			Atm×Med		Atm<High*
			Atm×High		Med<High*
			Med×High		Atm=Med
		Righting to 90°	AtmxMedxHigh	Recovery	H=1.49, df=2, p=0.476
			Atm×Med		Atm<Med
			Atm×High		Atm<High
			Med×High		Med<High
				AfterxRecovery	After<Recovery*
					H=16.63, df=1, p=<0.001*
		Righting to 180°	AtmxMedxHigh	Recovery	H=1.09, df=2, p=0.579
			Atm×Med		Atm<Med
			Atm×High		Atm<High
			Med×High		Med>High
				AfterxRecovery	After<Recovery*
					H=31.91, df=1, p=<0.001*
72		Righting to 90°	AtmxHigh	PrexPostxRecovery	Interaction between time and pressure* $F_{2, 35}$ =4.71, p=0.017
				Post	Atm<High*
					t=-3.86, df=10 p=0.003
				Pre	Atm=High
					t=0.20, df=10, p=0.848
				Recovery	Atm=High
					U=7.00, df=10, p=0.093
			Atm	PrexPost	Pre<Post*
					U=5.00, df=10, p=0.041
				PostxRecovery	Atm=High
					t=1.82, df=10, p=0.099
				PrexRecovery	Atm=High
					t=0.70, df=10, p=0.500
			High	PrexPost	Atm<High*
					t=5.72, df=10, p=<0.001
				PrexRecovery	Pre<Recovery*
					U=2.00, df=10, p=0.009
				PostxRecovery	Pre<Recovery*
					t=2.42, df=10, p=0.036
		Righting to 180°	AtmxHigh	PrexPostxRecovery	Interaction between pressure and time*
					$F_{2, 35}$ =4.71, p=0.017
				Post	Atm<High*
					t=-3.75, df=10 p=0.04
				Pre	Atm=High
					t=-0.162, df=10, p=0.875

				Recovery	Atm<High* U=4.00, df=10, p=0.026
			Atm	PrexPost	Pre<Post* t=-2.45, df=10, p=0.034
				PostxRecovery	Post=Recovery t=-2.10, df=10, p=0.062
				PrexRecovery	Pre=Post t=0.01, df=10, p=0.094
			High	PrexPost	Pre<Post* t=-5.31, df=10, p=<0.001
				PrexRecovery	Pre=Recovery U=3.00, df=10, p=0.015
				PostxRecovery	Post=Recovery t=2.21, df=10, p=0.052
216x24[c]		Feeding	AtmxHigh	Post	No interaction between pressure and time $F_{1, 43}$=1.564, p=0.218 Effect of time* $F_{1, 43}$=9.45, p=0.004
			Atm		24<216*
			High		24=216
			AtmxHigh		Effect of pressure $F_{1, 43}$=2.63, p=0.113
24	Ambient ×Acidified	Feeding Indices	Atm×Med	Post	No interaction between pressure and pH $F_{1, 56}$=1.33, p=0.254 Effect of pressure* $F_{1, 56}$=5.09, p=0.028
	Ambient				Atm=Med
	Acidified				Atm<Med*
	Ambient ×Acidified				Effect of pH* $F_{1, 56}$=9.36, p=0.003
			Atm		Ambient>Acidified*
			High		Ambient=Acidified
		Feeding Positions	Atm×Med		No interaction between pressure and pH $F_{1, 44}$=2.54, p=0.118 No effect of pressure $F_{1, 44}$=1.02, p=0.751 No effect of pH $F_{1, 44}$=0.28, p=0.598
		Righting to 90°			No effect of pressure $F_{1, 56}$= 2.20, p=0.143 No effect of pH $F_{1, 56}$=0.50, p=0.482
		Righting to 180°			No effect of pressure $F_{1, 56}$=0.36, p=0.553 No effect of pH $F_{1, 56}$=0.07, p=0.789
		Righting to 90°	AtmxHigh	Recovery	No effect of pressure $F_{1, 56}$=1.97, p=0.166 Effect of pH* $F_{1, 56}$=1.36, p=0.007
			High		Acidified<Ambient*
			Atm		Acidified=Ambient
		Righting to 180°	AtmxHigh		No effect of pressure $F_{1, 56}$=1.36, p=0.248 No effect of pH $F_{1, 56}$=2.67, p=0.108

[a] Pressure atm (atmospheric), med (medium) and high refer to 0, 6 and 24 MPa, respectively
[b] Time of measurement: Pre (activity prior to pressure exposure <2 h), During (while being exposed to pressure) Post (immediately after pressure exposure), Recovery (7-d after pressure exposure)
[c] 216 h experimental pressure duration experiment was replicated once so results were tested with 24 h

Table C3. Complete statistical results for *Cucumaria frondosa* health responses for the following experimental conditions: ambient pH/24 h, ambient pH/72 h and acidified pH/24 h. Asterisks indicate significance ($p<0.05$).

Time (h)	pH	Experiment	Pressure (atm, med, high)[a]	Time relative to trial (pre, post and recovery)[b]	Results
24	Ambient	Feeding response	AtmxMedxHigh	Post	H=9.96, df=2, p=0.007
			Atm×Med		Atm<Med*
			MedxHigh		Med=High
			Atm×High		Atm<High*
72			MedxHigh		U=12.00, df=2, p=0.394
24		Cloacal movement	AtmxMedxHigh		H=8.96, df=2, p=0.011
			Atm×High		Atm>High*
			Atm×Med		Atm=Med
			MedxHigh		Atm=High
			AtmxMedxHigh	Recovery	$F_{2, 17}$= 1.56, p= 0.238
				PostxRecovery	Interaction between pressure and time*
					$F_{2, 35}$ = 8.85, p= <0.001
					Effect of pressure*
					$F_{3, 35}$ = 11.72, p=<0.001
			AtmxHigh	Post	Atm>High*
			MedxHigh		Med>High*
			AtmxMed		Atm=Med
			AtmxHigh	Recovery	Atm=High
			MedxHigh		Med=High
			AtmxMed		Atm=Med
			AtmxMedxHigh	PostxRecovery	Effect of time*
					$F_{1, 35}$ = 9.33, p= 0.005
			Atm		Final=Recovery
			Med		Final=Recovery
			High		Final<Recovery
			AtmxMedxHigh	PrexPostxRecovery	H=10.71, df=2, p=0.005
				PrexPost	Pre<Post*
				PrexRecovery	Pre=Recovery
				PostxRecovery	Post=Recovery
72			MedxHigh	PrexPostxRecovery	Interaction between pressure and time*
					$F_{2, 35}$ =4.21, p=0.025
					Effect of pressure*
					$F_{1, 35}$ =4.60, p=0.040
				Pre	Atm=High
					U=13.00, df=10, p=0.485
				Post	Atm>High*
					t=5.41, df=10 p=<0.001
				Recovery	Atm=High
					t=-1.57, df=10, p=0.147
				PrexPostxRecovery	Effect of time*
					$F_{2, 35}$ =6.08, p=0.006
			Atm	PrexPost	Pre=Post
					U=13.00, df=10, p=0.466
				PrexRecovery	Pre=Recovery
					t=13.00, df=10, p=0.485
				PostxRecovery	Post=Recovery
					U=10.00, df=10, p=0.240
			High	PrexPost	Pre>Post*
					t=-7.94, df=10, p=<0.001
				PrexRecovery	Pre>Recovery*
					U=2.00, df=10, p=0.009
				PostxRecovery	Post=Recovery
					U=12.00 df=10, p=0.394
24		Predator-escape response	AtmxMedxHigh	Post	$F_{2, 17}$=2.89, p=0.087
			AtmxMed		Atm=Med
			AtmxHigh		Atm=High

			MedxHigh		Med=High
72			MedxHigh		Atm=High t=-0.571, df=10, p=0.580
24		Anchorage time	AtmxMedxHigh	Post	H=8.06, df=2, p=0.018
			AtmxHigh		Atm<High*
			AtmxMed		Atm=Med
			MedxHigh		Med=High
			AtmxMedxHigh	Recovery	H=1.99, df=2, p=0.370
				PrexPostxRecovery	No effect of pressure $F_{2, 35}$ = 2.66, p=0.087 No effect of time $F_{1, 35}$ = 0.07, p= 0.795
72			AtmxHigh	PrexPostxRecovery	No interactions between pressure and time $F_{2, 35}$ =1.64, p=0.210 No effect of time $F_{2, 35}$ =1.96, p=0.159 Effect of pressure $F_{1, 35}$ =4.55, p=0.041
				Pre	Atm<High*
				Post	Atm=High
				Recovery	Atm=High
24	Ambientx Acidified	Cloacal movement	AtmxMed	Post	No interaction between pressure and pH $F_{1, 23}$=0.04, p=0.856 No effect of pressure $F_{1, 23}$=3.62, p=0.072 No effect of pH $F_{1, 23}$=0.003, p=0.952
				PrexPostxRecovery	No interaction between time and pH $F_{2, 66}$ = 0.36, p= 0.697 Effect of time $F_{2, 66}$ = 3.54, p= 0.035 Effect of pH $F_{2, 66}$ = 1.73, p= 0.193
				PrexPost	Pre=Post t=1.99, df=2, p=0.014
				PrexRecovery	Pre>Recovery* t=1.99, df=2, p=0.051
				PostxRecovery	Post=Recovery t=0.54, df=2, p=0.537
				PrexPostxRecovery	No effect of pH $F_{1, 66}$ = 1.73, p= 0.193
		Anchorage time	AtmxMed	Post	No interaction between pressure and pH $F_{1, 23}$=1.92, p=0.181 No effect of pressure $F_{1, 23}$=0.32, p=0.576 Effect of pH* $F_{1, 23}$=5.33, p=0.032
			Atm		Ambient<Acidified*
			Med		Ambient=Acidified
				PrexPostxRecovery	Interaction between pH and time* $F_{2, 63}$=5.09, p=0.009 Effect of pH* $F_{1, 63}$=6.67, p=0.012
	Ambient				Pre=Post=Recovery H=4.24, df=2, p=0.120
	Acidified				Pre<Post* $F_{2, 21}$=4.50, p=0.047 Post>Recovery* $F_{2, 21}$=5.91, p=0.025 Pre=Recovery $F_{2, 21}$=0.11, p=0.748 H=4.24, df=2, p=0.120
					Effect of time* $F_{2, 63}$=3.55, p=0.035
	Ambient			Pre	Ambient=Acidified

xAcidified				H=2.77, df=1, p=0.096
			Post	Ambient>Acidified* H=4.02, df=1, p=0.045
			Recovery	Ambient=Acidified H=3.56, df=1, p=0.551
Ambient xAcidified	Feeding		Post	Interaction between pressure and pH* $F_{1, 23}$ = 7.28, p=0.014
Ambient		AtmxMed		Atm<Med * t=-5.72, df=10, p=<0.001
Acidified				Atm=Med U=17.0, df=10, p=0.937
Ambient xAcidified		Med		Ambient=Acidified t=-2.11, df=10, p=0.061
		Atm		Ambient=Acidified U=10.0, df=10, p=0.240
	Predator escape response	AtmxMed		No interaction between pressure and pH $F_{2, 17}$=2.89, p=0.087 Effect of pressure* $F_{1, 23}$=4.75, p=0.041
Ambient				0<6.5*
Acidified				0=6.5
Ambient xAcidified				No effect of pH $F_{1, 23}$=1.03, p=0.322

[a] Pressure atm (atmospheric), med (medium), high refer to 0, 6 and 24 MPa, respectively
[b] Time of measurement: Pre (activity prior to pressure exposure <2 h), During (while being exposed to pressure) Post (immediately after pressure exposure), Recovery (7-d after pressure exposure)