UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Ocean and Earth Science

Physiological thresholds through early ontogeny: the effects of temperature and hydrostatic pressure on the common whelk *Buccinum undatum* (Linnaeus 1758)

by

Kathryn Elizabeth Smith

Thesis for the degree of Doctor of Philosophy

January 2013

‘It is not the strongest of the species that survives, nor the most intelligent.

It is the one that is the most adaptable to change’

- Clarence Darrow (1857-1938)

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ABSTRACT

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PHYSIOLOGICAL THRESHOLDS THROUGH EARLY ONTOGENY: THE EFFECTS OF TEMPERATURE AND HYDROSTATIC PRESSURE ON THE COMMON WHELK *BUCCINUM UNDATUM* (LINNAEUS 1758)

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The eco-physiological thresholds controlling the distribution of marine invertebrates are of significance in understanding the evolution of marine diversity. This includes the direction of species radiation throughout the oceans. Range expansions occur as a result of evolutionary adaptations, or through environmentally or anthropogenically driven shifts in distribution. The success of such events is centred around a species ability to adapt; in order for a migration to be successful, all life history stages must tolerate the conditions of the new habitat. This thesis examines the thermal and hyperbaric thresholds affecting range extension in the marine environment. It focuses on the larval development of the shallow-water North Atlantic gastropod *Buccinum undatum* (Linnaeus 1758). The Buccinidae family consists of a wide range of shallow and deep-water species distributed globally. Improved knowledge on this topic will contribute to our understanding of the adaptations influencing both historical and modern shifts in the distribution of species.

The thermal and hyperbaric ranges observed during development indicate *B. undatum* to have the capacity to develop at both temperatures and pressures outside its current distribution. Thermal acclimation to low temperature was also found to increase pressure tolerance during development. A shift in number of embryos developing and nurse egg partitioning *per* embryo indicate a decrease in developmental success at temperatures above those it is naturally exposed to. An increase in energetic expenditure, with both increasing temperature and pressure, relates to a rise in the metabolic cost associated with development under either condition. These results, combined with the known life history of *B. undatum*, suggest range expansion into deep water may be a plausible scenario, but tolerance of warmer conditions remains questionable due to the cold-induced spawning observed in this species. The results of this thesis support theories of high-latitude migrations into the deep sea *via* cold, isothermal waters, and indeed, suggest polar temperatures may promote the rate at which such range expansions occur. Additionally, the observed metabolic cost associated with development suggests hydrostatic pressure may induce bathymetric limits, which explain patterns observed in reproductive trends, eco-physiological adaptations, and faunal distribution in marine invertebrates throughout the oceans.

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DECLARATION OF AUTHORSHIP

I, Kathryn Elizabeth Smith declare that the thesis entitled

> 'Physiological thresholds through early ontogeny: the effects of temperature and hydrostatic pressure on the common whelk *Buccinum undatum* (Linnaeus 1758)'

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University;
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- Where I have consulted the published work of others, this is always clearly attributed;
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- Parts of this work have been published as:

Smith KE, Thatje S (2013) Nurse egg consumption and intracapsular development in the common whelk *Buccinum undatum* (Linnaeus 1758). Helgoland Marine Research 67:109-120

Smith KE, Thatje S (2012) The secret to successful deep-sea invasion: does low temperature hold the key? PLoS ONE. 7(12): e51219. doi:10.1371/journal.pone.0051219

Smith KE, Thatje S, Hauton C (2013) Thermal tolerance during early ontogeny in the common whelk *Buccinum undatum* (Linnaeus 1785): bioenergetics, nurse egg partitioning and developmental success. Journal of Sea Research 79:32-39

Smith KE, Thatje S (*in press*) The subtle intracapsular survival of the fittest: maternal investment, sibling conflict or environmental effects? Ecology http://dx.doi.org/10.1890/12-1701.1

Signed: ……………………………………………………………………..

Date:……………………………………………………………………..

ACKNOWLEDGEMENTS

During this PhD I have had the honour of working alongside many inspiring scientist at all levels of career. The encouragement and enthusiasm of each has stimulated the ideas and interests which I have built. I would like to thank my supervisors Sven Thatje and Chris Hauton for the support they have given me throughout the past three and a half years. In particular Sven, with his endless enthusiasm and drive, has been a constant source of encouragement and has provided both guidance and friendship throughout.

I would like to thank the skippers and crew of *RV Callista* and *RV Bill Conway* (University of Southampton) for help with collection of whelk eggs from the Solent, UK over the past three winters, and the fishmongers Viviers UK, who provided me with adult whelks during my PhD. Thanks also go to Erla Björk Örnólfsdóttir and the team at Vör Marine Research Center, Breiðafjörður, Iceland for helping with collection of whelk eggs from Iceland in 2011. Erla has continued to be a fantastic source for information throughout my PhD. Thanks also go to Shir Akbari at the University of Southampton for help with elemental analysis, and to other technical staff at the University of Southampton, including Matt O'Shaughnessy, Neil Jenkinson, Stephen Hayward and Jenny Mallinson, for help with endless queries, and equipment provisions and repair.

I thank every person who has read parts of this thesis, and in particular the reviewers and editors of the journals to which I have submitted published work; all of your comments have been invaluable in refining the finished product of this thesis. I would also like to thank both the Total Foundation (Abyss2100) and the Malacological Society, who provided funding for this PhD and without whom the work carried out here would not have been possible.

The Thatje lab., in which I have worked throughout my PhD, has provided an exceptional foundation for inspiring discussions, formulating ideas and preserving sanity. For that, I would like to thank each and every scientist who has been a part of the team. In particular this incudes Adam Reed, Andrew Oliphant and Alastair Brown, all of whom I have had many invaluable debates, and who have helped on numerous occasions with animal maintenance. I would like to express a special thank you to Alastair Brown, who carried out with me, the work discussed in Chapter 7. Without his enthusiasm and commitment, the work completed in this chapter would not have been possible. I would also like to thank my office mates, who have provided a sounding board throughout my PhD. In particular, Leigh Marsh; I could not have completed this without you!

Finally, I would like to thank my friends and family, all of whom have been pillars of support. Thanks go to all of you, all over the world, for tolerating me throughout, I couldn’t ask for a better bunch! Last but definitely not least, I would like to thank Mike, whom I definitely owe more than a couple of ‘dark and stormys’ to when we reach the Caribbean. You have been an absolute hero putting up with me and filling me with confidence and encouragement, especially over the last few months of write up. Thank you for helping me achieve my goal!

Chapter 1: Introduction

This thesis examines the thermal and hyperbaric thresholds affecting range extension in the marine environment. As an introduction to the topic, the first chapter reviews evolution and colonisation of the oceans, from both a historical and modern perspective. Modern species distributions are then discussed, with regard to global patterns and the variables affecting these patterns. This is followed with an examination into range expansion, with consideration for the importance of larval development, maternal investment and dispersal in marine invertebrates. Temperature and hydrostatic pressure are then reviewed, as dominant factors affecting range shifts in species. Next, Neogastropoda, and the study species for this thesis (*Buccinum undatum*) are described. Finally, the rationale, hypotheses and objectives for the body of the thesis are formulated.

1.1. Evolution and colonisation of the oceans: a historical perspective

Throughout the Phanerozoic eon (~541 Ma to present), the oceans have been subject to a number of mass extinction events (Raup and Sepkoski 1982, 1984; Jablonski 2005b). Five major events have been observed through fossil records, hypothesised to have occurred as a result of meteor impacts (Raup and Sepkoski 1984; Zachos et al 2001), solar or galactic influences (Raup and Spekoski 1984), mass volcanism (Hesselbo et al. 2002; Benton and Twitchett 2003) or severe climatic cooling (Stanley 1988). Each one, lasting approximately 1 to 10 million years each has resulted in a loss of at least 11 % of all marine invertebrate and vertebrate families (Raup and Sepkoski 1982). The largest, taking place during the late Permian (~251 Ma) eradicated 52 % of these families, resulting in a loss of more than 95 % of all marine species (Raup 1979; Benton and Twitchett 2003). The subsequent evolutionary migrations and speciations which occurred, gave rise to the marine fauna observed today throughout latitudinal and bathymetric ranges (Jablonski and Bottjer 1991; Jablonski 2005a, 2005b). Through such radiations, biodiversity continues to increase and with this, organism complexity. This has resulted in what is arguably the most diverse array of marine taxa to ever have been in existence (McShea 1996; Knoll and Bambach 2000; Carroll 2001). Recent examination of fossil records and molecular phylogeny, have revealed the evolutionary paths followed during recolonisation events, occurring over geological time periods.

These findings have led to the development of several theories concerning the evolutionary expansion of marine fauna, indicating paths primarily to exist along bathymetric or latitudinal gradients. Bathymetric migrations and speciations are understood to have occurred predominantly *via* isothermal water columns (warm or cold), through adaptation to different hydrostatic pressures (Wilson 1980; Sepkoski 1988; Young et al. 1997; Tyler and Young 1998; Thatje et al. 2005). Colonisations to new latitudes are instead thought to have occurred either by tracking of existing environmental niches during geological periods of cooling or warming or through gradual adaptation of thermal tolerance ranges (Pfenninger et al. 2007).

1.1.1. Bathymetric range expansions

Peak shifts in marine biodiversity between shallow-water and deep-sea, appear in-line with certain geological periods when isothermal waters were widespread (Kussakin 1973; Wilson 1980; Sepkoski 1988). Warm, homogenous waters existed globally throughout the Mesozoic and early Cenozoic eras (Lear et al. 2000; Zachos et al. 2001; Cramer et al. 2011). For example, during the early Cenozoic (~ 55 Ma), deep sea temperatures averaged 12 °C (maximum 16 °C), and shallow water temperatures ranged 20 to 26 °C at low latitudes and averaged 15 °C at high latitudes. The maximum vertical temperature gradient at this time was 14 °C, and across much of the surface of the earth, it was 2 to 4 °C. In comparison, the maximum vertical temperature gradient found in the modern ocean is 27 °C (Fig. 1.1) (Zachos et al. 1994; Lear et al. 2000). Although less

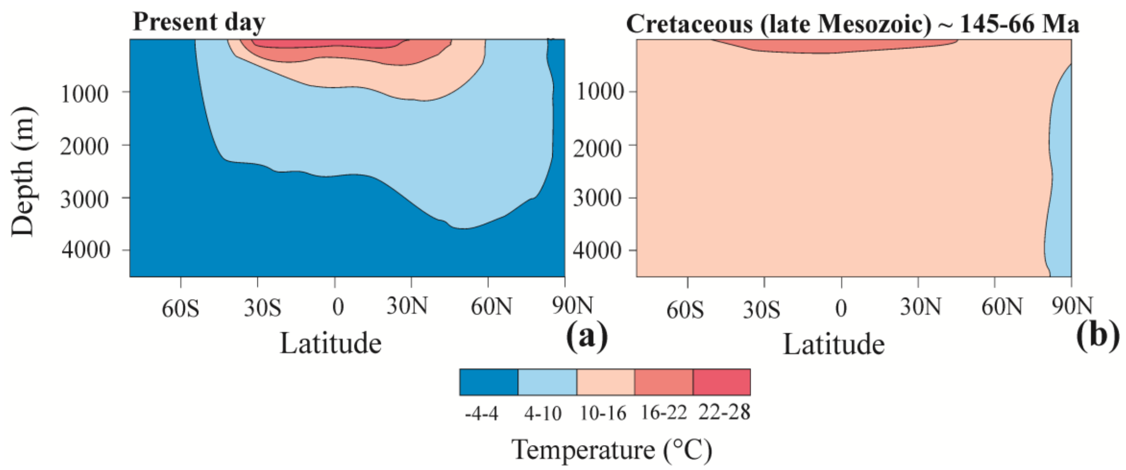

Figure 1.1. (a) present day and (b) cretaceous ocean temperatures. Profile represents temperatures at 30° west, through the centre of the Atlantic ocean. Temperature profiles are adapted from Otto-Bliesner et al. (2002).

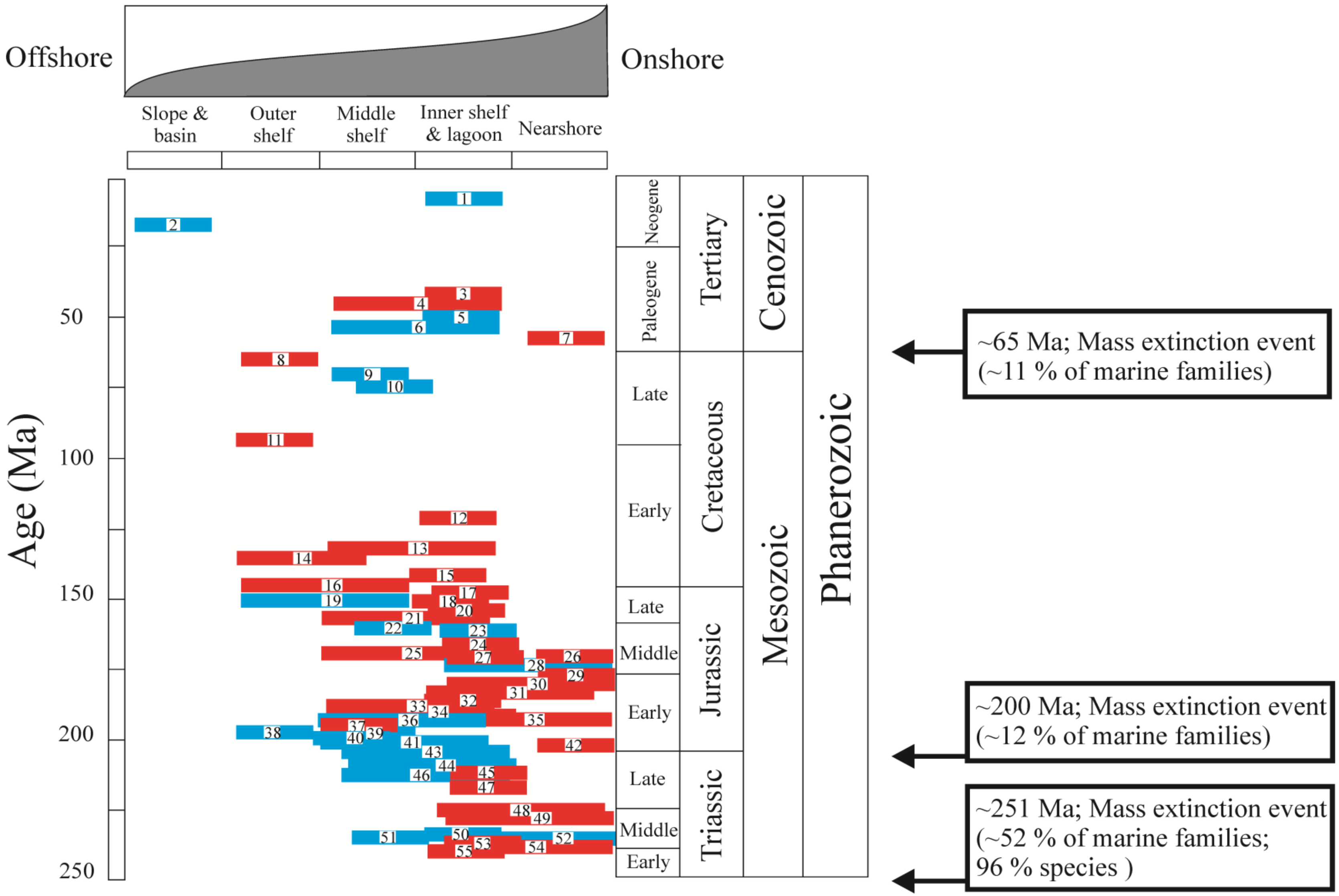
Offshore
Onshore
Slope & basin
Outer shelf
Middle shelf
Inner shelf & lagoon
Nearshore
Age (Ma)
50
100
150
200
250
Neogene
Paleogene
Late
Early
Late
Middle
Early
Late
Middle
Early
Tertiary
Cretaceous
Jurassic
Triassic
Cenozoic
Mesozoic
Phanerozoic
~65 Ma; Mass extinction event (~11 % of marine families)
~200 Ma; Mass extinction event (~12 % of marine families)
~251 Ma; Mass extinction event (~52 % of marine families; 96 % species)

Figure 1.2. Environments of first occurrence of benthic marine invertebrate orders in the fossil record. Red represents well preserved orders, blue represents poorly preserved orders. Data compiled from Jablonski and Bottjer (1991) and Jablonski (2005a). Depth estimates from Sepkoski (1988). Geological periods and mass extinction events are listed (from Raup and Sepkoski 1982; Zachos et al. 2001). 1, Stolonifera; 2, Brisingida; 3, Echinoidea; 4, Oligopygoida; 5, Nostapidea; 6, Sacoglossa; 7, Clypeasteroida; 8, Stylasterina; 9, Pennatulacea; 10, Spinulosida; 11, Bourgueticida; 12, Coenothecalia; 13, Temnopleuroida; 14, Neogastropoda; 15, Holasteroida; 16, Spatangoida; 17, Milleporina; 18, Cheliostomata; 19, Coloniales; 20, Sphaerocoelida; 21, Echinoneina; 22, Micropygoida; 23, Anaspidea; 24, Lychniscosida; 25, Arbacoida; 26, Lithonida; 27, Disasteroida; 28, Echinothuroidea; 29, Calycina (Salenioida); 30, Cassiduloida; 31, Superorder Microstomata; 32, Holectypoida; 33, Orthopsida; 34, Cyrtocrinida; 35, Cephalaspidea; 36, Velatida; 37, Pygasteroida; 38, Dactylorchirotida; 39, Paxillosida; 40, Notomyoida; 41, Forcipulatida; 42, Phymosomatoida; 43, Diadematoida; 44, Comatulida; 45, Tetralithistida; 46, Valvatida; 47, Pedinoida; 48, Thecidida, 49; Isocrinida; 50, Molpadida; 51, Aspidochirotida; 52, Trichasteropsida; 53, Scleractinia; 54, Millericrinida; 55, Encrinida.

common, isothermal water columns do continue to prevail today, for example in some isolated seas and through regions of deep-water formation in high latitude areas (Sverdrup et al. 1942; Tyler et al. 2000; Gage and Tyler 1991). In the Mediterranean, water temperatures are consistently 13 °C from 250-400 to 5000 m (Carney 2005) and in high latitude areas, temperatures typically range between 1 and 4 °C throughout the water column (Gage and Tyler 1991). Molecular phylogenetic analyses reveal close relationships between shallow-water and deep-sea species, indicating multiple high latitude radiations to have occurred (e.g. Zinsmeister and Feldmann 1984; Strugnell et al. 2008; Raupach et al. 2009; Tsang et al. 2009). For example, three independent historical bathymetric radiations have been observed in anomuran decapods (family Lithodidae) alone (Hall and Thatje 2009).

Amongs other taxa, phylogenetic relationships have also been found between shallow-water and deep-sea isopods (Raupach et al. 2009), decapods (Tokuda et al. 2006) and bivalves (Distel et al. 2000). Additionally, patterns of onshore-offshore radiations by marine benthic communities, evident through fossil records (Jablonski et al. 1983; Jablonski and Bottjer 1991) and molecular phylogeny (Weetman et al. 2006), support the proposed bathymetric migrations by shallow-water species. Many benthic marine invertebrate orders appear to have originated near-shore before radiating to deeper, offshore environments (Jablonski and Bottjer 1991; Jablonski 2005a). Onshore-offshore radiations have been observed repeatedly following mass extinction events (Jablonski et al. 1983), including in some groups which today are exclusively deep-sea (Jablonski 2005a).

While it is widely understood that existing deep-sea fauna originated primarily from shallow-water ancestry (e.g. Horne 1999; Wilson 1999), the opposite pattern has also been observed (Fig. 1.2). Neogastropoda and a handful of other benthic marine invertebrate orders first appear in the fossil record on the 'deep' outer shelf, before radiating into shallow water (Sepkoski 1988; Jablonski and Bottjer 1991; Jablonski 2005a). Phylogenetic analysis, although limited, confirms these findings, indicating several species of shallow-water stylasterid and scleratinian corals and pennatulid sea pens to have multiple deep-water origins (Dolan 2008; Lindner et al. 2008; Stolarski et al. 2011). Both origins have, however, subsequently given rise to recurrent bathyal migrations and emergences (e.g. Hall and Thatje 2009; Raupach et al. 2009).

1.1.2. Latitudinal range expansions

Latitudinal trends in speciations and colonisations in the oceans have also been identified globally. Species richness today predominantly increases from the poles to the tropics, throughout the oceans (Rex et al. 1993, 2000; Culver and Buzas 2000; Briggs 2003; Hillebrand 2004; Jablonski et al. 2006). These patterns were, however, not always evident; when the oceans were homogenous throughout, such gradients did not prevail. Latitudinal shifts in species diversity are thought to be climate-driven, occurring in parallel to global cooling events such as that in the early Cenozoic (~65 Ma) (Thomas and Gooday 1996; Culver and Buzas 2000). During these periods, latitudinal thermal gradients developed; high latitudes cooled to as low as 4 °C while low latitudes reduced only to temperatures ranging 18 to 22 °C (Zachos et al. 1994). The available habitat for existing species became limited, resulting in major niche contraction (Fig. 1.3). The speciation events which followed have given rise to the related, but latitudinally distinct, taxa found globally today (Williams et al. 2003). Over the geological period that followed, an 'out of the tropics' pattern was observed, indicating taxa to primarily originate at lower latitudes and radiate towards the poles (Jablonski 1993; Crame 2001; Jablonski et al. 2006). In particular this pattern is evident in the accelerated tropical radiation events, which took place periodically throughout the Cenozoic era, during which the speciation of many clades occurred (Crame 2001). Tropical diversifications of reef corals (Rosen 1988), bivalves (Crame 2000; Jablonski et al. 2006) and foraminiferans (Wei and Kennett 1986) are all evident in fossil records from these periods.

Although less common, inverse latitudinal diversity gradients have also been observed, showing patterns of increasing species diversity towards the poles (e.g. around

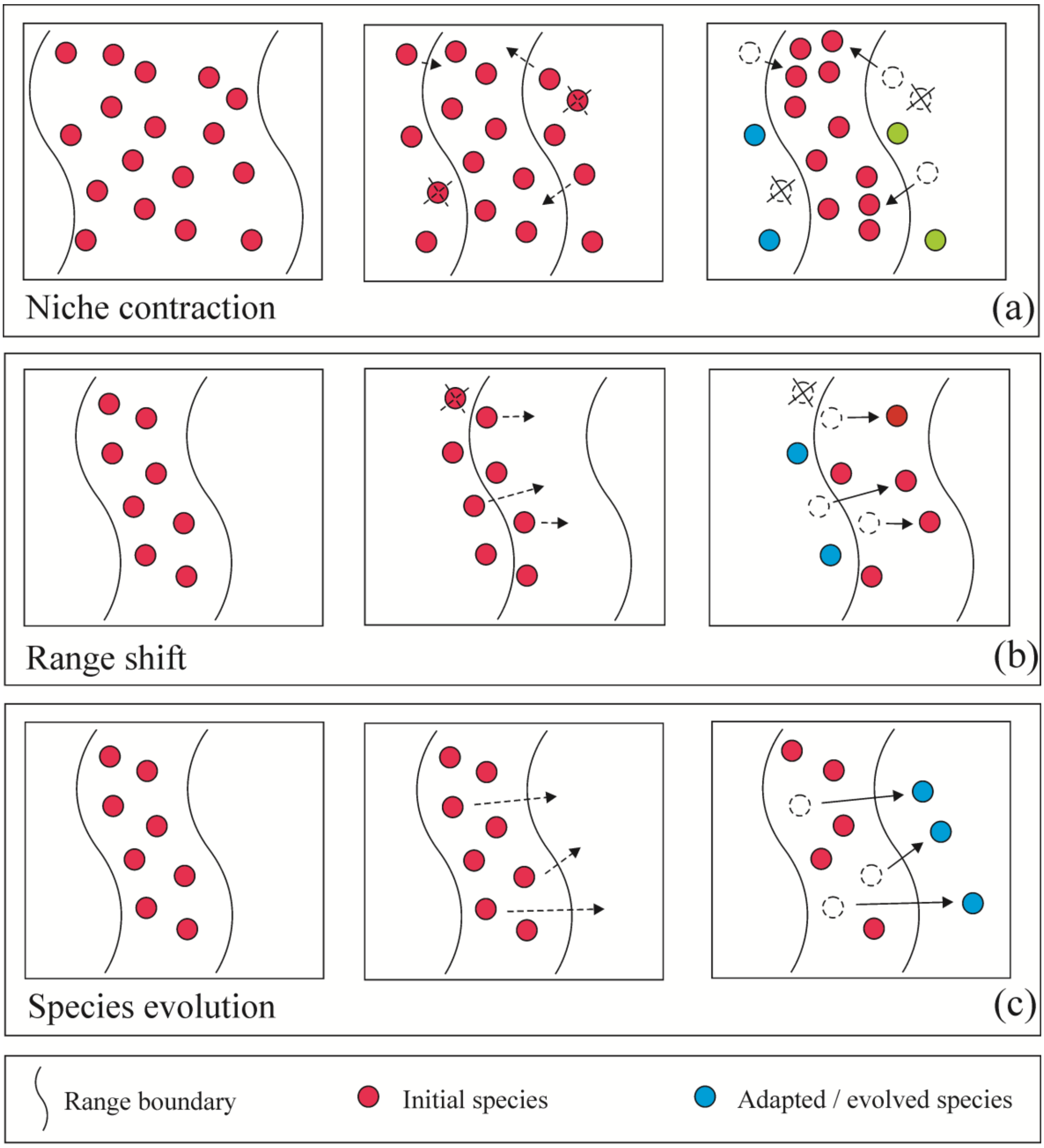

Figure 1.3. The effects of (a) niche contraction, (b) range shift and (c) species evolution. In (a) and (b), as range boundaries shift, species either migrate into a suitable niche (red circles), adapt to tolerate the conditions outside the niche (blue circles) or do not survive (crossed circles). In (c), species remain within their niche (red circles) or evolve and migrate out of the niche (blue circles). Broken lines indicate migrations which are about to occur. Solid lines indicate migrations which have occurred. Broken circles indicates absence of species.

Patagonia; Kiel and Nielsen 2010). Amongst other taxa, this trend is evident in southern hemisphere gastropods and bivalves (Kiel and Nielsen 2010). In some taxa, including southern hemisphere isopods, no latitudinal diversity gradient is seen (Rex et al. 1993). Additionally, tropical origins are not evident in all taxa. Neogastropods, although having completed multiple tropical radiations (Crame 2001 and references therein) are thought to have a mid-latitude origin (Sohl 1987; Crame 2001), and high-latitude origins of some genera of bivalves, gastropods, asteroids, crinoids, and decapods have also been shown to exist (Zinsmeister and Feldmann 1984; Briggs 2003).

1.1.3. Simultaneous bathymetric and latitudinal range expansions

Although often discussed independently, evolutionary paths identified through fossil records and molecular phylogeny indicate taxa to often radiate bathymetrically and latitudinally during the same period. Groups originating in shallow-water, high latitude areas of the southern hemisphere during the early Cenozoic radiated along both gradients simultaneously; fossil records indicate migrations to both deep-sea environments and shallow-water, lower latitude environments to have occurred concurrently (Zinsmeister and Feldmann 1984). Additionally, during major environmental events, when rapid range contractions occur, species may follow any available path in order to seek refuge. Glacial retreat theories hypothesise multiple species expansions to have occurred during periods of glacial maxima, the directions of which were both latitudinal and bathymetric (Thatje et al. 2005a, 2008; Campo et al. 2009; Kettle et al. 2011; Panova et al. 2011; Albaina et al. 2012). Phylogeographic evidence indicates some gastropods to have taken advantage of adjacent deep-water habitats (Albaina et al. 2012), and both gastropods (Panova et al. 2011) and cirripede (Campo et al. 2009) to have invaded lower latitudes in order to avoid advancing ice sheets. Following glacial retreat, these areas were recolonised through rapid adaptive radiations and increased rates of speciation, occurring along both bathymetric and latitudinal gradients simultaneously (Kiel and Nielsen 2010; Albaina et al. 2012).

1.2. Species range limits and modern range expansions

The capacity for new radiations within the oceans continues to persist today. Genetic diversification and speciations are on-going evolutionary processes and it may take taxa tens, or hundreds of millions of years to spread from the tropics to the poles, or from surface waters to the deep-ocean floor (Crame 2000; Šlapeta et al. 2006). Consequently, it is likely that many species are still in the process of radiating. Both cryptic species and sister species are understood to occur as a result of recent diversifications (Knowlton 1986; Bickford et al. 2007; Puritz et al. 2012). Modern range expansions, migrations and speciations may occur either as a result of evolutionary adaptations to changing conditions or through an environmentally or anthropogenically driven shifts of a current range (Fig. 1.3) (Pfenninger et al. 2007). Regardless, as a shift occurs, the factors determining a species range limit will change to some level.

1.2.1. Species range limits

A species range is defined by its physiological tolerance of the ecological factors that it is exposed to throughout its lifetime (Bohonak 1999; Sexton et al. 2009; Bozinovic et al. 2011). These may include a number of physical, chemical and biological variables which collectively create invisible barriers that mark the boundaries of the potential niche. Initially defined by Elton (1927), the niche concept was later popularised by Hutchinson (1957), who described a niche as a multi-dimensional hypervolume. On a global scale, each species generally has a range in which it can survive (fundamental niche) and a narrower tolerance window in which it thrives (realised niche) (Hutchinson 1957; Pörtner 2001). On a local scale, a range may vary between populations of the same species, or between ontogenetic stages, within one population (e.g. deRivera et al. 2007; Storch et al. 2009). Subsequently, tolerance levels can fluctuate between life history stages or across a species distribution. Population-level differences in thermal tolerance (Storch et al. 2009; Zippay and Hofmann 2009; Sorte et al. 2011), depth range (Macpherson 2003), host selection (Trowbridge and Todd 2000; Sotka 2005), and predation response (Stachowicz and Hay 2000; Cotton et al. 2004; Freeman and Byers 2006) have been observed in a range of marine invertebrates (reviewed in Sanford and Kelly 2011). Variations in depth range (Tyler and Young 1998; Howell et al. 2002) and thermal (Dawirs 1985; deRivera et al. 2007; Gosselin and Chia 1995; Weiss et al. 2009b), CO_2 (Kurihara and Shirayama 2004; Kurihara et al. 2007) and salinity tolerance (Qiu et al. 2002) with ontogenetic stages have also been reported. The scope of these variations differs between species and between populations; for example, populations of the edible mussel *Mytilus edulis* (Linnaeus 1758) show differences in shell-thickening responses to predator cues (Freeman and Byers 2006). When considering depth ranges, a wider bathymetric distribution is observed during early ontogeny in some echinoderms, while in others, it occurs during adult life (Howell et al. 2002). Similarly, many copepods undergo ontogenetic vertical migration (OVM) during their life history, whereby the eggs of deep-water species complete development in shallow waters. While in some species, females migrate to the surface in order to spawn, in others, eggs are laid at depths of 1000 to 2000 m before transportation to the surface for development (Yoshiki et al. 2011). The opposite pattern has been observed in Antarctic krill; adults inhabit shallow water but eggs sink to depths of 2000 m to hatch (George 1984). Patterns of vertical migrations to shallow water have been hypothesised to occur to allow larvae to develop in warmer, food rich waters and to aid dispersal (e.g. Kobari and Ikeda 1999, 2001; Yoshiki et al. 2011). Ontogenetic migrations to deep water have instead been suggested to occur as a result of low pressure tolerance during early ontogeny (George 1984).

1.2.2. Modern marine range expansions

Many modern marine migrations occur in response to anthropogenic impacts, which are taking place globally. These have the potential to destroy or create marine habitats, affecting both shallow-water and deep-sea environments. Impacts may include pollution, resource exploitation and introduction of alien species, each of which can result in a shift in niche availability and faunal composition (McClain and Hardy 2010; Aronson et al. 2011; Connell et al. 2011). Over recent decades, the effects of overfishing have led to shifts in fish populations (e.g. Rudstam et al. 1994; Sparholt 1994), and habitat destruction of coral reefs, kelp forests and seagrass beds have led to rapid recolonisation of such areas with new faunal assemblages (Connell et al. 2011). Regardless of the cause, these shifts have the potential to cause major ecosystem perturbation as new niches become available and faunal assemblages change (Cheung et al. 2008, 2009). Additionally, rates of migration vary between species; range shifts in introduced species (i.e. species who's introduction into a particular location which they would be unlikely to reach on their own, has been directly mediated by humans, either deliberately or inadvertently) occur at approximately double the rate of native species, potentially filling available habitat and preventing native species from successfully migrating (Sorte et al. 2011).

Recently, a period of rapid species radiation has become evident in response to current trends of ocean warming. The world's climate is currently warming, resulting in global increases in sea water temperatures (Barnett et al. 2005; Harley et al. 2006; IPCC 2007). Median increases in sea surface temperature are currently 0.07 °C *per* decade (Burrows et al. 2011), with some areas being more affected than others. In the UK and north-east Atlantic for example, temperatures have risen by 0.2 – 0.8 °C *per* decade (Hughes et al. 2010; MCCIP 2010). Recent predictions suggest the oceans will continue to warm until at least 2080 (Hughes et al. 2010; MCCIP 2010). Many recent migrations have been observed in response to these current trends, and some migrations already occurring in response to anthropogenic impacts have been enhanced (Walther et al. 2009; Sorte et al. 2010). Climate-driven range shifts have been observed over periods of decades, with a variety of echinoderms (Ling et al. 2008), crustaceans (Southward et al. 1995; Beaugrand et al. 2002) and gastropods (Zacherl et al. 2003) showing poleward shifts in species distributions. Additionally, both latitudinal and bathymetric shifts have been observed in marine fish over a period of just 25 years; while some species have moved polewards, others have migrated to deeper water in order to avoid increasing temperatures (Perry et al. 2005; Dulvy et al. 2008; Nye et al. 2009). Bathymetric range expansion or speciation is also thought to be occurring today in the echinoid *Gracilechinus acutus* (Lamark 1816) (Tyler and Young 1998; Minin 2012). In this species, both shallow (< 100 m) and deep (~ 1000 m) water populations exist. The particularly wide pressure tolerance observed in embryos from the deep-water population has led authors to hypothesise this species to be in the process of radiating into the deep-sea. In summary, species expansions continue to occur in multiple directions. The dynamics dictating the environmental gradient tracked during such migrations remain poorly understood.

1.3. Modern species distributions: significant variables and global patterns

Global patterns in species distribution are dependent on many variables (Sexton et al. 2009; Bozinovic et al. 2011). Over geological time periods the dominant factors affecting distribution have changed. For example, temperatures have shifted from being latitudinally and bathymetrically near isothermal during the Mesozoic and early

Cenozoic (Fig. 1.1) (Lear et al. 2000; Zachos et al. 2001; Cramer et al. 2011), to varying by up to 27 °C between high and low latitude surface waters today (Nikolaev et al. 1998). With this thermal shift, surface productivity also changed, from being relatively continuous during periods of global isothermy to seasonally fluctuating today (Thomas and Gooday 1996; Culver and Buzas 2000). In the modern ocean, these variables, and others (e.g. O_2 levels; Rhoads and Morse 1971; Brewer and Peltzer 2009), have led to distinct trends in bathymetric and latitudinal species assemblages which are evident globally. Of the many factors affecting this, two stand out as prominent in today's oceans; temperature and hydrostatic pressure. Temperature shifts with latitude, and both temperature and pressure change with depth.

Hydrostatic pressure increases by 1 atmosphere (atm) with every 10 m of water depth. This not only creates the longest continuous environmental gradient on the planet (Tyler et al. 2000), but is also the only variable in the ocean to change consistently with depth (Carney 2005). Excluding variations in sea level, pressure gradients have remained the most constant variable throughout the evolution of fauna. Thermal gradients in shallow water currently vary substantially (Nikolaev et al. 1998), and although relatively homogenous at high latitudes, throughout equatorial areas, shift by as much as 20 °C in the top 1000 m of water depth (Fig. 1.1b) (Sévellec and Fedorov 2011). Below 1000 m water temperatures average less than 4 °C (Tyler 2003). Both temperature and hydrostatic pressure create substantial physiological challenges throughout the oceans. Each factor is a prominent ecological variable, and therefore, should be considered significant in defining latitudinal and bathymetric shifts in marine fauna. Traditionally, however, temperature has been regarded as the most important factor affecting species adaptations. Consequently, emphasis has been placed on understanding the physiological effects of temperature on marine invertebrates (e.g. Pörtner et al. 2005; Pörtner 2001, 2006, 2010; Somero 2010; Tomanek 2010; Hoffman and Sgrò 2011; Sunday et al. 2011), with much less consideration being given to the effects of hydrostatic pressure (e.g. Somero 1992; Pradillon and Gaill 2007; Pradillon 2012).

1.3.1. Global patterns in latitudinal distribution and thermal tolerance

A gradient in latitudinal species diversity is evident globally, peaking at the tropics and continually reducing towards the poles (Jablonski et al. 2006; Roy et al. 1998). This cline is stronger in the northern hemisphere than the southern hemisphere (e.g. Gray 2001). Across this gradient, species range sizes vary and a poleward increase in range size has been hypothesised to occur (Rapoport's rule; Stevens 1989). This rule continues to be widely debated and today, is thought to be accurate only on a local scale (e.g. Roy et al. 1994; Gaston 1996; Rohde 1996; Gaston et al. 1998; Addo-Bediako et al. 2000; Macpherson 2003; Witman et al. 2004; Calosi et al. 2010). Latitudinal trends in thermal tolerance ranges do, however, appear to be evident in ectotherms. Thermal tolerance ranges generally shift with latitude, with tropical species predictably tolerating higher temperatures than polar species (Somero 2005; Peck et al. 2009; Somero 2010; Sunday et al. 2011). Typically, high- and low-latitude species, which annually experience low temperature variability, live close to their lower and upper thermal limits respectively. Consequently, such species often have a narrow thermal capacity outside this (Stillman 2003; Gilman et al. 2006; Calosi et al. 2008; Deutsch et al. 2008). In contrast, mid-latitude species which are annually exposed to much larger temperature fluctuations, have a wider thermal range (Somero 2010). A narrowing of range breadth has also been observed during early ontogeny (Thorson 1950). For example, larvae of the common shore crab *Carcinus maenas* (Linnaeus 1758) exhibit a narrower thermal tolerance than adults from the same population (deRivera et al. 2007). Regardless, few ectotherms can tolerate temperatures much beyond their natural thermal range; outside this, the onset of physiological disruption tends to be rapid. Patterns exist both globally (between species) and locally of a positive relationship between temperature and a range of physiological processes including growth (Lima and Pechenik 1985; Hoegh-Guldberg and Pearse 1995; Pörtner 2002), development (Johns 1981; Anger et al. 2003) and metabolism (Childress 1995). Within a species distribution, population specific thermal tolerance ranges are regularly observed in marine invertebrates. Such patterns have been seen in a range of species including bivalves (Jansen et al. 2007) gastropods (Gosselin and Chia 1995; Kuo and Sanford 2009; Zippay and Hofmann 2009), crustaceans (Anger et al. 2003; Storch et al. 2009) and echinoderms (Stanwell-Smith and Peck 1998; Sewell and Young 1999).

While latitudinal trends in thermal tolerance ranges indicate mid-latitude adults to have a higher range-shift capacity than species from other areas or life history stages, a narrow thermal tolerance range does not necessarily limit the potential for a species to exhibit range shift. Seasonal variations in thermal response indicate some species to be capable of achieving thermal acclimation as habitat temperatures change (Jansen et al. 2007). Thermal phenotypic plasticity may in turn allow a species the capacity to shift its thermal limit (Stillman 2003; Ravaux et al. 2012). However, this capacity varies with species, and possibly with latitude; while thermal acclimation was successfully achieved in temperate decapods over a 4 month period, positively shifting thermal limits (Ravaux et al. 2012), acclimation ability has been reported to vary between several polar marine invertebrates, with some species showing a reduction in thermal tolerance over equivalent durations (Peck et al. 2009). Although giving different responses, both studies highlight the importance of thermal acclimation and of long experimental duration, without which, such results may not have been evident.

1.3.2. Global patterns in bathymetric distribution

Although less widely researched, bathymetric ranges appear to shift with depth, in a similar manner to thermal tolerance with latitude. Most marine species have upper and lower depth limits, of which the size may vary from tens of metres to several hundred metres (Tyler and Young 1998; Pradillon and Gaill 2007). For example, a study of 44 species of North Atlantic asteroids indicated each one to have a depth range which typically covered 500 to 1000 m in width. Within this, a narrower (200-300 m) band of maximum abundance was observed for each species (Howell et al. 2002). Distinct bathymetric patterns of faunal assemblage have been reported on many occasions (Carney 2005). In the North Atlantic, a unimodal trend of species diversity has been observed, reaching a maximum at approximately 1250 m (Etter and Grassle 1992). This correlates well with areas of high species turnover which have been consistently reported to occur between approximately 1000 and 1700 m, in the North Atlantic (Billett 1991; Gage et al. 2000), Western Mediterranean (Cartes 1993) and South Pacific (Hayward et al. 2002). Additional smaller areas of faunal change have been reported at depths of between 2000 and 3000 m or deeper. Carney (2005) conceptualised zonation boundaries comprising of 3 separate species domains for continental margin (100 to 4000 m) fauna. These included upper boundary biota which occurred predominantly in the top 2000 m, lower boundary biota which appeared in large numbers at 1250 m, but predominantly existed below 3250 m, and inter-boundary biota which occurred between, but not extending to, upper or lower boundaries. Unlike latitudinal trends, where thermal tolerance ranges of species are relatively well identified, patterns of depth range have principally been established through the absence or presence of species, and the pressure tolerance of species from different depths remains poorly understood. Hydrostatic pressure is rarely used to explain patterns of species abundance and because bathymetric faunal shifts fit, in part at least, to bathymetric thermal shifts, changes in species assemblage are typically attributed to temperature alone. In areas of isothermy, for example the Mediterranean, patterns have instead been hypothesised to occur in response to food availability and sediment type (for review see Carney 2005).

Research into pressure physiology remains limited and consequently, evidence of shifts in pressure tolerance with bathymetric range are largely unknown. Pressure tolerances vary with both species and ontogenetic stage (Young and Tyler 1993; Tyler and Young 1998; Tyler et al. 2000; Howell et al. 2002; Pradillon and Gaill 2007; Pradillon 2012) and although data are rare, evidence also indicates population specific tolerances to exist amongst species. For example, the pressure tolerance of embryos of *G. acutus* is outside the adult bathymetric distribution for both shallow and deep water populations. Amongst the embryos, the greatest pressure tolerance of all is shown in those from the deeper living population (Tyler and Young 1998). Pressure tolerances during early ontogeny which exceed the adult distribution range for the same species (and population), have been observed in several echinoderms (e.g. Young et al. 1997; Tyler and Young 1998; Benitez-Vilallobos et al. 2006) and molluscs (Mestre et al. 2009, 2012). These ranges may give important clues about both a species ancestry, and its potential for future range expansion. In many species, the optimum period for dispersal is during the larval stages. Consequently, the wider pressure tolerance observed during this stage has been hypothesised to indicate a high potential for bathymetric range expansion (e.g. Tyler et al. 2000; Benitez-Vilallobos et al. 2006; Aquino-Souza et al. 2008; Mestre et al. 2009).

Interestingly, a latitudinal comparison of species depth ranges indicates a widening of bathymetric ranges in cold, isothermal, high latitude areas (Macpherson 2003). Although this trend is predominantly based on fish it may lend support for hypotheses indicating bathymetric range expansions to occur predominantly through isothermal water columns (e.g. Sepkoski et al. 1988; Tyler and Young 1998; Thatje et al. 2005a).

The dynamics behind bathymetric migrations and emergences remain poorly understood. In contrast, factors affecting latitudinal shifts are well researched. While thermal tolerance is an important factor affecting bathymetric shifts, it is essential that emphasis is also placed on understanding the significance of hydrostatic pressure and the limits it may impose on bathymetric trends.

1.4. Means of range expansion: the importance of larval development, maternal investment, and dispersal

A species potential for range expansion is in part related to its ability to disperse. In many species dispersal is significantly influenced by the developmental strategy employed. While mobile species may migrate throughout their life history, in benthic sedentary species, the dispersal potential of an adult is limited. Consequently, the capacity for range expansion is typically greatest during larval development (Cowen and Sponaugle 2009).

1.4.1. Modes of larval development

Although many intermediate modes of development have been recognised in marine invertebrates to date, two primary modes of development are know; pelagic and benthic. These modes differ in egg-to-juvenile development time, reproductive effort *per* offspring, relative fecundity and dispersal capacities (Jablonski 1986; Pechenik 1999; Grantham et al. 2003; Fernández et al. 2009).

Species undergoing pelagic development generally have a long larval stage, during which extensive dispersal by ocean currents, over broad geographic ranges may occur (Strathmann 1985; Pechenik 1999; Thatje 2012; Young et al. 2012). Typically, each mother produces a large number of small offspring, which develop, swimming or floating in the plankton, until metamorphosing to the juvenile stage (Pechenik 1999). These larvae most commonly obtain nutrition from consumption of smaller plankton (planktotrophic), but may also rely on resources allocated by the mother, direct to the egg (lecithotrophic) (Vance 1973; Pearse 1994; Poulin et al. 2001; Young 2003; Kamel et al. 2010). The passive dispersal mechanism of this developmental mode infers larvae are likely to be exposed to a broader range of environmental conditions than are benthic larvae from a similar environment. Consequently, pelagic larvae are typically more resilient to environmental stresses and exhibit stronger phenotypic plasticity (Sotka

2012). Survival, however, is often reduced and many individuals are lost in the plankton during development.

Benthic larvae are essentially non-motile during development. Offspring are typically brooded by an adult, or develop inside a benthic egg mass or individual egg capsules of some description attached to a hard substrate (Vance 1973; Hoskin 1997; Poulin et al. 2001). Often, no pelagic stage occurs (direct development) and any which does is brief (mixed development). Although this typically results in a limited larval dispersal capacity (Jablonski 1986; Poulin et al. 2001), embryos develop with some level of protection, usually from capsule walls or from a parent. Therefore, the chance of survival is often higher for benthic larvae than for pelagic larvae. Benthic larvae are lecithotrophic, typically with nutrition supplied either in the form of nurse eggs stored inside a capsule with developing embryos, or as yolk, provisioned directly to each egg upon production (Thorson 1950; Poulin et al. 2001; Kamel et al. 2010). Some nutrition may also be gained from intracapsular fluid (Bayne 1968; Moran 1999; Pechenik et al. 1984; Stöckmann-Bosbach 1988) or capsule walls (Ojeda and Chaparro 2004). Larval dispersal, and consequently range shift potential, is usually reduced under non-planktotrophic development, but offspring typically hatch at a larger size, potentially improving their chances for survival (Bernardo 1996; Moran and Emlet 2001; Marshall and Keough 2006).

Both pelagic and benthic embryos develop through morphologically distinct larval stages, which are typically different to their adult form. Regardless of whether they are feeding or non-feeding, the rapid growth, periods of metamorphosis and production of exoskeleton which occur during development are energetically expensive, suggesting ontogeny to be a ‘costly’ life history stage. The number and duration of these stages vary between species, and both bathymetric and latitudinal trends exist globally. In shallow water, latitudinal trends indicate rates of development to largely increase from the poles to the tropics, as temperature increases. Similar bathymetric patterns are observed, with developmental rates typically decreasing with depth (Clarke 1983, 1992; Hoegh-Guldberg and Pearse 1995; Stanwell-Smith and Peck 1998; Anger et al. 2003, 2004). Additionally, in high-latitude and deep-sea environments, larval development may be abbreviated, with larvae developing through a reduced number of stages before reaching metamorphosis (Wilson and Hessler 1987; Anger et al. 2003; Lovrich et al. 2003; Thatje et al. 2003, 2004; Buhl-Mortensen and Hoeg 2006).

1.4.2. Maternal investment

While mode of development has the potential to affect range shift, offspring fitness is also of fundamental importance. This is related to maternal investment, and survival is traditionally understood to increase with increased allocation of resources to offspring (Rivest 1983; Moran and Emlet 2001; Marshall et al. 2006). Here, an energetic trade-off occurs between offspring size and number, and life history theory principally suggests a negative correlation to exist between the two (Vance 1973; Smith and Fretwell 1974). Initially, this was simplified and showed mothers to produce either many small, or few large offspring (Smith and Fretwell 1974). This simplification can be related to benthic and pelagic reproductive traits; although not universal, trends indicate species exhibiting pelagic development to produce a greater number of smaller offspring, each of which is allocated a small amount of reserves. Conversely, in species exhibiting benthic development, maternal investment *per* egg is greater, resulting in a smaller number of larger offspring (Vance 1973; Pearse 1994). There is a great deal of flexibility in this, however, and contemporary life history theory recognises that 'optimal' offspring size varies with environmental conditions (McGinley et al. 1987; Bernardo 1996; Mousseau and Fox 1998). Even within a species, the size and number of offspring produced has been shown to differ. For example, a linear relationship is typically observed between female size, and number and size of offspring, and across a species range, a larger number of smaller offspring are usually found with increased developmental temperature (Hadfield 1989; Marshall and Keough 2007; Crean and Marshall 2009; Kamel et al. 2010). Consequently, global patterns exist, for example in both high-latitude and deep-sea environments, a trend of increasing egg size (or energetic investment *per* egg) have been observed in a range of species. This has been reported across all reproductive modes, indicating an increase in maternal investment *per* offspring (Fig. 1.4) (Thorson 1950; King and Butler 1985; King 1987; Van Dover and Williams 1991; Young et al. 1997; Young 2003). In these particularly hostile environments, an increase in lipid supply to the offspring helps facilitate successful development and survival (Anger et al. 2002). Such trends are typically attributed to shifts in temperature, which occur with both increasing depth and latitude (Killingley and Rex 1985; King and Butler 1985; Kobari and Ikeda 2001; Anger et al. 2003; Young 2003; Morley et al. 2006; Rosa et al. 2007).

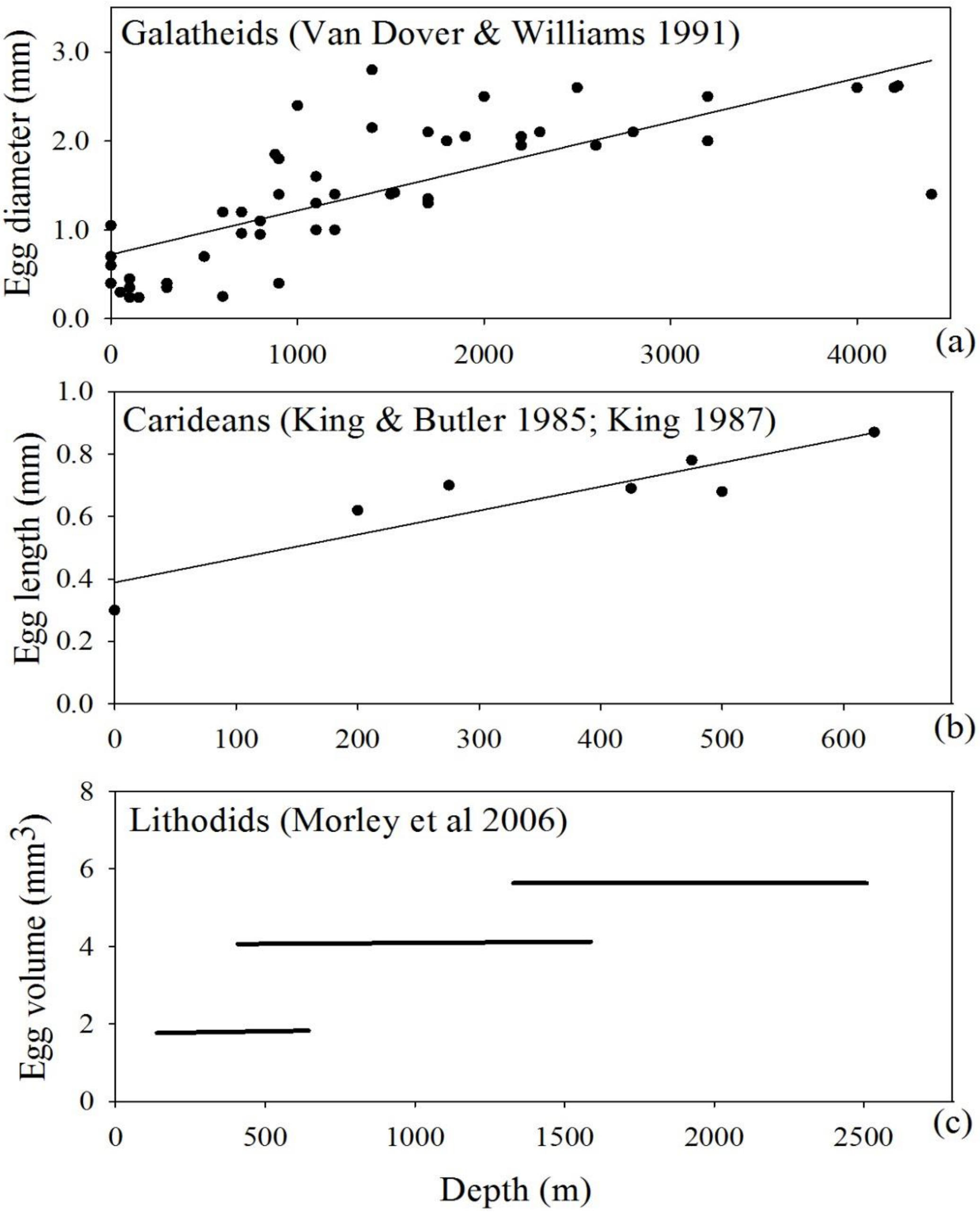

Figure 1.4. Trends of egg size with depth for crustaceans. (a) galatheid crabs, (b) caridean shrimp, (c) lithodid crabs. Data compiled from referenced studies. NB: note differing scales *per* plot on x-axis.

1.4.3. Dispersal

The relationship between developmental mode and dispersal potential is often reflected in a species geographic distribution. While benthic larvae generally have a reduced dispersal capacity due to limited mobility during development, pelagic larvae in contrast typically have a high dispersal potential as a result of time spent in the plankton. Consequently, patterns suggest species exhibiting benthic larval forms to have a much smaller geographic range than pelagic larval forms (Jablonski and Lutz 1983; Young 2003). However, although less common, the opposite pattern can also be found. For example, a broader geographic distribution has been shown in brooding gastropods when compared to closely related species exhibiting pelagic development (Johannesson 1988). Additionally, a study examining 215 species of echinoderm found range size to differ between species exhibiting pelagic feeding and pelagic lecithotrophic developmental modes but not between those exhibiting pelagic lecithotrophy and benthic development (Emlet 1995).

Patterns linking developmental mode and dispersal potential, although widely observed, are not universal. In reality, there are large variations in dispersal capacity between species exhibiting benthic and pelagic larval forms. Pelagic development increases the likelihood of a broad dispersal, but it does not guarantee it. Distances travelled by larvae are dependent on time spent in the plankton and local water movement. For example models indicate pelagic larval duration to vary from 3 weeks to 2 years between different species in the Gulf of Mexico alone, allowing for dispersal distances of 300 km to more than 5000 km (Young et al. 2012). In any area, however, the actual distance travelled by larvae varies depending on annual conditions and position in the water column. The scope for variety is huge, with some species covering thousands of kilometres, while others cover only a few metres before metamorphosing (for review see Shanks et al. 2003). Additionally, the total distance travelled may not reflect the horizontal range covered. For example some deep-living species have pelagic larvae which travel several km up and down the water column during development but cover only a limited horizontal area (Kobari and Ikeda 1999, 2001).

While species exhibiting benthic larval development typically have a reduced dispersal potential, this reproductive strategy does not completely inhibit dispersal during development. Brooded larvae inevitably cover the same geographic range as that travelled by the parent through development and in gastropods, the same mobilisation may occur through attachment of egg capsules to the shell of a parent or another species (Kamel and Grosberg 2012). Broader geographic dispersal may also be achieved through rafting of adults or egg masses (Highsmith 1985, Higgs et al. 2009); one species of brooding bivalve has been observed to travel up to 2000 km using this method of dispersal (Helmuth et al. 1994). Additional methods of dispersal by species exhibiting benthic larval development include drifting of juveniles in currents using extruded mucus threads (Martel and Chia 1991) and hitchhiking inside fish before passing alive through the guts (Domaneschi et al. 2002).

Globally, trends in reproductive strategy and dispersal are evident within the oceans, varying both latitudinally and bathymetrically. Gunnar Thorson first suggested such patterns (1936, 1950). His most famous proposal, of pelagic development being rare, if not absent in high latitude and deep-sea regions (Thorson's rule), where temperatures and productivity are typically low, has sparked numerous discussions and all subsequent theories explaining patterns of larval development, have been built on these initial suppositions. Trends recognised today show pelagic development (feeding or non-feeding) to occur throughout the oceans, although the incidence of pelagic larval forms remain most prevalent in tropical and temperate shallow waters (Clarke 1992; Pearse 1994; Marshall et al. 2012). In these areas, the rapid growth rates and short generation times, which are typical of warm waters aid speciation rates, lending support for the 'out of the tropics' diversification theory (Jablonski 1993; Jablonski et al. 2006; Mittelbach et al. 2007). In contrast, the slow development and extended dispersal periods typical of cold water development is likely to increase generation time and reduce speciation rates (Young et al. 1997; Young 2003).

In high-latitude and deep-sea environments, the occurrence of benthic larvae typically continues to outweigh the occurrence of pelagic larvae. Echinoderms, which appear to be the one group to not fit this pattern, instead have a particularly high proportion of non-feeding larvae relative to feeding larvae (Stanwell-Smith et al. 1999). Recent observations indicate pelagic lecithotrophy, such as that observed in echinoderms, to be more common in the deep sea than previously realised. Consequently, this has become increasingly recognised as a dominant reproductive mode in this area (Pearse 1994; Sewell et al. 1997; Young et al. 1997; Young 2003). Pelagic lecithotrophy in both high-latitude and deep-sea species, reduces risk of starvation during development, whilst still allowing the potential for dispersal between geographic ranges. In echinoderms, the lecithotrophic eggs are buoyant, and potentially move extended distances through the water column during development (OVM) (Young and Cameron 1987; Cameron et al. 1988; Young 2003). Ontogenetic vertical migration is also often employed as a reproductive strategy in species with feeding planktotrophic larvae in the deep sea

(Bouchet and Warén 1979, 1994; Killingley and Rex 1985; Kobari and Ikeda 1999, 2001; Yoshiki et al. 2011). Larvae are typically exported as much as several kilometres up through the water column to a depth where plankton is available for feeding, and often develop in the top 100 of water. These patterns indicate significant links to exist between shallow and deep-water habitats, with species occupying both environments during their life history. This highlights the importance of understanding the dynamics contributing to range shifts and species distribution limits.

1.5. The dominant factors affecting species range shifts: temperature and hydrostatic pressure

When considering evolutionary migrations and speciations, temperature is the dominant factor affecting latitudinal radiations while both temperature and pressure affect bathymetric radiations.

1.5.1. Physiological effects of temperature and hydrostatic pressure

Temperature and pressure both have the potential to cause critical physiological disruptions in marine fauna, at exposures outside their specific tolerance levels. The effects of these two variables are often antagonistic and physiological disruptions caused by increases in one, are similar to those caused by decreases in the other (Pradillon and Gaill 2007). As an example, a temperature increase of 2.7 °C has been shown to reverse the effects of a pressure increase of 100 atm on membrane fluidity (deSmedt et al. 1979), and a temperature increase of 13 to 21 °C, of counteracting a pressure increase of 1000 atm (Somero 1992). The effects of temperature and pressure may shift with population (Tyler and Young 1998; Storch et al. 2009; Zippay and Hofmann 2009; Sorte et al. 2011), and with ontogenetic stage (Howell et al 2002; Anger et al. 2004; Weiss et al. 2009; Yoshiki et al. 2011). Amongst other things, these factors may impact aerobic scope (Frederich and Pörtner 2000; Pörtner 2001, 2002), metabolic processes (Clarke 1993; Childress 1995; Pörtner 2004; Peck et al. 2008), enzymatic reactions (Macdonald 1984; Balny et al. 1989; Somero 1992), gene expression (Bartlett et al. 1995; Pradillon and Gaill 2007), and membrane based processes such as membrane fluidity (Behan et al. 1992; Pradillon and Gaill 2007). For example, as an organism approaches the limits of its thermal tolerance range, aerobic scope is often quickly impaired, through a mismatch of oxygen supply and demand (Frederich and Pörtner 2000; Pörtner 2001, 2002). In response to high pressure, a rapid onset of high pressure neurological syndrome is regularly seen; motor activity becomes compromised leading to the onset of tremors, spasms and paralysis, which become irreversible over time (Macdonald 1997). Even if the initial physiological impacts of temperature and pressure are not lethal, they generally affect processes such as growth and reproductive success, potentially influencing the survival of a population (Pörtner et al. 2004).

1.5.2. Adaptations to temperature and hydrostatic pressure

When any environmental variable shifts within a habitat, adaptation is necessary for survival and for a species migration to be successful all life history stages must tolerate the conditions of the new habitat. Considering this, shifting ecological factors can either promote, or inhibit speciation events. Adaptations may occur on the basic cellular to whole animal level. Amongst other things this may include changes in growth and metabolic rate, reproductive strategy and predator defence mechanism (Jablonski 1986; Clarke 1993; Childress 1995; Cotton et al. 2004). While most species show some degree of plasticity in their physiological tolerances within one generation, essentially the rate at which adaptations occur may vary by years, decades or millennia, and over multiple generations.

Studies comparing related fauna inhabiting different bathymetric and latitudinal ranges, have allowed us an understanding of the adaptations that enable species to inhabit such environments. Since low temperature and high pressure are comparable in the physiological impairments that they cause, similar adaptations occur in response to either variable. A general delay in physiological processes (Thiel et al. 1996) is evident in both low temperature and high pressure (deep sea) environments, including a decrease in metabolic rate (Childress 1995; Seibel et al. 1997; Company and Sardà 1998; Gillooly 2001; Drazen and Seibel 2007), and in relation to this, a lower energy requirement (Mickel and Childress 1982). Such shifts predominantly occur as a function of temperature (Childress et al. 1990; Childress 1995) or locomotory capacity (Ikeda 1988; Childress et al. 1990; Company and Sardà 1998; Seibel and Drazen 2007) and pressure is rarely regarded as a significant factor affecting evolutionary adaptations (but see Seibel and Drazen 2007). While it is beneficial to understand such adaptations, the physiological impacts from which they have arisen can be appreciated by examining the effects of temperature and pressure on organisms at levels which are outside the range of natural exposure. Measures of physiological performance in response to changes in temperature or pressure typically include analysis of behaviour or respiration rates over short experimental durations. Over longer durations, developmental stage or weight are commonly used as a proxy for growth, and offspring number and survival as a proxy for fecundity.

1.5.3. Previous investigations into the combined effects of temperature and pressure: a brief overview

The short-term effects of temperature and pressure are regularly observed to be antagonistic, with increases in temperature and decreases in pressure showing similar responses (Somero 1992; Balny et al. 1997; Pradillon and Gaill 2007). Consequently, a species combined tolerance to these two factors may be vital in determining the upper and lower limits in its vertical distribution (Thatje et al. 2010). Current data examining the combined effects of temperature and pressure on marine invertebrates, which have predominantly been carried out on shallow-water species, support these findings; pressure sensitivity has typically been noted to increase with decreasing temperature. In addition, temperature has been consistently observed to be the dominant of the two factors (e.g. Thatje et al. 2010; Oliphant et al. 2011).

A number of investigations have been carried out examining the combined effects of temperature and pressure on marine invertebrates. Here, I consider investigations which have been carried out on shallow-water species. Early studies examining the effects of hydrostatic pressure on marine invertebrates investigated pressures greater than those experienced in the oceans (≥ 2000 atm; ≥ 20000 m depth) over short periods of seconds or minutes (e.g. Macdonald et al. 1972; Menzies and George 1972). Whilst an achievement at the time, such studies gave unrealistic responses when compared to more recent studies and therefore only those with duration of one hour or more are discussed. Typically, studies have focused on echinoderms and crustaceans, and have generally exercised a small number of acute treatments (temperature and pressure), not exceeding 50 hours in duration (Table 1.1). Only one study has significantly exceeded this, completing one impressive 28 day exposure to 100 atm (10 °C) (Cottin et al. 2012) in an adult crustacean. To date, both experimental duration and the potential for acclimation to experimental pressure, has been considerably restricted by the limited availability of high pressure aquariums. Small, isolated pressure vessels attached to manual hydraulic pumps only allow rapid build-up and release of pressure, and although still widely used, levels of accuracy and ability to retain pressure have varied considerably throughout their lifetime (for review see Pradillon and Gaill 2007). Additionally, such acute treatments likely lead to either an underestimation or overestimation of physiological tolerance levels. It is only during recent decades that re-circulating pressure systems have been developed allowing the exchange of water under controlled thermal and hyperbaric conditions (Pradillon and Gaill 2007). Modern systems (e.g. IPOCAMP; Shillito et al. 2001) provide increased pressure stability and experimental duration, and allow a gradual build-up and release of pressure.

The phenotypic plasticity associated with thermal acclimation, which may have the capacity to shift tolerance (Ravaux et al. 2012), and pressure acclimation are both highly relevant from an ecological perspective, but have only been considered in a handful of studies (Thatje et al. 2010; Oliphant et al. 2011). A maximum thermal acclimation period of 3 days prior to experimentation and a maximum pressure acclimation of 10 atm *per* 5 mins have been reported previously (Oliphant et al. 2011). Both adults and larvae have been studied, although only on planktotrophic larvae. Typically, fertilisation and developmental success have been used as a measure of tolerance during early ontogeny (e.g. Tyler and Young 1998; Aquino-Souza et al. 2008; Mestre et al. 2009, 2012), and respiration and behaviour as a measure of tolerance in adults (e.g. Thatje et al. 2010; Oliphant et al. 2011; Cottin et al. 2012). Recently, molecular approaches have also been used to investigate the combined effects of temperature and pressure on gene expression (Cottin et al. 2012). The full physiological scope of a shallow-water invertebrate with regard to both factors has only been investigated in one species of adult crustacean (Oliphant et al. 2011), the same species (the variable shrimp *Palaemonetes varians* (Leach 1813)) in which extended (7 or 28 day) pressure exposure has been investigated (Cottin et al. 2012).

Results of these studies have repeatedly indicated the fauna examined to be capable of tolerating hydrostatic pressure outside the bathyal ranges they are exposed to across their distribution (Young et al. 1997; Tyler and Young 1998; Benitez-Villalobos 2006; Aquino-Souza et al. 2008; Mestre et al. 2009; Thatje et al. 2010; Oliphant et al. 2011; Cottin et al. 2012). Tolerances are influenced by temperature, and within a species

Table 1.1. Previous investigations into the effects of temperature and pressure on shallow-water marine invertebrates. Only investigations with a minimum duration of 1 hour, and examining a maximum pressure of 600 atm are reported. Larval types are reported as planktotrophic (P) or non-planktotrophic (NP). Pressure vessels used were either small independent pressure vessels (Simple) or a pressurised incubator (IPOCAMP).

Species	Stage	Temperatures investigated (°C); acclimation	Pressures investigated (atm); acclimation	Duration (hours)	Variable measured	Climate zone	PV type	Reference
CRUSTACEA								
Crangon crangon	Adult	8	1,10,30,40,50,60,100,120; Stepwise (20-600 atm h^{-1})	≤ 7.5	Behaviour	Temperate	Simple	Wilcock et al. 1978
Pagurus cuanensis	Adult	5,10,15,20; Stepwise (5 days)	20,50,100; Stepwise (100 atm h^{-1})	2	Behaviour	Temperate	IPOCAMP	Thatje et al. 2010
Pagurus cuanensis	Adult	5,10,15,20; Stepwise (5 days)	1,100; acute	1	Respiration	Temperate	Simple	Thatje et al. 2010
Palaemonetes varians	Adult	5,10,20,30; Stepwise (3 days)	1-300; stepwise (120 atm h^{-1})	5	Behaviour	Temperate	IPOCAMP	Oliphant et al. 2011
Palaemonetes varians	Adult	5,10,20,30; Stepwise (3 days)	1,50,100,150,200,250,300; acute	≤ 1.5	Respiration	Temperate	Simple	Oliphant et al. 2011
Palaemonetes varians	Adult	5,10,27; Stepwise (3 days)	1,100; stepwise (120 atm h^{-1})	168,720	Behaviour, genetic	Temperate	IPOCAMP	Cottin et al. 2012
ECHINODERMATA								
Asterias rubens	Larvae (P)	5,10,15,20; acute	1,50,150,200; acute	6,12,24,48	Behaviour	Temperate	Simple	Benitez-Vilallobos et al. 2006
Arbacia lixula	Larvae (P)	5,10,15; acute	1,50,150,250; acute	6,12,20,24	Behaviour	Warm temp.	Simple	Young et al. 1997
*Gracilechinus acutus**	Larvae (P)	4,7,11,15; acute	1,50,150,200; acute	6,12,24	Behaviour	Cold temp.	Simple	Tyler & Young 1998
*Echinus esculentus**	Larvae (P)	4,7,11,15; acute	1,50,150,200; acute	6,12,24	Behaviour	Cold temp.	Simple	Tyler & Young 1998
Paracentrotus lividus	Larvae (P)	5,10,15; acute	1,50,150,250; acute	6,12,20,24	Behaviour	Warm temp.	Simple	Young et al. 1997
Marthasterias glacialis	Larvae (P)	5,10,15,20; acute	1,50,150,200; acute	6,12,24,48	Behaviour	Temperate	Simple	Benitez-Vilallobos et al. 2006
*Psammechinus miliaris**	Larvae (P)	5,10,15,20; acute	1,50,150,200; acute	3,6,12,24	Behaviour	Temperate	Simple	Aquino-Souza et al. 2008
Sphaerechinus granularis	Larvae (P)	5,10,15; acute	1,50,150,250; acute	6,12,20,24	Behaviour	Warm temp.	Simple	Young et al. 1997
Sterechinus neumayeri	Larvae (P)	-1.2,0.9,2.5; acute	1,50,150,200; acute	24,48	Behaviour	Polar	Simple	Tyler et al. 2000
MOLLUSCA								
Crepidula fornicata	Larvae (P)	5,10,20,25; acute	1,50,100,150,200,250,300,350, 400; acute	24	Behaviour	Temperate	Simple	Mestre et al. 2012
*Mytilus edulis**	Larvae (P)	5,10,15,20,25; acute	1,100,200,300,400,500; acute	4,24,50	Development	Temperate	Simple	Mestre et al. 2009
Patella ulyssiponensis	Larvae (P)	5,10,15,20; acute	1,50,100; acute	24h	Behaviour	Temperate	Simple	Aquino-Souza 2006
Patella vulgata	Larvae (P)	5,10,15,20; acute	1,50,100; acute	24h	Behaviour	Temperate	Simple	Aquino-Souza 2006

thermal tolerance range, susceptibility to pressure typically increases as temperature decreases. Additionally, tolerances appear to vary with life history stage; pressure tolerance increases briefly following early cleavage but then appears to decrease at later life history stages (e.g. Tyler et al. 2000; Benitez-Villalobos 2006). Authors have speculated that the investigations carried out to date indicate the species examined to be theoretically capable of deep-sea penetration (e.g. Young et al. 1997; Tyler et al. 2000). The lack of duration of thermal or pressure exposure, and the short duration of these studies require caution to be exercised when interpreting the results. In particular, short experimental duration increases the likelihood of subtle, but potentially lethal, effects of pressure to be overlooked; these effects may be enhanced in longer experiments.

1.6. Neogastropoda

The Neogastropoda is an extremely successful and highly diverse group of shelled gastropods, comprising predominantly of active predators or scavengers (Fretter and Graham 1985; Bouchet et al. 2002). The group is relatively young, first appearing in the fossil record during the early Cretaceous with an offshore, cold, deep-water origin (Sepkoski 1988; Jablonski and Bottjer 1991; Jablonski 2005a). Today, the six superfamilies and 16,000 living species making up the Neogastropoda (Cunha et al. 2009), have a broad global distribution (Fig. 1.5a). They are entirely aquatic, predominantly marine species (World Register of Marine Species (WoRMS), http://www.marinespecies.org), with a small number which have diversified to inhabit freshwater environments (Strong et al. 2008). Regularly occurring in large numbers, the neogastropoda form a dominant part of many benthic marine communities (Fretter and Graham 1985; Bouchet et al. 2002). Adults are gonochoric (single sex), and following copulation, egg capsules are commonly laid by females. Within each capsule nurse eggs are typically consumed for nutrition and development is direct (Fretter and Graham 1985). The cold, deep-water origin of the Neogastropoda suggest that shallow-water species may be likely candidates for re-emergence into deep-water.

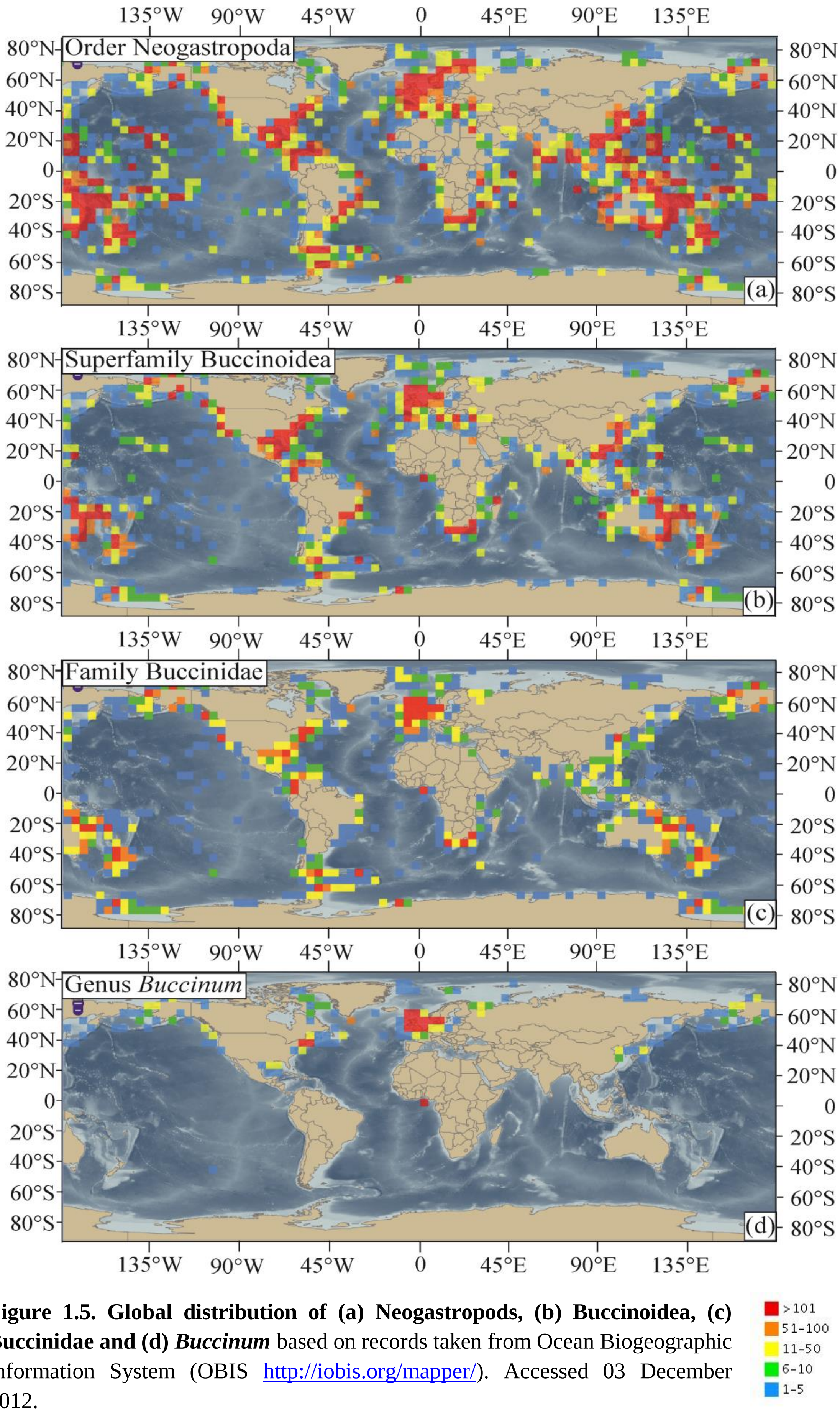

Figure 1.5. Global distribution of (a) Neogastropods, (b) Buccinoidea, (c) Buccinidae and (d) *Buccinum* based on records taken from Ocean Biogeographic Information System (OBIS http://iobis.org/mapper/). Accessed 03 December 2012.

The six superfamilies comprise Buccinoidea, Cancellarioidea, Conoidea, Muricoidea, Olivoidea and Pseudolivoidea (Bouchet and Rocroi 2005). Amongst them, global distribution varies; Buccinoidea, Cancellarioidea, Conoidea and Muricoidea are found globally whereas Olivoidea and Pseudolivoidea are located relatively equatorially (Ocean Biogeographic Information System; OBIS http://iobis.org/mapper/). Buccinoidea, the whelks, in particular are present throughout every ocean and across bathymetric ranges (Fig. 1.5b) (Martell et al. 2002). They make up among the most species rich and widely distributed of the superfamilies, and comprising of almost 7,500 species, they form nearly half of the Neogastropoda. Within this superfamily it is exclusively the family Buccinidae (~ 2200 species) which although predominantly cold-water, is present across every latitude (Fig. 1.5c) (WoRMS). Buccinidae generally comprise of large, carnivorous gastropods, with feeding being aided by the presence of a long proboscis. They typically inhabit soft sediment as adults, and egg capsules are laid on adjacent hard substrate (Fretter and Graham 1985). The genus *Buccinum*, comprising of almost 600 shallow and deep-water species, falls into the family Buccinidae. This genus is particularly abundant in northern hemisphere cold-waters (Fig. 1.5d) (WoRMS) and includes a wide range of shallow and deep-water species. Shallow-water species include *Buccinum frustulatum* (Golikov 1980), *Buccinum glaciale* (Linnaeus 1761), *Buccinum micropoma* (Thorson 1944) and *Buccinum undatum*. Deep-water species include *Buccinum abyssorum* (Verril and Smith 1884), *Beringius turtoni* (Bean 1834), *Belomitra quadruplex* (Watson 1882) and *Buccinum thermophilum* (Harasewych and Kantor 2002) (Martell et al. 2002; Rosenberg 2009). Within this genus, *Buccinum undatum*, which is present across polar and temperate regions, is by far the most widely distributed of the species (Fig. 1.6).

The common whelk *Buccinum undatum* (Linnaeus 1758) is commonly found in soft-bottomed areas of the north Atlantic and Arctic oceans, from the shallow subtidal down to approximately 250m water depth (Rosenberg 2009). Across the distribution, annual temperatures vary considerably. This species shows a level of plasticity across its distribution and different populations are adapted to different temperature ranges (Hancock 1967; Martel et al. 1986a; Kideys et al. 1993). In the south eastern area of the range, around UK waters, temperatures reach above 22 °C and around western areas (Gulf of St Lawrence, Canada) and Northern areas (Iceland), below 0 °C. This species is

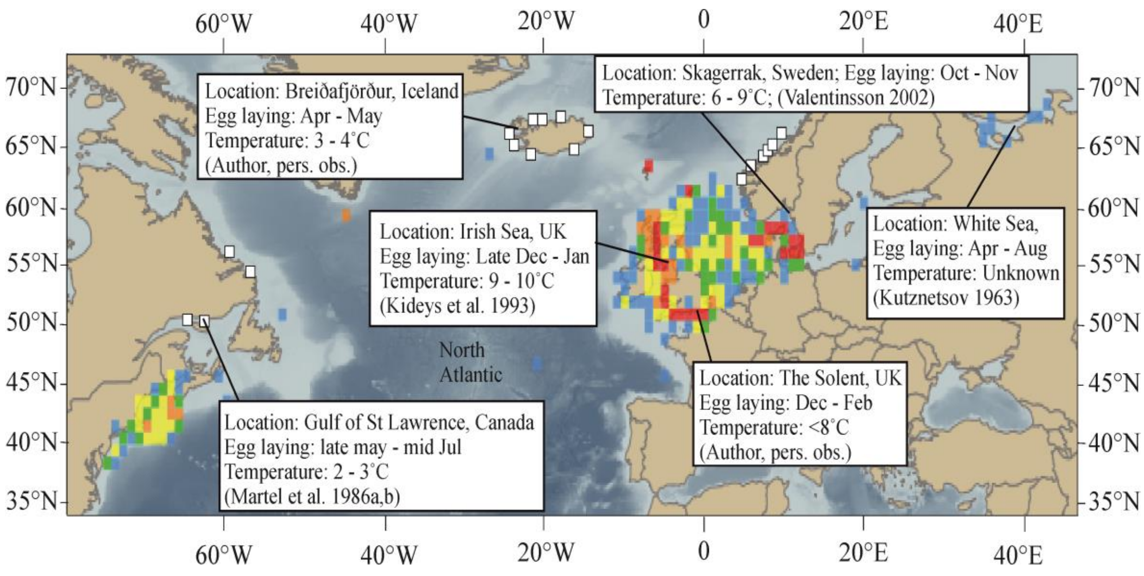

Figure 1.6. Current distribution and known reproductive patterns for *Buccinum undatum*. Distribution records obtained from the Ocean Biogeographic Information Systems (coloured squares) (OBIS http://iobis.org/mapper/?taxon=Buccinum undatum) and from scientific literature (white squares). Reproductive patterns are obtained from the referenced literature.

a large gastropod (≤ 14 cm; Henderson and Simpson 2006), possessing a tough shell, a large, fleshy foot and muscular penis (Fig. 1.7) (Hancock 1967).

Buccinum undatum is becoming increasingly popular for human consumption, and provides locally valuable fisheries in several areas around the North Atlantic including the UK, the USA and Canada (Hancock 1967; Morel and Bossy 2004). It has been suggested as a good candidate for aquaculture (Nasution and Roberts 2004) and globally, demand for it is continuously increasing (Department of Marine Resources www.maine.gov/dmr/rm/whelks.html). In the UK, adults are easily caught using baited traps; circular pots containing carrion of some description, opening with an inward-facing netted funnel to prevent escape (Hancock 1967).

Buccinum undatum is a cold water spawner and copulation and egg laying are temperature induced (Hancock 1967; Martel et al. 1986a; 1986b; Kideys et al. 1993; Valentinsson 2002). Consequently, the reproductive cycle varies across the species distribution, dependent on local temperature conditions (Fig. 1.6). In the coastal waters of the UK, at the south eastern end of the range where the warmest water population are found, copulation and egg laying occur as temperatures drop below 9 °C, predominantly between late November and January (Hancock 1967; Kideys et al. 1993). In the northwest Atlantic, where annual water temperature rarely exceed 4 °C, copulation and egg laying instead take place in spring (April to mid-July) as water temperatures warm to approximately 2-3 °C (Martel et al. 1986a, 1986b). In both areas, reproduction is induced when critical water temperatures are reached (Fig. 1.8). At spawning, females deposit small creamy coloured spherical egg capsules (6 to 12mm length), containing nurse eggs and developing embryos, onto hard substrate (Fretter and Graham 1985; Martel et al. 1986a). The action of one female spawning induces spawning in other females, probably through the release of pheromones (Martel et al. 1986b). Females then group, contributing egg capsules to the same egg mass simultaneously. Each female lays approximately 80 to 150, which collectively can create large egg masses of hundreds to thousands of capsules. Masses have been observed up to 50cm in length and 25cm in height, containing several thousand capsules (Fretter and Graham 1985, Valentinsson 2002).

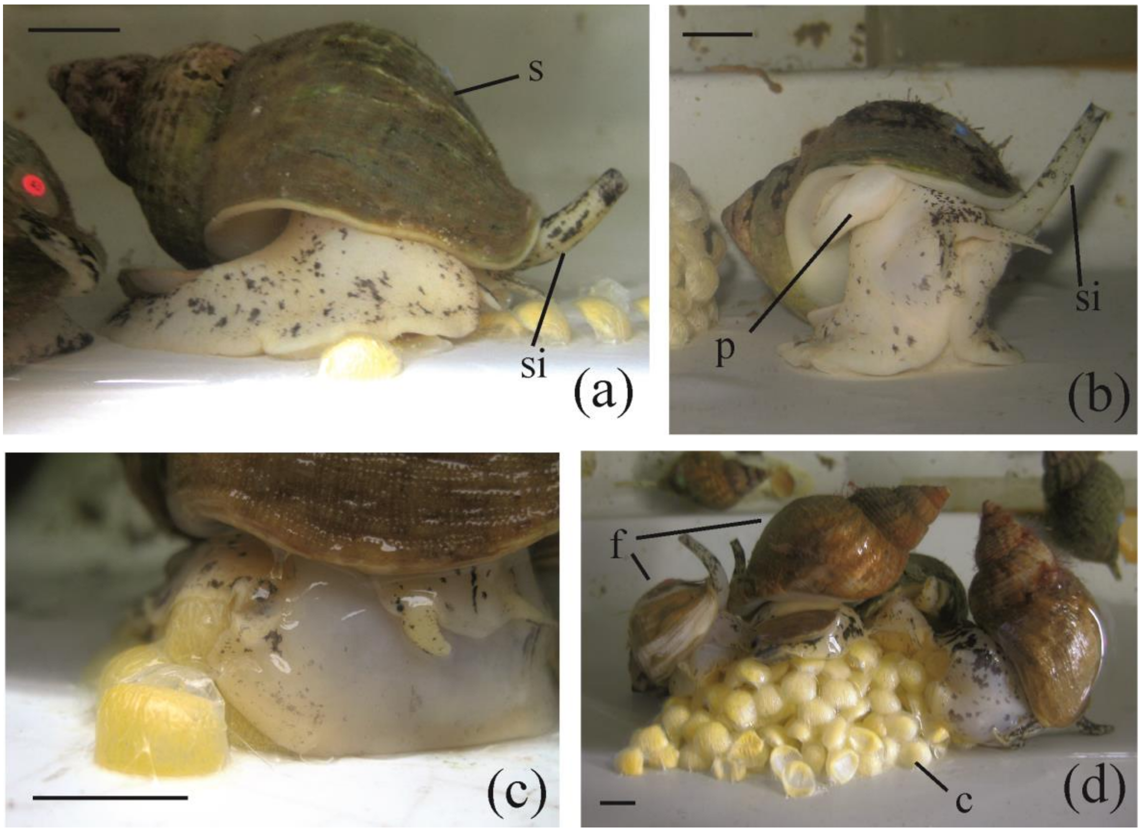

Figure 1.7. Pictures of *B. undatum* adults. (a) adult with foot, thick shell and siphon evident. (b) Male adult with penis obvious. (c) Female laying egg capsules. (d) egg mass being contributed to by many females. Scale bars represent 1000μm. *s* shell, *si* siphon, *p* penis, *c* capsule, *f* females laying.

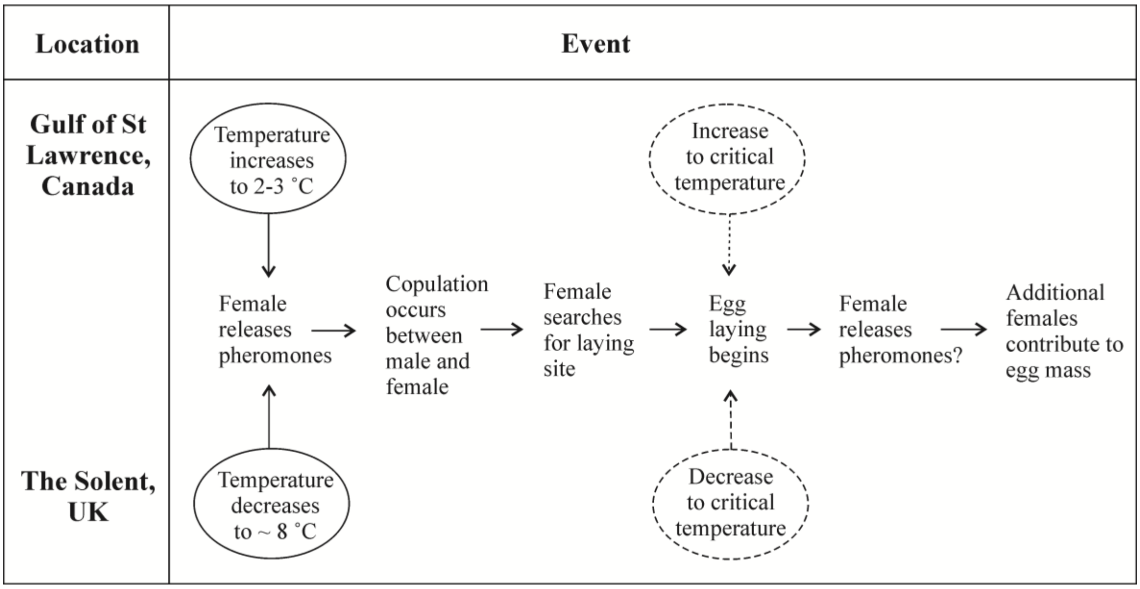

Figure 1.8. Model describing reproductive cycle in *B. undatum*, adapted from Martel et al. (1986b). Environmental cues are indicated in circles. Factors encircled with solid line have been observed (Author, pers. obs.; Martel et al. 1986b), and those with dashed lines are hypothetical.

Intracapsular development is direct, with larvae being food independent until hatching as juveniles. It takes between 2.5 and 9 months across the species range, depending on local water temperatures (Fretter and Graham 1985; Martel et al. 1986a; Kideys et al. 1993; Nasution 2003). For example, around the UK, development has been observed to take between 2.5 and 5 months (Hancock, 1967; Kideys 1993; Nasution 2003) and in the Gulf of St Lawrence, Canada, it has been observed to take between 5 and 8 months (Martel et al. 1986a). Across the species distribution, temperatures during development are regularly in the region of 2 to 6 °C and typically do not exceed 10 °C.

Previous descriptions indicate each capsule to contain between 500 and 3000 eggs (Fretter and Graham 1985), of which approximately 10 to 20 develop into embryos (Hancock 1967; Nasution 2003; Smith and Thatje 2013). The embryos pass through a veliger stage during which they consume the remainder of the eggs (nurse eggs) for nutrition. Encapsulated development continues until the juvenile stage is reached, indicated by the presence of a fully developed head, foot and shell. Juveniles depart the capsule *via* a hole created by radula scraping, then remain on the egg mass for a couple of days before moving off to feed (Nasution 2003; Smith and Thatje 20113). More detailed accounts of encapsulated development have been described by Portmann (1925) and Nasution (2003), but reports to date are incomplete. The developmental stages used throughout these works were therefore determined during the investigation discussed in chapter 3 (Section 3.5.1). The identified stages were as follows; egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile, hatching juvenile.

1.7. Rationale, hypotheses and objectives

The physiological thresholds controlling species distribution within the oceans, in particular with regard to bathymetric radiations, remain poorly understood. Improved knowledge on the physiological thresholds in marine invertebrates will contribute to our understanding of the adaptations influencing historical migrations and colonisations, and will give insight into the directions of potential future species range expansions.

This thesis proposes to investigate the thermal and hyperbaric thresholds affecting range extension in the marine environment. In order to generate accurate theories and predict the direction in which such migrations may take, it is important that knowledge is accumulated on the effects of temperature and pressure on a wide range of marine fauna across a range of life history stages. The north Atlantic gastropod *Buccinum undatum* is an ideal study species in which to examine physiological thresholds during non-planktotrophic development. The cold, deep-water origin of neogastropods makes this species a likely candidate for re-emergence into the deep-sea. Additionally, the broad distribution of this species, covering polar and temperate climates allows for exposure to a wide thermal range.

The specific aim of this thesis is to investigate the physiological tolerance to temperature and hydrostatic pressure of *B. undatum* during development. This aim has led to developing the following hypotheses:

Hypothesis 1: Developmental rate will increase positively with temperature in *B. undatum* egg masses.

Hypothesis 2: *Buccinum undatum* embryos will be less tolerant of high hydrostatic pressure at later development stages, when complexity is greater.

Hypothesis 3: Thermal acclimation will result in an increase in pressure tolerance in *B. undatum* embryos with decreasing temperature.

Hypothesis 4: An increase in energetic expenditure during development will be observed with increasing pressure in *B. undatum* embryos.

The following objectives were developed in order to test the above hypotheses:

Objective 1: Investigate the full intracapsular development of *B. undatum*, describe ontogenetic stages and assess developmental timing (Chapters 3 and 5).

Objective 2: Examine the full thermal tolerance range of *B. undatum* for development using a population from the south eastern end of the species distribution (test of hypothesis 1; Chapter 4).

Objective 3: Interpret the combined effects of temperature and pressure on development in *B. undatum*, using egg masses collected from across the species range and acclimated to temperatures which are ecologically relevant throughout the species distribution (test of hypotheses 2 and 3; Chapter 6).

Objective 4: Analyse the effects of sustained exposure to high pressure on development of *B. undatum* egg masses (test of hypothesis 4; Chapter 7).

In the following chapter, the experimental design, methodology and equipment are first discussed (Chapter 2). Subsequently, the objectives of the thesis are carried out (Chapters 3 to 7), ending with a final synthesis of the complete works (Chapter 8).

Chapter 2: Experimental design, methodology and equipment

In this chapter, the experimental design of Chapters 3 to 7 is explained, general methodology including sample collection and maintenance is discussed and the equipment and protocols used during these works are described. All study specific information is included in the respective chapters.

2.1. Experimental design

Following the aims and objectives defined in Chapter 1, the general experimental design for each of the following chapters is described below. In addition to the experiments described, an inter-annual comparison of egg mass quality was also carried out on all egg masses collected from the Solent (Section 2.4). All experimental work was carried out at the National Oceanography Centre, Southampton, UK (NOCS). All nomenclature used in this thesis is written according to the World Register of Marine Species (www.marinespecies.org/)

Chapter 3: Intracapsular development in the common whelk *Buccinum undatum* (Linnaeus 1758)

Intracapsular development in the common whelk was investigated using egg masses from the Solent, UK. Three egg masses laboratory reared at the NOCS (Section 2.2.1.2; Table 2.2), were developed at 6 °C (Sections 2.3, 2.6) for the duration of encapsulation. This temperature is within the local developmental temperature range for the Solent population of *B. undatum.* Egg masses were examined invasively (three capsules *per* egg mass) and non-invasively each week (Section 2.5) and ontogenetic stages described. An additional thirty five egg masses collected *via* trawl from the Solent and stored in 4 % formalin (Section 2.3) were used to investigate the relationship between capsule volume and number of eggs or embryos *per* capsule, and change in number of embryos *per* capsule through development (Section 2.5). An inter-population comparison of capsule content was carried out using seven egg masses collected from Breiðafjörður, Iceland (Section 2.2.2).

Chapter 4: Thermal tolerance during early ontogeny in the common whelk *Buccinum undatum* (Linnaeus 1758)

Once intracapsular development at 6 °C had been assessed (Chapter 3), thermal tolerance during early ontogeny was investigated using seven trawled and 14 laboratory reared egg masses collected from the Solent, UK (Section 2.2.1; Table 2.2). Only egg masses containing capsules with volumes between 100 and 150 mm^3 were used (Section 2.5.1). Development was assessed across seven temperatures (0, 2, 6, 10, 14, 18 and 22 °C; Section 2.6), with one trawled and two laboratory reared egg masses maintained under each temperature (Section 2.3). Egg masses were examined invasively (three capsules *per* egg mass) and non-invasively (Section 2.5) each week for the first 14 weeks and then each fortnight for the remainder of the developmental period. Capsule content was counted and the ontogenetic stage was determined according to the findings from Chapter 3. At the early veliger stage of development (Chapter 3), 10 capsules were dissected (Section 2.5.2) and sampled for dry weight (Section 2.8.2) and elemental analysis (Section 2.8.3). At hatching, juveniles were also sampled for dry weight (shell, flesh and total; Section 2.8.2) and elemental composition (Section 2.8.3). An inter-population comparison of number of early veligers *per* capsule (Section 2.5) and early veliger dry weight (Section 2.8.2) was carried out using two egg masses with equal capsule volume, collected from Breiðafjörður, Iceland (Section 2.2.2).

Chapter 5: The subtle intracapsular survival of the fittest: maternal investment, sibling rivalry or environmental factors?

Following the investigation of developmental stages (Chapter 3) and thermal tolerance during development (Chapter 4), nurse egg consumption in *B. undatum* was examined using five laboratory reared egg masses collected from the Solent, UK (Section 2.2.1.2; Table 2.2). Egg masses with small (< 100 mm^3) and large (> 200 mm^3) capsules were developed at 10 °C, and egg masses with medium (100 – 200 mm^3) were developed at 6, 10, 14 and 18 °C (Sections 2.3, 2.5.1, 2.6). Each mass was inspected non-invasively twice each week (section 2.5.) until the early veliger stage became visible (Chapter 3). Once the early veliger stage was complete, 15 capsules from each condition were dissected and the contents counted (Section 2.5; details in Chapter 5) and the contents of five capsules were sampled for dry weight (Section 2.8.2) and elemental composition

(Section 2.8.3). Embryos from the remaining 10 capsules were dissected to determine the number of nurse eggs consumed (Section 2.5.2). An inter-population comparison of number of early veligers *per* capsule (Section 2.5) and early veliger dry weight (Section 2.8.2) was carried out using four egg masses with medium volume capsules collected from Breiðafjörður, Iceland (Section 2.2.2).

Chapter 6: The secret to successful deep-sea invasion: does low temperature hold the key?

The combined temperature and pressure tolerance of *B. undatum* during early development was investigated, based on the previously determined thermal tolerance of egg masses (Chapter 4). Seven trawled and nine laboratory reared egg masses from the Solent, UK and six egg masses from Breiðafjörður, Iceland were used in this investigation (Section 2.2; Table 2.2). Egg masses were maintained throughout development at temperatures ranging 3 to 18 °C (Sections 2.3, 2.6). At two developmental stages (veliger and hatching juvenile; Chapter 3), embryos were subjected to four h pressure treatments, in 2.8 ml vials (1 to 400 atm; Section 2.7.1). A total of nine vials were examined for each temperature - pressure combination. Respiration measurements were taken from each vial and compared across all temperature and pressure treatments for each developmental stage (Section 2.8.1, 2.8.2).

Chapter 7: Metabolic cost of development under pressure: the limiting factor for bathymetric distribution?

Upon completion of exploration into temperature and pressure tolerance in *B. undatum* during early ontogeny using four h exposures (Chapter 6), egg masses trawled from the Solent, UK (Section 2.2.1.1; Table 2.2), were subjected to longer pressure exposures of 16 to 20 days. Twelve egg masses at the egg stage of development (Chapter 3), were split into two halves (details in Chapter 7) and maintained under either control (ambient pressure) or experimental conditions (Sections 2.3, 2.7.2). Experimental samples were subjected to pressure treatments ranging 1 to 300 atm. Both control and experimental conditions were maintained at 10 °C. This temperature is within the natural developmental thermal range for the Solent population of *B. undatum* (Smith and Thatje 2013), and is the sea water temperature at 1000 m off the southern coast of the UK.

Control egg masses were examined non-invasively every day (Section 2.5.). Once all control egg masses had completed early veliger development (Chapter 3 for descriptions), experimental treatments were ended (Section 2.7.2.). Ten egg capsules from each egg mass (control and experimental) were dissected and sampled for dry weights and elemental analysis (Sections 2.5.2, 2.8.2, 2.8.3). For each condition, direct comparisons were made between control and experimental egg mass halves.

2.2. Sample collection

Buccinum undatum egg masses were obtained from two locations, at the south eastern (the Solent, UK) and northern (Breiðafjörður, Iceland) ends of the species distribution in the Eastern Atlantic (Fig. 2.1). These locations were chosen as representative of the warmest (the Solent) and coldest (Breiðafjörður) areas of the species distribution Breiðafjörður is located approximately 1200 miles north north west of the Solent. Most egg masses collected had been contributed to by more than one female (Fig. 2.2a), and some contained egg capsules of a variety of ages. All egg masses were maintained in a similar fashion (Section 2.3). Egg mass collection took place over several periods and using a variety of methods (Table 2.1). Collection methods are described below.

2.2.1. The Solent, UK (50° 47’ N, 001° 15’ W)

Egg masses were collected from the Solent, UK (at the south eastern end of the species distribution) in January and March 2009, between December 2009 and March 2010, December 2010 and February 2011, and during January and February 2012. Two methods of collection were used. Since adult whelks were obtained from the same population, water temperatures at time of egg mass collection were similar, and no difference was observed in egg capsule size, number of eggs *per* capsule or egg capsule energetics (Section 2.4) between egg masses collected *via* trawl and those laboratory reared in the aquarium, all egg masses collected from the Solent, UK, were classified as a single population.

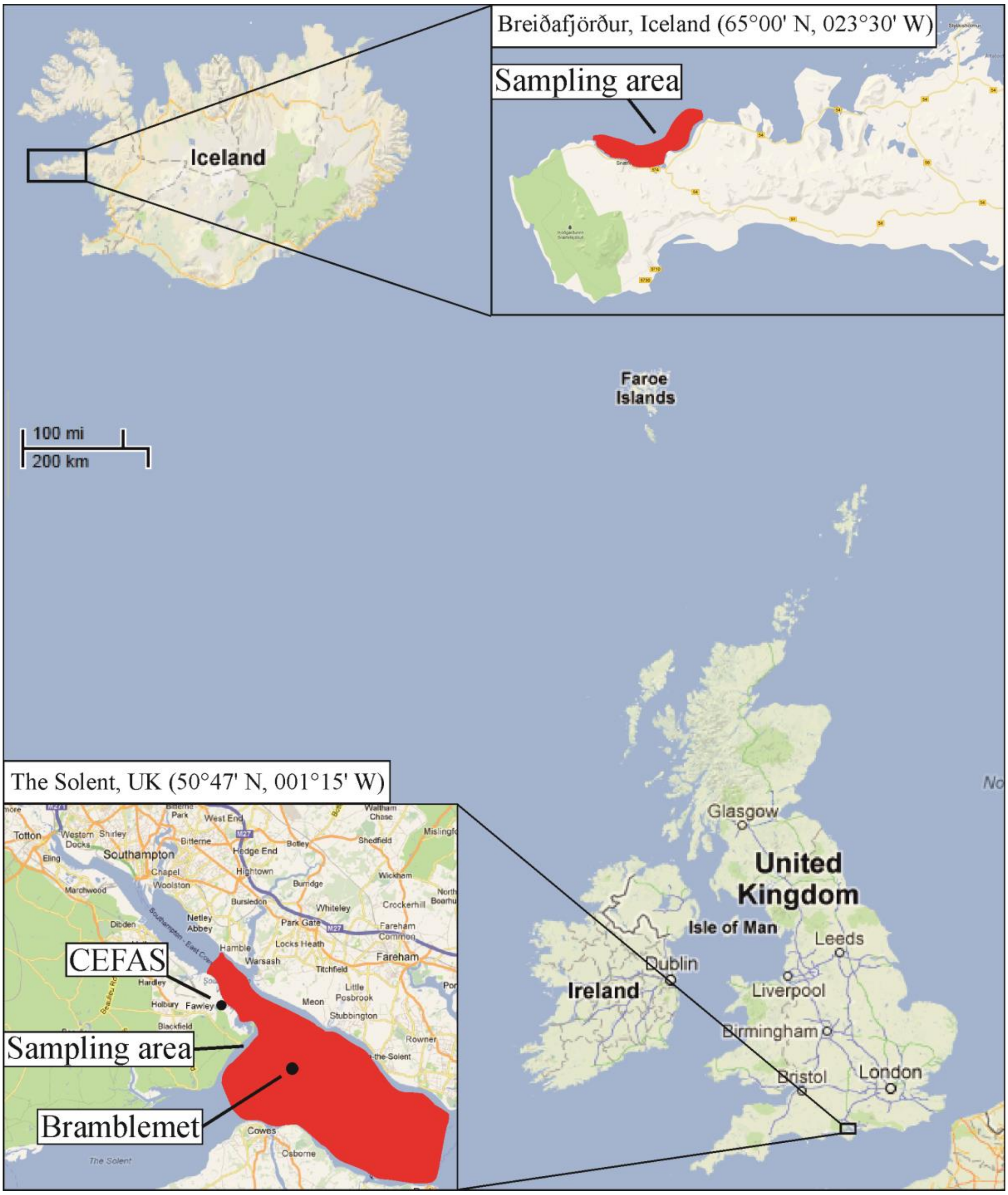

Figure 2.1. Overview of *Buccinum undatum* sampling areas. The Solent area lies between Southampton and the Isle of Wight, off the south coast of the UK. The Breiðafjörður area lies on the northern side of the Snæfellsnes Peninsula, off the western side of Iceland. Areas represent the Northern- and South-eastern-most points of the species distribution, in the Eastern Atlantic. Images courtesy of google maps (www.maps.google.com). The weather information stations 'Bramblemet' and 'CEFAS' are shown on the inlay for the Solent.

2.2.1.1. Trawling

Egg masses were collected using beam trawls deployed by the University of Southampton research vessels *RV Callista* and *RV Bill Conway*. Sea water temperatures at the time of collection were monitored using a laboratory thermometer. Each trawl was on the seabed for approximately 5 mins, at depths of approximately 5 to 10 m, before being recovered. The content of a trawl was sorted on the back deck of the research vessel; egg masses were removed and placed in 10 L buckets filled with sea water collected on-site. Egg masses were returned to the NOCS and transferred to the Aquarium to be maintained at their collection temperature until experimentation began. During January and March 2009 and March 2010 a total of 34 egg masses were collected by trawling. A further 44 egg masses were collected between December 2009 and February 2010, December 2010 and February 2011, and January and February 2012.

During the collection periods, sea water temperatures ranged 4.0 to 9.4 °C (Fig. 2.3). Local temperature data were obtained from long-term monitoring programmes, predominantly from Bramblemet (www.bramblemet.co.uk/), but also from Chimet (www.chimet.co.uk/) and CEFAS (www.cefas.defra.gov.uk/our-science/observing-and-modelling/monitoring-programmes/sea-temperature-and-salinity-trends/presentation-of-results/station-22-fawley-ps.aspx). Bramblemet and Chimet are both comprehensive weather information systems, giving realtime (and historic) information in 5 min intervals. Both are located on piles, offshore. Bramblemet is central in the Solent (50° 47’. 41N, 001° 17’. 15W) (Fig. 2.1) and was the main source for sea surface temperature information throughout these works. Chimet is located approximately 14 nautical miles due east of Bramblemet, 1 nautical mile outside the entrance to Chichester Harbour (50° 45’. 45N, 000° 56’. 59W). This system was used as back-up during an 11 week period (August to October 2011) when data collection failure occurred at Bramblemet. The CEFAS long term monitoring station is located at Fawley Power Station (50° 50’N, 001° 20’W) (Fig. 2.1), giving monthly sea temperature averages dated back to 1984. This information was used to confirm data collected from Bramblemet, and to examine long term temperature trends in this area.

During winter 2011 / 2012, sea water temperatures remained relatively high compared to the previous two years. Temperatures below 8 °C were only recorded from late

January (2012), and lasted for the subsequent seven weeks. In contrast, during the previous two winters (2009 / 2010 and 2010 / 2011), sea water temperatures dropped below 8 °C during early or mid-December, and remained at this level for 15 or 16 consecutive weeks (Fig. 2.3). During this final year (2011 / 2012) a reduction in the number of *B. undatum* egg masses present in the Solent was noted. The effort which went into trawling increased, and the frequency and size of egg masses collected decreased. Several authors have indicated spawning in this species to be induced by low temperature (Hancock, 1967; Valentinsson 2002; Smith and Thatje 2013). Although not investigated in these works, it is possible that the reduced number of egg masses noted here is linked to this.

2.2.1.2. Laboratory reared in sea water aquarium

Approximately 150 adult *B. undatum* were collected by the fishmongers Viviers UK in late November 2009 and 2010 (www.fishmarketportsmouth.co.uk). Adults were originally gathered from the Solent by Viviers using whelk traps (~15 m water depth). Following trapping, Viviers stored adult whelks out of water, in a refrigerated room (5 °C) for approximately 10 to 12 h prior to collection. Whelks were then returned to the hatchery at the NOCS, in a 10 L bucket, without water. They were placed in a large outdoor tank with continuous sea water flow through, at approximately 10 to 12 °C (similar to water temperatures at the time of adult collection) and allowed to recover. Adults were initially checked twice daily, and any dead specimens were removed. Approximately 80 % of the adults collected by Vivers survived. After about three days, all remaining whelks appeared to have recovered. At this point, each was sexed and measured in length (Section 2.5.1). Whelks were sexed by being held out of water, operculum facing upwards. As the whelk attempts to turn itself over by extending its body out of its shell, the length of the body becomes visible. At this point, a relatively large penis is obvious beneath the outer lip of the shell of male whelks (Chapter 1, Fig 1.7b). A bee tag (Imkerei Rudolph, Bremen-Oberneuland, Germany) was then glued (Super glue, Loctite, Westlake, Ohio) to the shell of each whelk in order to distinguish it. Scrap fish was fed to them *ad libitum* three times a week. The tank was checked daily for laying activity. Water temperature in the tank was checked randomly when no laying was occurring, and daily when it was. Egg laying took place between early December 2009 and mid February 2010, and mid December 2010 and mid February 2011. It predominantly occurred when water temperatures fell below 8 °C. All egg masses were

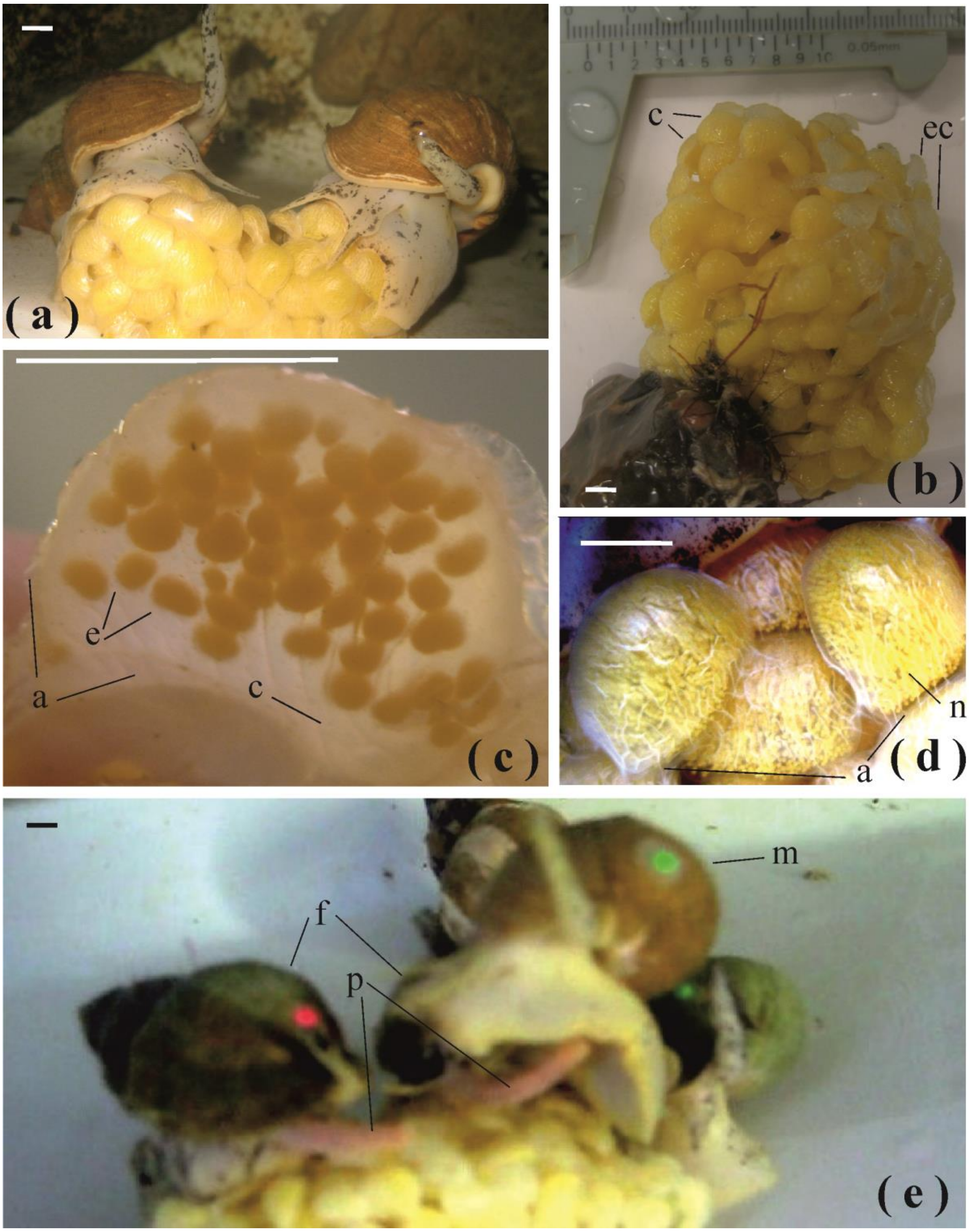

Figure 2.2. Photos of *B. undatum* egg masses. (a) female *B. undatum* group laying an egg mass. **(b)** egg mass of *B. undatum* showing individual capsules. **(c)** a large individual egg capsule showing many developing embryos inside, post nurse egg consumption. **(d)** close-up of capsules containing eggs prior to development. **(e)** video still displaying two females forcing a male off an egg mass using their proboscis (image acquired from video footage). Scale bars represent 5 mm. *c* capsule, *ec* empty capsule, *n* egg (nurse egg or developing embryo), *a* capsule edge attached to other capsules, *e* developing embryo, *f* female, *m* male, *p* proboscis.

laid on aquarium walls within a few cm of the water line, except for one egg mass which was laid inside a bucket on the floor of the tank. Once egg laying was complete, each egg mass was left undisturbed for 24 h before being removed from the aquarium walls. Each mass was removed from the aquarium wall using a scalpel; the blade was carefully inserted between the base of each capsule and the aquarium wall in order to remove the egg mass causing minimal damage. A total of 24 egg masses were laboratory reared during these works.

In order to confirm past studies which indicate that capsule size is positively related to female size, when females were observed laying, their identity was recorded, and 10 capsules laid by each, were measured in length (Section 2.5.1). Regression analysis indicated a positive relationship between female length and capsule length (y = - 0.027 + 0.129 x; R^2 = 0.781; d.f. = 1; $p \leq 0.001$) (Fig. 2.4a).

Females laid capsules piled up on each other to form a ball-shaped mass (Fig. 2.2a, b). Each female began laying capsules directly onto a hard substrate, or onto another egg mass. Each capsule measured approximately 5 to 11 mm across. Capsules were laid over an initial area of approximately 4 to 7 cm^2, with the female moving across the area to lay capsules evenly. More capsules were then laid on top of this area, and each mass grew outwards, away from the point of attachment to form the ball shape. Each female laid approximately 80 to 150 capsules. Females were regularly observed group laying. Of the 24 egg masses laboratory reared, only one was laid by a single female. Every other was contributed to by between two and five females. Each female was only observed to go through one laying period *per* season (approximately one week in duration), but during this time, often contributed to more than one egg mass. Each season, the first female to begin laying induced this in other females, who always initially contributed to the same egg mass. On one occasion, the first female to lay was surrounded by a wire cage in an attempt to keep her separate from other females in the tank. Within a few h of laying beginning, several females had crowded around the cage, and over the following days, attempted to enter the cage by pushing at the edges and corners. Females regularly extended their proboscis through the cage during this time. During the laying period, males occasionally crawled on to the egg masses and appeared to be attempting to consume the newly laid egg capsules. On these occasions, females extended their proboscis under the foot of the male, using it to lever them off the egg mass (Fig 2.2e).

2.2.2. Breiðafjörður, Iceland (65° 00’ N, 023° 30’ W)

Prior to collection of egg masses from Breiðafjörður, Iceland, confirmation was obtained from the Centre for Environmental, Fisheries and Aquaculture Services (CEFAS), for acceptance of *B. undatum* live into the UK, from Iceland (Section 2.10).

Egg masses which had been detached from substrate by heavy weather were collected during April and May 2011 from the intertidal area by hand, from beaches around Breiðafjörður. Masses were generally found being deposited on the beach, in the surf zone. During the collection period, sea water temperatures ranged from 3 to 4 °C (Personal observations; Erla Björk Örnólfsdóttir (Personal Communication), Vör Marine Research Center, Breiðafjörður, Iceland). Following collection, egg masses were returned to Vör Marine Research Center in 10 L buckets filled with sea water collected on-site, and maintained in a refrigerator at approximately 4 °C, until being returned to the UK. During this period, complete water changes were carried out every 48 h. Otherwise, egg masses were maintained as described below (Section 2.3). A total of 9 egg masses were collected using this method. In May 2011, egg masses were returned to the UK by flight, to be maintained at the NOCS. The egg masses were placed in a sealed plastic box, and kept damp using a piece of towelling soaked in 4 °C sea water. These boxes were kept inside a vacuum box (MiniVaqCase, Delta T, Giessen, Germany) containing a cooling element chilled to 4 °C. This vacuum box is designed to maintain internal temperatures for approximately 48 h if not opened. During transportation, a laboratory thermometer was placed inside the vacuum box in order to confirm that the internal temperature was still 4 °C upon arrival at the NOCS.

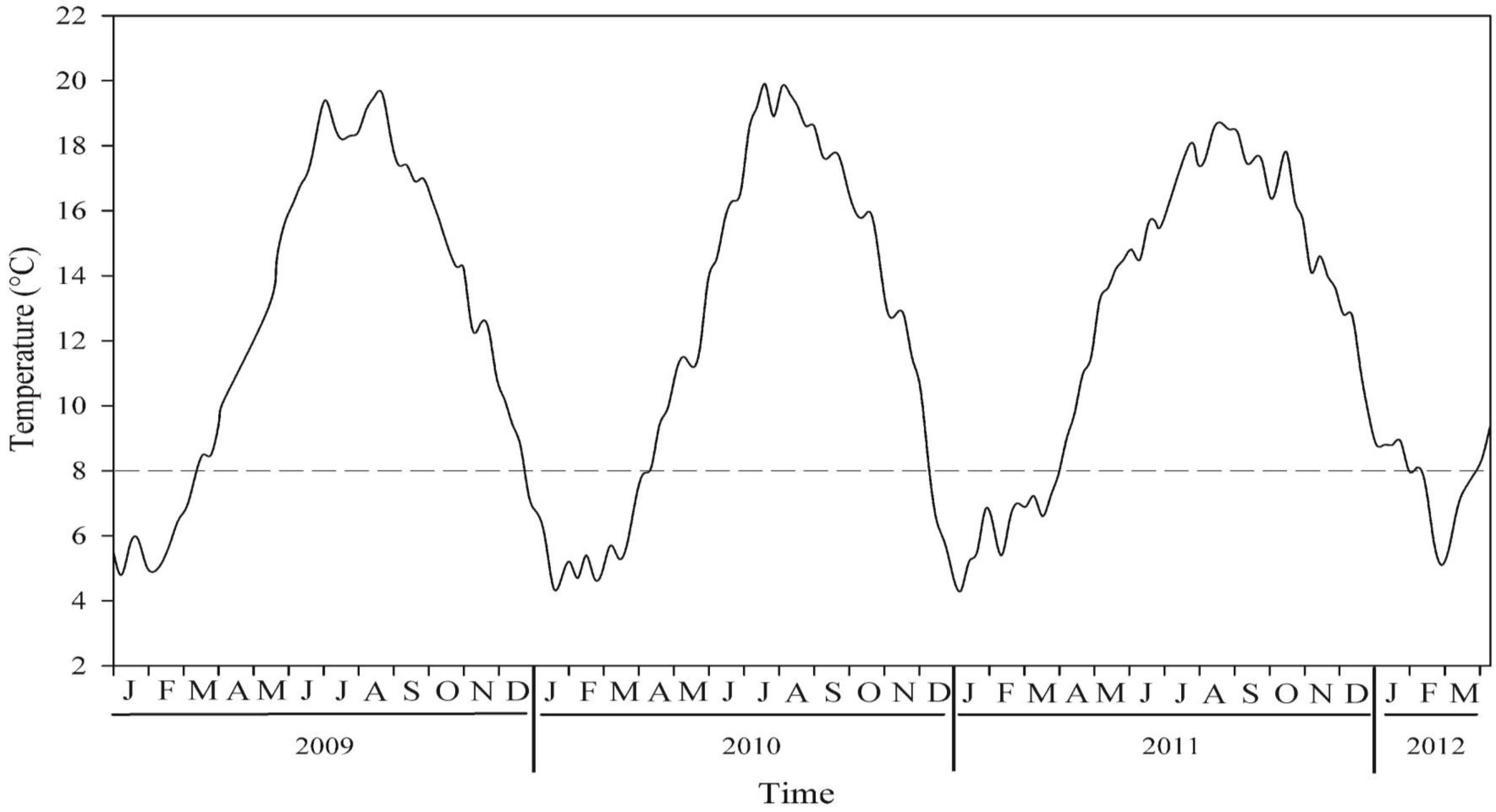

Figure 2.3. Sea temperature trends for the Solent, UK from 1st January 2009 to 31st March 2012. Temperatures based on weekly readings taken from Bramblemet (50° 47'. 41N, 001° 17'. 15W; www.bramblemet.co.uk/). Between 22nd August 2011 and 31st October 2011, data collection failure occurred at Bramblemet. Temperatures for this period are taken from Chimet (50° 45'. 45N, 000° 56'. 59W; www.chimet.co.uk/), situated approximately 14 miles due east of Bramblemet. The dash line indicates 8 °C, the temperature below which, egg mass laying was predominantly observed.

Table 2.1. *Buccinum undatum* egg masses collected during this thesis work. Collected from two populations at the south eastern (the Solent, UK) and northern (Breiðafjörður, Iceland) ends of the species distribution in the northeast Atlantic. Methods of collection and collection periods are indicated. *n* indicates number of masses collected using each method during each period.

Population	Collection method	Collection period	*n*	Egg mass ID	Stored / maintained in
The Solent, UK	Trawled	January and March 2009	20	1 – 20	4 % formalin
		December 2009 to February 2010	14	FM1 – FM14	sea water
		January 2010	1	21	4 % formalin
		March 2010	14	22-35	4 % formalin
		December 2010 to February 2011	15	FM1a – FM15a	sea water
		January 2012 to February 2012	14	FM1b – FM21b	sea water
	Lab. reared	December 2009 to February 2010	10	AM1 – AM10	sea water
		December 2010 to February 2011	14	AM1a – AM14a	sea water
Breiðafjörður, Iceland	Hand	April to May 2011	8	IM1 – IM9	sea water

2.3. Sample maintenance and egg mass use

All egg masses were maintained at the NOCS. Upon collection, the outer layer of each egg mass was removed since capsules in this layer were often empty or held a very low number of eggs (Fig 2.2b). All egg masses from the south eastern location (the Solent), collected during January and March 2009 and March 2010, and one egg mass collected during January 2010 (a total of 35 egg masses) were fixed in 4 % formalin, regardless of age, for later investigation. All other egg masses were maintained individually in 1.8 L incubation tanks containing, 1 µm filtered sea water (three 100 % water changes *per* week). Each tank had a sealed lid, with a hole for an airline. Tanks were continuously aerated using air pumps and air stones. Only egg masses in which no development was observed, were maintained for these works (Section 2.2.5). Any additional egg masses were used for confirming descriptions of developmental stages, for trial protocols and for preliminary investigations.

Egg masses were used for the experiments described above (Section 2.1). The egg masses used for each experiment are detailed in table 2.2. Large egg masses were regularly split into separate segments and used across a variety of treatments or investigations. Only data from the egg masses listed in table 2.2 are included in work reported here

2.3.1. Developmental stages

A total of seven ontogenetic stages were identified (Smith and Thatje 2013). These are described in detail in Chapter 3. The identified stages were as follows: egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile, hatching juvenile. In short, the developmental stages are as follows. Egg: no development evident, nurse eggs and embryos look identical. Trochophore: very early developmental stage before nurse egg consumption occurs. Early veliger: mouthpart forms and all nurse egg consumption takes place. Veliger: thin larval shell and foot begin to develop. Pediveliger: shell thickens, foot develops and velar lobes shrink back and disappear. Pre-hatching juvenile: shell colours and features become more prominent, operculum develops. Hatching juvenile: juvenile exits capsule. Separate experiments focused on different developmental stages. The stages examined are outlined in table 2.2.

2.4. Egg mass variability

In order to compare egg mass quality, elemental analysis (Section 2.8.3) was carried out on all egg masses collected (both locations, all collection methods), which were at the egg stage of development (Table 2.2 and Chapter 3 for a description of the egg developmental stage). Elemental analysis examines the carbon (C) and nitrogen (N) content of a sample, giving an indication of how energetically 'well off' an embryo is. Elemental C and N correlate to lipid and protein levels, and therefore indicate the physiological condition of an organism. Changes in structure and metabolic machinery are reflected by changes in N content, and information on nutritional reserves, provided by C content (Anger 2001). C: N ratio can then be determined from the C and N values and it is often used as a proxy of a lipid: protein ratio. This analysis was therefore carried out on egg masses collected from the Solent, to compare the energetic status of eggs between different seasons, and between collection methods, in order to confirm they were energetically comparable. Egg masses from the Solent were also compared to egg masses from Breiðafjörður. A total of six samples were analysed from each egg mass (three capsules, two samples *per* capsule, approximately 200 eggs *per* sample). Small volumes of sea water were regularly sampled with eggs; this was unavoidable due to the small size of the eggs being collected. As a result, residues of salt remained

Table 2.2. Breakdown of usage of *B. undatum* egg masses. The egg masses used for each investigation are listed below, with reference to the developmental stages examined through the investigation and the collection method and location. Only data collected from the egg masses listed are reported in these works.

Chapter (topic)	Developmental stages used	Population	Collection method	Egg mass ID
2 (Egg mass variability)	Egg	The Solent, UK	Trawled	FM4 – FM8, FM10 – FM12, FM14 FM3a – FM15a FM1b – FM7b, FM9b – FM14b
			Lab. reared	AM4 – AM6, AM8 – AM10 AM1a – AM3a, AM5a, AM6a, AM8a, AM10a – AM13a, AM14a
		Breiðafjörður, Iceland	Hand	IM1 – IM3, IM8
3 (Development)	Egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile, hatching juvenile	The Solent, UK	Trawled	1 – 35
			Lab. reared	AM4 – AM6
		Breiðafjörður, Iceland	Hand	IM1 – IM8
4 (Thermal tolerance)	Egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile, hatching juvenile	The Solent, UK	Trawled	FM4 – FM8, FM10, FM12, FM14 FM1a
			Lab. reared	AM2 – AM10 AM1a – AM3a, AM7a
		Breiðafjörður, Iceland	Hand	IM3, IM6
5 (Nurse egg consumption)	Egg, trochophore, early veliger	The Solent, UK	Lab. reared	AM1a, AM2a, AM5a, AM9a, AM11a
		Breiðafjörður, Iceland	Hand	IM3 – IM6
6 (Short-term temperature and pressure tolerance)	Veliger, hatching juvenile	The Solent, UK	Trawled	FM5, FM8, FM10, FM12, FM14 FM1a, FM9a
			Lab. reared	AM4, AM6, AM8 – AM10 AM1a – AM3a, AM11a
		Breiðafjörður, Iceland	Hand	IM2 – IM6, IM9
7 (Long-term pressure tolerance)	Egg, trochophore, early veliger, veliger, pediveliger	The Solent, UK	Trawled	FM3a, FM5a, FM6a, FM11a, FM12a, FM13a FM4b, FM9b, FM13b, FM18b, FM19b, FM21b

within samples following freeze-drying, potentially affecting the proportions of C and N observed in analysis. Since C:N ratio should not be impacted, this value was used in analysis. Data were analysed using a one-way analysis of variance (UK samples, season versus C: N and collection method versus C: N; all samples, location versus C: N). Prior to analysis, data were subject to logarithmic transformation where necessary, in order to attain homoscedasticity (Levenes test, $p > 0.05$). For the Solent population, analysis indicated there to be no variation in C: N ratios inter-annually ($F = 2.920$; d.f. = 2; $p = 0.055$) or between collection methods ($F = 3.290$; d.f. = 1; $p = 0.071$). No difference was found in C: N ratios between the Solent and Breiðafjörður populations ($F = 3.460$; d.f. = 1; $p = 0.064$).

2.5. Measurements and capsule dissection

From December 2009 to February 2012, three capsules from every egg mass from the Solent, were measured and dissected upon collection. Egg masses to be maintained in sea water were only kept if no embryonic development had yet occurred (i.e. if all embryos were still at the egg stage). All egg masses to be preserved in 4 % formalin were preserved and used regardless of developmental stage. Following the initial sampling, egg capsules were measured and dissected at various points throughout experimental work. The methods used are described below.

2.5.1. Measurements

Adult whelk measurements were obtained in one dimension (length), and linear capsule measurements, in three dimensions (length, width, depth), using digital vernier callipers (500 196-20 digimatic calliper, Mitutoyo, Andover, UK) (Fig. 2.5a, b). *Buccinum undatum* egg capsules are relatively ellipsoid in shape, with a concave / convex face (Fig 2.5c). Measurements were taken in millimetres to an accuracy of two decimal places. All capsules which were subsequently dissected for experimentation were measured in three dimensions.

Egg capsule volume was estimated using the following equation taken from Smith and Thatje (2013);

$V = (\pi\ ab) \times (c)$

Where a = length / 2, b = width / 2 and c = depth.

The equation used to establish egg capsule volume was adapted from one initially used by Pechenik (1983) and Rawlings (1990). The original equation was that for determining the volume of a prolate ellipsoid: $V = 4/3\pi ab^2$, where a = half capsule height and b = half capsule width. The gastropod capsules for which this equation was used, were much longer, thinner and more rounded than *B. undatum* egg capsules. Instead, based on the shape of *B. undatum* egg capsules, the equation chosen was for the surface area of an ellipse*capsule depth. Using this equation, regression analysis indicated capsule volume to be positively related to capsule length; the alternative measurement which is regularly used to size capsules in encapsulation studies ($y = 5.11 + 0.0218\ x$; $R^2 = 0.776$; d.f. = 1; $p \leq 0.001$) (Fig. 2.4b).

2.5.2. Capsule dissection and capsule content examination

Where individual capsules were dissected from egg masses, this was carried out using fine scissors. The outer edge of an individual capsule was held with tweezers, and the inner (attached) edge gently severed (Fig. 2.2c, d). Incisions were made as close to the edge of the capsule as possible, without damaging the capsule below. The dissected capsule was then removed and placed in a petri dish or Bogorov counting chamber containing sea water. The content of the capsule was gently washed out using a glass pasteur pipette. Capsule content was examined under a microscope (Model S8AP0, Leica Microsystems UK, Milton Keynes, UK), and the number of individuals counted. All eggs or embryos were counted using a hand tally counter. Measurements of eggs or embryos were taken along the longest axis using a microscope eyepiece graticule. Where measurements were taken, when a capsule contained loose eggs, approximately 20 were measured (in diameter) *per* capsule. When embryos were present of any age, all were measured.

For every egg capsule dissected and examined upon collection, mean egg diameter was compared to capsule volume. Regression analysis indicated there to be a positive

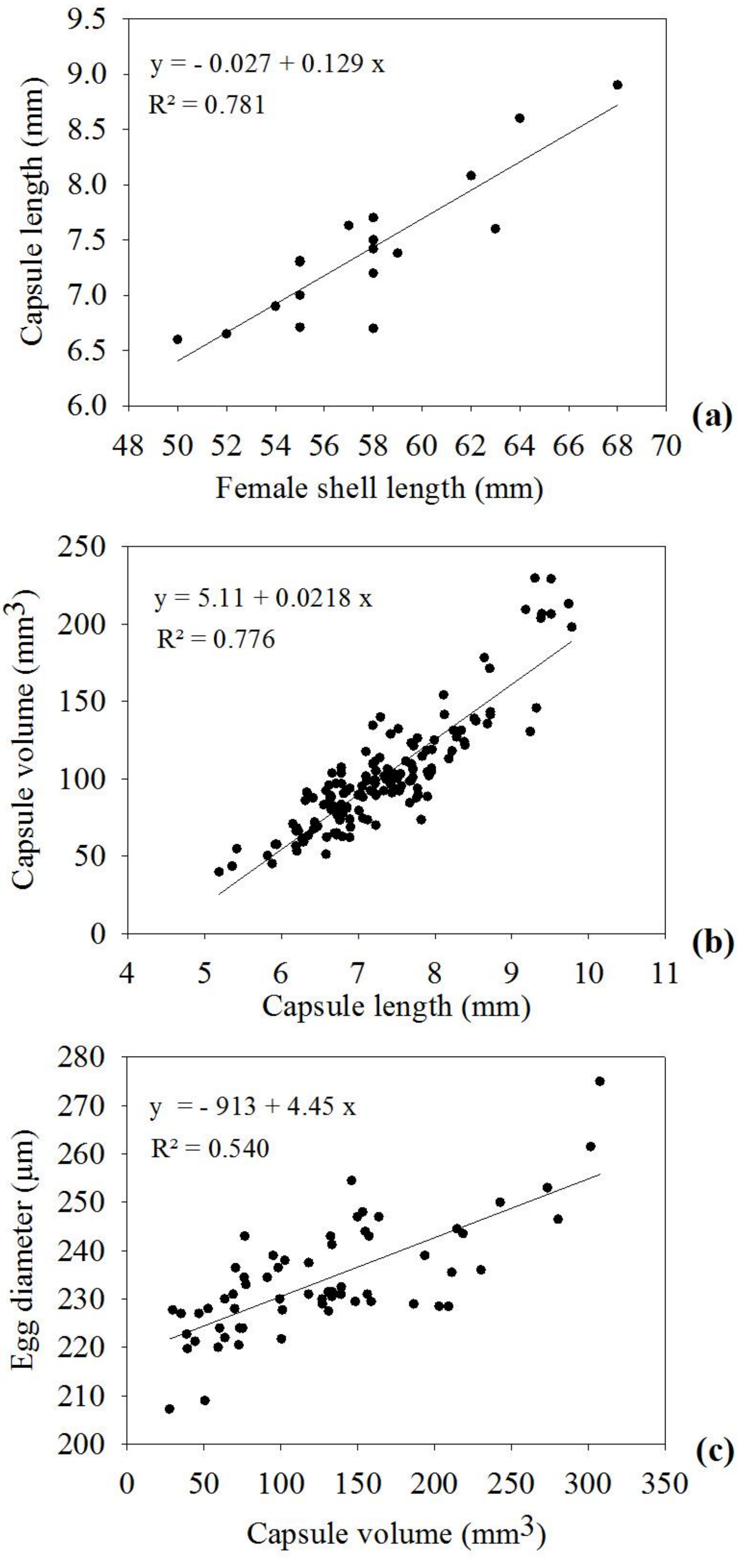

Figure 2.4. Regression analyses of reproductive data from *B. undatum*. **(a)** female length against capsule length, **(b)** capsule length against capsule volume and **(c)** egg diameter against capsule volume. Equations and R^2 values are displayed.

relationship between these two factors (y = - 913 + 4.45 x; R^2 = 0.540; d.f. = 1; p $\leq$ 0.001) (Fig. 2.4c).

Nurse egg consumption was examined by dissecting individual embryos (Chapter 5). In *B. undatum*, once consumed, nurse eggs are stored, undamaged in the mid-gut prior to use (Chapter 3). Early veligers can therefore be dissected, and the number of nurse eggs inside counted. In order to do this, an early veliger (Chapter 3) was transferred to a Bogorov counting chamber using a 3 ml pipette. The outer membrane surrounding the early veliger was pierced using a scalpel, allowing all the nurse eggs which had been consumed to spilled out. These eggs were then counted using a hand tally counter.

Often, non-invasive examinations were also carried out on egg masses. The transparency of *B. undatum* egg capsules (Fig. 2.2c, d) allows the internal content to be observed, and some estimate of age and number to be made. In particular, this allows nurse egg consumption to be determined, since individual eggs are easily discernible from developing embryos.

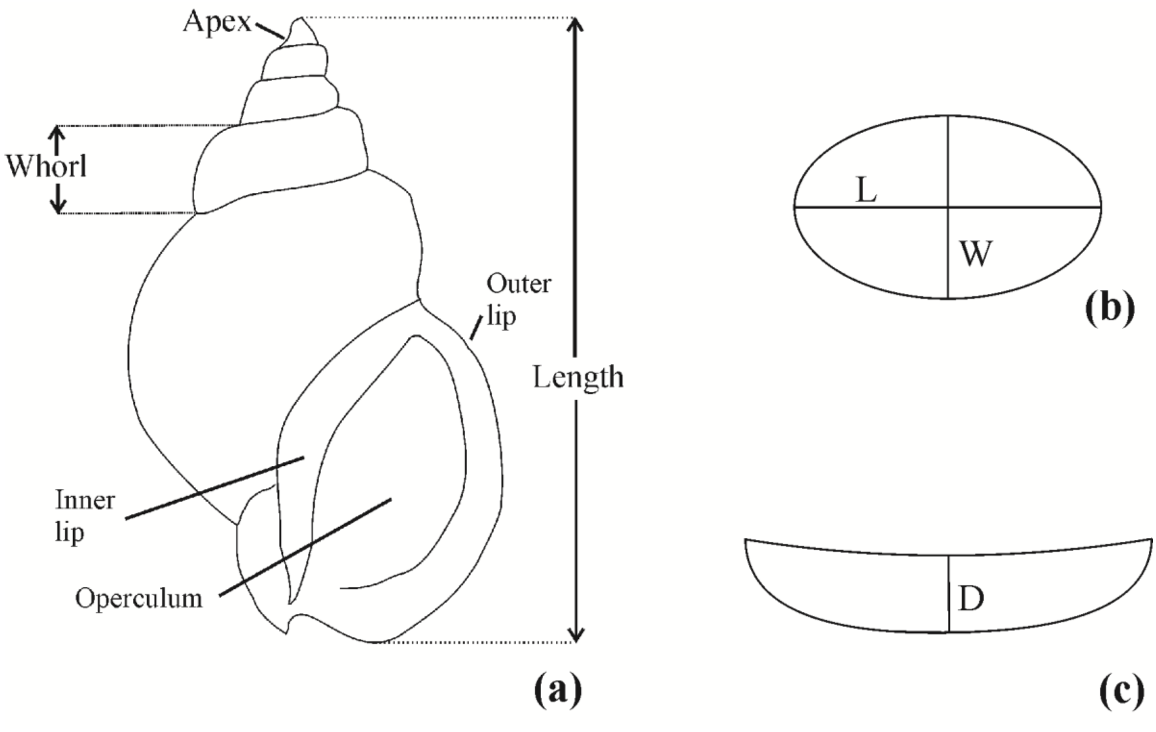

Figure 2.5. *Buccinum undatum* adult shell and capsule. (a) adult shell, **(b)** top profile of egg capsule and **(c)** side profile of egg capsule. Dimensions for measurements are displayed. L = length; W = width; D = depth.

Photos and video were also obtained using a Sony digital 8.1 megapixel camera focussed down the lens of the microscope. Photographs were also taken using a camera (model DFC280, Leica Microsystems UK, Milton Keynes, UK) mounted to a microscope.

2.6. Temperature manipulation

Egg masses were acclimated to different temperatures for the duration of development for all experimental work. In this section an overview is given of the thermal ranges to which egg masses were subjected to. Study specific designs are incorporated into each chapter.

Egg masses from the south eastern location (the Solent) were acclimated to 0, 2, 6, 10, 14, 18 or 22 °C, to include the full annual thermal range that adults from this population are exposed to annually. Egg masses from the northern location (Breiðafjörður) were acclimated to 3 or 6 °C, to include only the natural thermal range for development for this population. All acclimations were stepwise (1 °C *per* 24 h) from the initial water temperature at collection. Incubation tanks were maintained in water baths or cabinet incubators, for the duration of development, in order to retain experimental temperatures. Incubation tanks, water changes and aeration were as described in section 2.3. Throughout development, egg masses were examined on a weekly basis, and three capsules from each were dissected and preserved for later dry weight and elemental analysis.

2.7. Hydrostatic pressure manipulation

In the works carried out for this thesis, developing embryos were subject to high hydrostatic pressure for different durations during experiments (Chapters 6, 7). Egg masses were pressurised using two types of pressure system. Acute (4 h) pressure treatments were carried out using a cast iron pressure vessel and manual hydraulic pump (Chapter 6). Sustained pressure experiments (16 to 20 days) were carried out using a IPOCAMP (Incubateur Pressurisé pour l’Observation et la Culture d’Animaux Marins

Profonds, Autoclave France, Rantigny, France) pressurised incubator (Chapter 7). Both methods are described below.

2.7.1. Cast iron pressure vessel and manual hydraulic pump

A variety of pressure systems have been developed throughout the twentieth century (Pradillon and Gaill 2007). Typically, high pressure equipment consists of a pressure vessel which is pressurised using a manual hand pump. This equipment is relatively simple in design and easy to use but most vessels are very small, allowing only for rapid pressurisation and de-pressurisation. Older systems also often experienced problems with retaining pressure for the duration of experimentation. In the present investgations, pressure vessels were made up of a thick walled (cold-drawn steel body, cast iron head) pressure filters (model SF 030, Stauff, Sheffield, UK), equipped with stainless-steel pressure gauges (model EN 837-1, Stauff, Sheffield UK) (Fig. 2.6). Experimental pressures were obtained using a manual hydraulic pump (M series, Maximator GmbH, Nordhausen, Germany) (after Mestre et al. 2009; Thatje et al. 2010). A pressure vessel was connected to the hydraulic pump *via* a high pressure hose and quick release fitting. Once the desired pressure was reached, a lock-off valve enabled each vessel to be detached from the hydraulic pump, whilst retaining the experimental pressure. A total of 18 pressure vessels (volume 350 ml) were used.

Vessels were pressurised to 1, 100, 200, 300 or 400 atm. Pressures ranging 1 to 400 atm (equivalent to 0 to 4000 m water depth) were chosen to represent shallow water through to average ocean depths. Prior to experimentation, all pressure vessels were incubated to experimental temperature for a minimum of 12 h. Samples were placed inside 2.8 ml vials, filled with pre-incubated 1 µm filtered sea water. The base of a pressure vessel was then filled to the top with pre-incubated freshwater, and experimental vials were placed inside. The top of the pressure vessel was screwed on, taking care not to lose any water. The pressure vessel was then attached to the manual hydraulic pump *via* the high pressure hose, with the lock-off valve in the open position (parallel to the pressure vessel outlet pipe). The pump had a water tank integral to it. When pumped, water from the tank was forced through the high pressure hose, increasing pressure inside the vessel. Pressurisation was continuous and took a maximum of 30 sec. The pressure reached was confirmed in duplicate, using gauges on the pressure vessel and on the high pressure pump; when the lock-off valve was open, both gauges read the same. Once at the desired pressure, the lock-off valve was closed (90° to the pressure vessel outlet pipe). A relief valve on the pump allowed pressure to be released from the high pressure hose whilst being retained in the pressure vessel. The pressure vessel was then disconnected from the pump using the quick release fitting, and placed inside an incubator, set at experimental temperature. After four h, pressure was released from the vessels by opening the lock-off valve. Total de-pressurisation took approximately 1 to 2 sec. The top of the pressure vessel was then un-screwed, allowing access to the vials inside. Throughout experimentation, pressure remained constant in all vessels; any vessels which lost pressure were excluded.

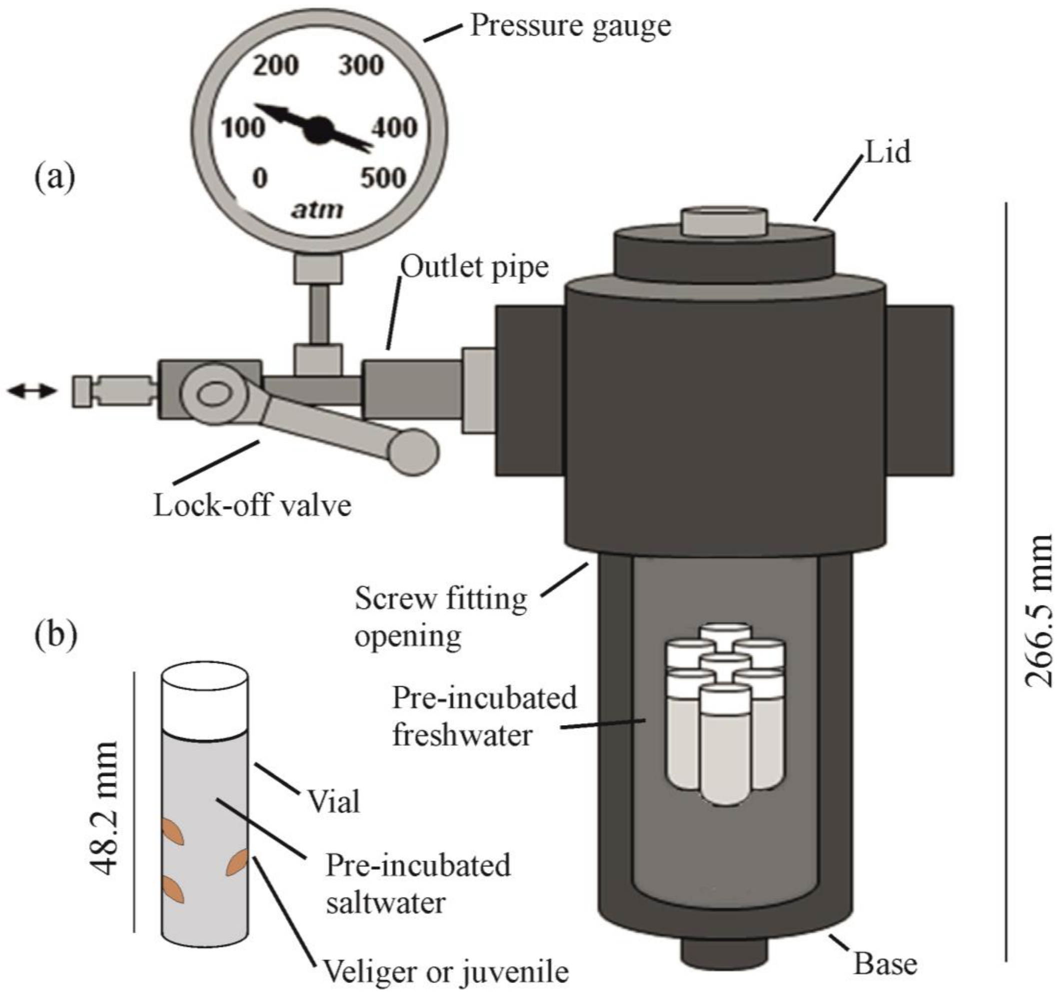

Figure 2.6. Pressure vessel used for shock pressure experiments (Chapter 6) (adapted from Mestre et al. 2009). **(a)** 350 ml cast iron pressure vessel containing sample vials. Two-way arrow indicates attachment to high pressure hose. **(b)** Experimental vial containing three *B. undatum* individuals.

2.7.2. IPOCAMP pressurised incubator

Continuous flow-through pressure systems have only recently been developed, which allow the exchange of water under controlled thermal and hyperbaric conditions (Pradillon and Gaill 2007). Such modern systems (e.g. IPOCAMP; Shillito et al. 2001, 2004, 2006; Ravaux et al. 2003) increase pressure stability, allow longer experimental duration, avoid difficulties with water O_2 levels and allow pressurisation to be carried out at a controlled rate. Some limitations continue to remain unresolved; the available space is limited, apart from the use of an endoscope camera, specimens can rarely be examined under pressure and in order to remove any animals, the entire system must be de-pressurised.

The IPOCAMP pressurised incubator is a 19 L stainless steel flow-through pressure system, attached to a header tank containing 40 L of water (Shillito et al. 2001, 2004, 2006; Ravaux et al. 2003). The re-circulating system allows continuous water exchange between the pressure vessel and head tank, whilst maintaining both experimental pressure and temperature. A constant temperature can be achieved through exchange of water *via* an incubator; temperature is constantly monitored at both the inlet and the outlet (± 1 °C). A high pressure pump allows pressure to be manipulated *via* a rotating dial. Sapphire glass windows in the top of the IPOCAMP allow the inside of the pressure vessel to be observed *via* an endoscope camera (Fig. 2.7).

All sustained pressure investigations carried out here were completed at 10 °C. The IPOCAMP and header tank were filled with 1 µm filtered sea water. The lid was placed into the IPOCAMP, and sealed, and the IPOCAMP system was run, in order to begin circulating temperature controlled water. Once the system was started, any remaining air was removed from the system using a release valve on the lid of the IPOCAMP. This set-up was run at 10 °C, 1 atm for 24 h prior to experimentation, in order to allow the temperature to stabilise. The high pressure pump was then turned off, and the IPOCAMP was opened, enabling samples to be placed inside. Following this, each experimental egg mass half was loosely attached using a cable tie to the inside of one leg of a tripod frame placed inside the IPOCAMP (Fig. 2.7). Each cable tie was attached to an egg mass by insertion through gaps between capsules. Once attached to the frame, each egg mass sat below a viewing port, which allowed it to be monitored using the endoscope camera. The IPOCAMP was then sealed again, and the pump re-started.

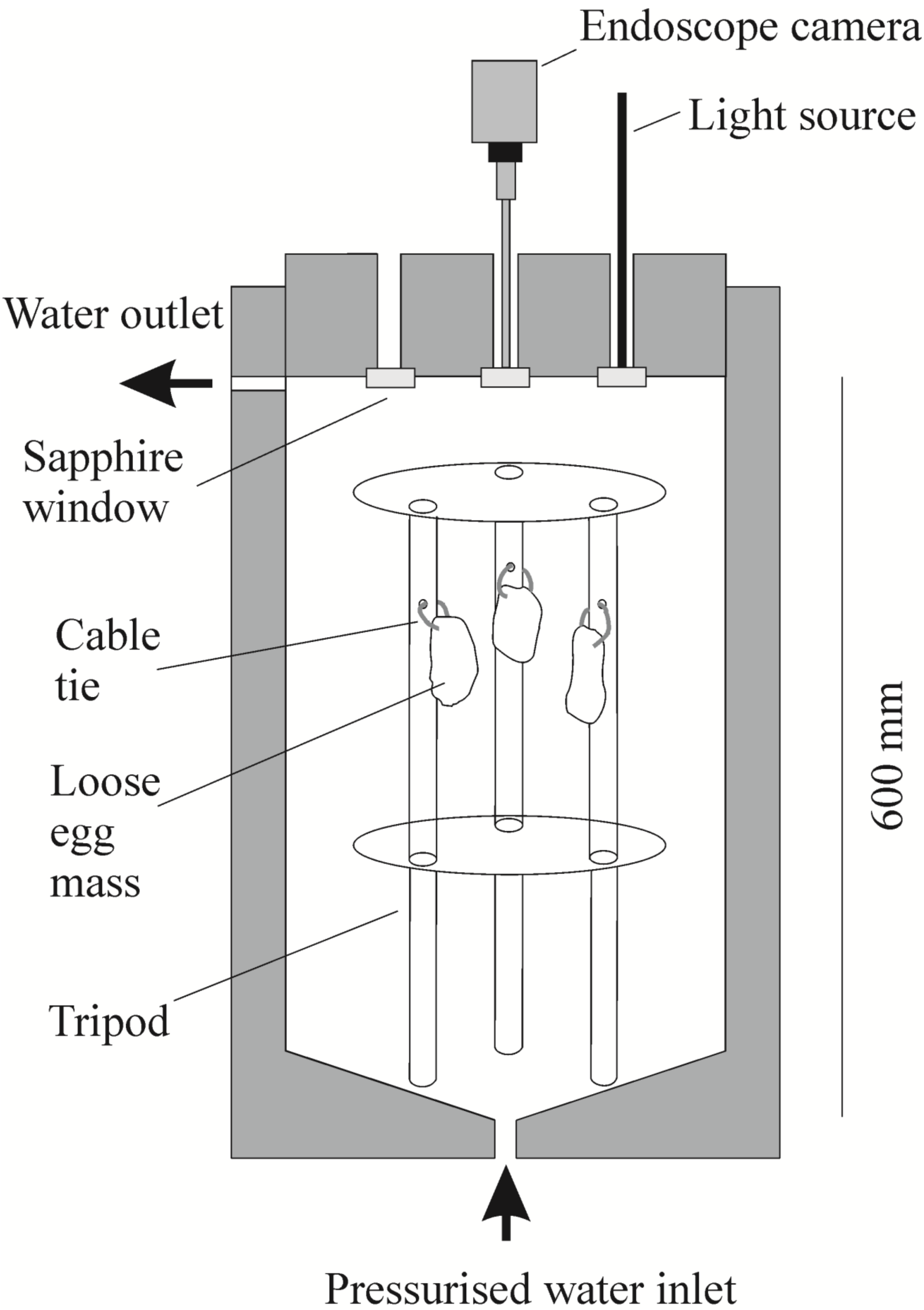

Figure 2.7. IPOCAMP (Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds) pressurised incubator used during experimentation (Chapter 7) (adapted from Shillito et al. 2004).

Pressurisation was stepwise to the desired experimental pressure (1, 100, 200 or 300 atm). Each pressure was obtained by rotating the dial until the intended pressure was reached. Pressure increases were stepwise; 10 atm every 10 mins, until the experimental pressure was achieved. In this fashion, total pressurisation was effective immediately at 1 atm, or took a total of 100, 200 or 300 mins to reach 100, 200 or 300 atm respectively. Each week, 10 L of pre-incubated 1 µm filtered sea water was added to the header tank, following the removal of an equal amount. Once pressurised, egg masses were observed weekly using the endoscope camera. At the end of the experiment, the IPOCAMP was de-pressurised stepwise at the same rate at which it was pressurised (10 atm pressure decrease every 10 mins). Once de-pressurised, the pump was turned off and the lid of the IPOCAMP removed in order to allow samples to be accessed.

2.8. Experimental protocols

2.8.1. Metabolic rate measurement

O_2 consumption (used as a proxy for metabolic rate) measurements were obtained for veligers and juveniles exposed to different temperature and pressure treatments (Chapter 6). For each measurement, three individuals were used in order to account for equipment sensitivity. The three individuals (either veligers or juveniles) were transferred into a 2.8 ml plastic vial containing pre-incubated 1 µm filtered sea water (Fig. 2.6b). The salinity of all sea water was measured and recorded prior to experimentation. Veligers were sampled by gently flushing the content of an open capsule into a petri dish (Section 2.5.2) and transferring individuals using a 3 ml plastic pipette. Individual veligers were randomly selected from three separate capsules for each vial. Juveniles were collected directly from the incubation tank walls, using a 3 ml plastic pipette with the end cut off, in order to increase the diameter of the pipette intake. This was done to ensure the intake was easily wide enough to comfortably sample juveniles. Care was taken to ensure that no samples were damaged during collection. For every three experimental vials (containing animals), a further four blank vials (containing no animals) were placed under the same experimental treatment. Blank vials were included in order to account for any loss of O_2 through microbial respiration.

Each vial was sealed under water to prevent air from being trapped inside. This allowed sea water to be pressurised without risking air being forced into the liquid, thus preventing the partial pressure of dissolved gases from being affected. In order to successfully avoid air from being trapped inside any vials, both the lid and the vial were submerged in sea water. Each was checked carefully for air bubbles, both inside, and throughout the screw fitting. Any bubbles observed were removed using a glass pasteur pipette. The exact time that each vial was closed, and later opened, was recorded, allowing the total time a vial was sealed for to be determined.

Experimental treatments lasted a total of 4 h. Prior to experimentation, preliminary investigations were carried out to ascertain the duration of the experiment (4 h), with regards to vial O_2 levels. Hypoxic conditions were considered to occur if levels fell below 60 % O_2 saturation. In order to avoid entering hypoxic conditions this figure therefore dictated experimental duration.

Following the experimental treatment, the O_2 concentration of the water inside each vial (% O_2 saturation) was recorded. Measurements were obtained using a temperature and atmospheric pressure adjusted O_2 meter (Microx TX3, Presens, Germany) and a micro-optode made of a fibre-optic cables (flat, broken tip, 140 µm) that were inserted into solution through a syringe. This equipment was calibrated daily using fully aerated sea water (100 % O_2 saturation), and sea water deoxygenated through oversaturation with sodium sulfite anhydrous (0 % O_2 saturation), both pre-incubated to temperature (after Thatje et al. 2010). Sample temperature and atmospheric pressure were both entered manually prior to calibration. Prior to O_2 measurements being recorded, each vial was gently agitated in order to ensure the O_2 level was constant throughout. O_2 saturation was either recorded inside a temperature controlled room, or with the sample inside a pre-incubated water bath, in order to ensure sample temperature did not vary during measurement. Experimental vials were opened in turn; a new vial was not opened until the reading had been completed for the preceding vial. Once a vial was opened, a micro-optode was inserted into the top 5 ml of the sea water and saturation measurements were continuously logged for a minimum of 1 min, or until a constant level of O_2 saturation was observed. Once a final figure had been obtained, the contents of the vial were transferred into a petri-dish and the animals were pooled together and sampled collectively for dry weight (Section 2.8.2). Once the O_2 saturation of all experimental vials had been determined, readings were obtained from blank vials in a similar fashion. The readings from all four blank vials were averaged for further analysis.

Final respiration rates were calculated following an adaptation of methods described by Thatje et al. (2010) and Brown and Thatje (2011). Here, the difference in readings between blank and experimental vials was determined and O_2 consumption assessed *per* unit of dry weight. This method accounts for any O_2 consumption occurring through microbial respiration.

In this method the concentration of O_2 in 100 % saturated sea water (µmol O_2 L^{-1}) was calculated according to Benson and Krause (1984), using the following equation:

$$\text{In}C^*_O = -135.90205 + 1.575701 \times 10^5 / T - 6.642308 \times 10^7 / T^2 + 1.243800 \times 10^{10} / T^3 - 8.621949 \times 10^{11} / T^4 - S(0.017674 - 10.754 / T + 2140.7 / T^2)$$

Where C^*_O is the concentration of O_2 in 100 % saturated filtered sea water (µmol O_2 L^{-1}), T is the temperature in Kelvin and S is the salinity.

The percentage of O_2 consumed in each experimental vial was then determined by subtracting the experimental reading ($\%O_2$exp) from the averaged blank reading ($\%O_2$cont). From this, the 100 % O_2 saturation figure (C^*_O) was used to determine the O_2 consumed (consO_2) in each sample (µmol O_2 L^{-1}), using the following equation:

$$(\text{cons}O_2) = (\%O_2\text{cont} - \%O_2\text{exp}) \text{ x } (C^*_O) / 100$$

Each vial was a closed system of a known volume (V) in L (the water volume displaced by veligers or juveniles was considered negligible and therefore was not considered). The time each vial was closed for in h (t) and the combined dry weight of samples in each vial in mg (DW) were also known. The O_2 consumption rate (rO_2) in each experimental vial (µmol O_2 mgDW^{-1} h^{-1}) was therefore determined using the following equation:

$$(\text{r}O_2) = (\text{cons}O_2) \text{ x } V / (t \text{ x } DW)$$

The results from this equation were the final rates used for further analysis of respiration measurements.

2.8.2. Sample dry weights

When it was necessary for sample dry weights to be established (for direct comparison between conditions, respiration calculations or for later elemental analysis), the following procedure was carried out. Generally, samples were stored individually in pre-weighed 6 mm x 4 mm tin capsules (Model D1007, Elemental Microanalysis, Okehampton, UK) and then frozen to -80 °C. Tin capsules were pre-weighed in milligrams on a microbalance, accurate to three decimal places. Developing embryos or juveniles were first transferred to a petri dish using a 3 ml pipette. They were removed from the petri dish using tweezers, gently blotted to remove excess moisture and then manipulated into a tin capsule. Where no development had occurred within a capsule and only loose eggs were present, approximately 200 were sampled together. These were collected using a glass pasteur pipette; once settled in the bottom of the pipette, they were placed carefully into a tin capsule with as little sea water as possible.

To obtain dry weights, samples were removed from the -80 °C freezer and placed into a freeze drier for 24 h. Each capsule and its related sample were then re-weighed before being maintained inside a desiccator. Sample dry weight was established by subtracting the initial capsule weight from the combined capsule and sample weight. For respiration workings (Section 2.8.1), sample weights were expressed in mg. At all other points throughout these works sample weights are expressed in µg.

For all respiration workings, pooled whole animal weights were used. For elemental analysis, it was necessary to remove any shell growth from samples prior to analysis. In order to achieve this, dried samples were rapidly de-calcified using a de-calcifying solution (RDC rapid decalcifier, Cellpath Ltd, Newtown, UK). De-calcification was complete when a sample stopped fizzing. Samples were then rinsed in distilled water before being re-frozen to -80 °C and dried again as described. Once the new sample dry weight had been established, individual shell weight and flesh weight were also determined by subtracting the new (shell free) weight from the whole (shelled) animal weight. Subsequently, shell: flesh ratio was also determined.

2.8.3. Elemental analysis

To complete elemental (carbon and nitrogen) analysis, the following procedure was carried out. Analysis was carried out through combustion at 1030 °C (Model 1108 elemental analyser, Carlo Erba, Milan, Italy). The elemental analyser was first calibrated using Chitin as a standard (%C = 44.71; %N = 6.79). Calibration was carried out using the same tin capsules as those used for samples (Section 2.8.2). It entailed running a sequence of empty, clean capsules and standard-filled capsules through the analyser. Standard-filled capsules were obtained by weighing 1 mg of the standard into a pre-weighed tin capsule. The capsule was then compressed into a small cube using tweezers, ensuring no air space remained inside the package. Empty capsules used for calibration, and capsules containing dried samples were compressed in a similar manner. Only samples weighing 200 µg or more were use in analysis; there is potential for weights below this to give inaccurate results. Following initial calibration, one standard was run before, and one after, every 10 samples to confirm calibration. These values were then used to correct for any deviation. Final values for sample C and sample N (%*n*) were therefore obtained using the following equation:

$$\%n = (\text{std } \%n \times \text{obs } \%n) / (½S^1\ \%n + ½S^2\ \%n)$$

Where (std %*n*) is the expected value for the standard (%C = 44.71; %N = 6.79), (obs %*n*) is the observed reading obtained from the sample, (S^1 %*n*) is the reading obtained from the standard run before the samples and (S^2 %*n*) is the reading obtained from the standard run after the samples.

C and N values obtained from analysis were initially expressed as percentages. These figures were then converted to µg weight using the sample DW, and C: N ratios were determined.

2.9. Data representation analysis and statistics

Throughout these works, statistical analysis was carried out using the software *Sigmaplot 11.0* and *Minitab 16*. All figures used created using the software *CorelDRAW X5* and *Sigmaplot 11.0.* An overview of the statistical tests used is given in table 2.3; each is discussed in more detail within each chapter. Where possible, parametric tests were used. Prior to this, all data were checked for normality of distribution, and homoscedasticity using Levenes test ($p > 0.05$). Any transformations made to data in order to obtain homoscedasticity are stated in the text within each chapter.

2.10. Importation legislation

In order to import *B. undatum* into the UK from Iceland, a strict procedure was followed under the guidance of CEFAS. Initially, the NOCS was certified by CEFAS to operate an Aquaculture Production Business (Authorisation number EW065-F-083A) (Appendix A), a certification which is essential in order to import live shellfish into the UK. Certification was carried out by completion of a CEFAS AAH2 form (Application to become an aquaculture production business (APB) to import live fish and shellfish into England and Wales; http://www.defra.gov.uk/aahm/files/Form-AAH2.pdf). Prior to field work commencing, it was confirmed with the Fish Health Inspectorate (CEFAS) that the species *B. undatum* was not a known disease carrier or a susceptible vector species for disease (http://www.scotland.gov.uk/Topics/marine/Fish-Shellfish/FHI/importexport/vectorspecies). This allowed the species to be imported within the EU without a health certificate. Following this, and 24 h prior to importing egg masses, a CEFAS AAH1 form (notification to import live fish and shellfish into England and Wales from another EU territory; http://www.defra.gov.uk/aahm/files/Form-AAH1.pdf) (Appendix B) was completed and sent to the Fish Health Inspectorate (CEFAS) to notify of the import; this was granted on the knowledge that the NOCS was certified to operate an Aquaculture Production Business.

Table 2.3. Statistical tests used in different experimental designs. For all parametric testing, Levenes test ($p > 0.05$) was used to confirm homoscedasticity. Where data transformation was necessary, the test used is indicated. ANOVA analysis of variance, ANCOVA analysis of covariance, C carbon, N nitrogen.

Chapter	Data	Equal variance?	Data transformed?	Parametric or non-parametric	Statistical test used
2	Female length vs capsule length Capsule length vs capsule volume	n/a	n/a	n/a	Regression analysis
	Inter-annual, collection method and population comparisons of C: N ratios	Yes	Yes, Logarithmic	Parametric	One-way ANOVA
3	Capsule volume vs number of eggs or veligers	n/a	n/a	n/a	Regression analysis
	Change in number of embryos *per* capsule Population comparison – eggs or veligers *per* capsule (Capsule size used as covariate for both)	Yes	No	Parametric	ANCOVA
4	Temperature vs number, weight or elemental composition of veligers and juveniles	No	n/a	Non-parametric	Kruskal-Wallis
	Population comparison – veliger numbers and weights	Yes	Yes, Fourth root	Parametric	One-way ANOVA
5	Capsule volume vs number and weight and resource partitioning in embryos Temperature vs number and weight and resource partitioning in embryos Nurse egg maximum consumption vs natural consumption	No	n/a	Non-parametric	Kruskal-Wallis
	Capsule volume vs elemental composition	Yes	Yes, 1/x	Parametric	ANCOVA
	Number of embryos *per* capsule and capsule volume vs resource partitioning in embryos	n/a	n/a	n/a	Regression analysis
6	Effects of temperature and pressure on veliger and juvenile respiration	Yes	Yes, square root	Parametric	General linear model ANOVA
7	Effects of pressure on embryo weight and elemental composition Effects of pressure on number of embryos *per* capsule	Yes	No	Parametric	One-way ANOVA

Chapter 3: Intracapsular development in the common whelk *Buccinum undatum* (Linnaeus 1758)

Published as: Smith KE, Thatje S (2013) Nurse egg consumption and intracapsular development in the common whelk *Buccinum undatum* (Linnaeus 1758). Helgoland Marine Research 67:109-120 (Appendix C)

3.1. Abstract

Intracapsular development is common in marine gastropods. In many species embryos develop alongside nurse eggs, which provide nutrition during ontogeny. The common whelk *Buccinum undatum* (Linnaeus 1758) is a commercially important North Atlantic shallow-water gastropod. Development is intracapsular in this species, with individuals hatching as crawling juveniles. While its reproductive cycle has been well documented, further work is necessary to provide a complete description of encapsulated development. Here, using laboratory reared *B. undatum* egg masses from the Solent, on the south coast of England, intracapsular development at 6 °C is described. This temperature is within the natural thermal range which this population is exposed to during development. Nurse egg partitioning, timing of nurse egg consumption and intracapsular size differences through development are discussed. Number of eggs, veligers and juveniles *per* capsule are compared using egg masses collected from Northern (Breiðafjörður) and Southern (the Solent) extremes of the species distribution. Total development took between 133 and 140 days, over which seven ontogenetic stages were identified. The number of both eggs and veligers were positively related to capsule volume in both populations, but number of eggs *per* capsule was lower in the Breiðafjörður population than in the Solent population. Approximately 1 to 1.5 % of eggs developed *per* capsule. Each early veliger consumed nurse eggs rapidly over just 3 to 7 days. Within each capsule initial development was asynchronous, but it became synchronous during the veliger stage. No evidence for cannibalism was found during development but large size differences between embryos developing within each capsule were observed, and occasionally ‘empty’ veligers were seen which had not successfully consumed any nurse eggs. These results indicate a high level of competition for nurse eggs within each capsule during development in the common whelk. The initial differences observed in nurse egg uptake may affect individual predisposition in later life.

3.2. Introduction

Many marine gastropods undergo intracapsular development inside egg capsules (Thorson 1950; Natarajan 1957; D’Asaro 1970; Fretter and Graham 1985). Embryos develop within the protective walls of a capsule which safeguards against factors such as physical stress, predation, infection and salinity changes (Thorson 1950; Pechenik 1983; 1999; Strathmann 1985; Rawlings 1995; 1999). Periods of encapsulation vary; some species are released as veligers and undergo a planktonic stage before reaching adult life (mixed development), while others display direct development, hatching from capsules as crawling juveniles (Natarajan 1957; D'Asaro 1970; Pechenik 1979). When direct development occurs, embryos are often accompanied in a capsule by nurse eggs, non-developing food eggs, which provide nutrition during development (Thorson 1950; Spight 1976b; Rivest 1983; Lahbib et al. 2010). These are usually indistinguishable from embryos in the very early stage of ontogeny and are consumed during development, potentially increasing size of juveniles at hatching (Thorson 1950). In some species, nutrition may also be provided by intracapsular fluid or protein from capsule walls (Bayne 1968; Stöckmann-Bosbach 1988; Moran 1999; Ojeda and Chaparro 2004).

Generally speaking, nurse egg consumption occurs over a period of several weeks or months. It commences some weeks into development as embryos form and nurse eggs are then slowly consumed throughout much of development (Chaparro and Paschke 1990; Ilano et al. 2004; Lahbib et al. 2010). The number of nurse eggs consumed during this period varies across species. Ratios range from 1.7 nurse eggs *per* embryo in the Pacific shallow-water muricid *Acanthinucella spirata* (Blainville 1832) (Spight 1976a), to between 50000 and 100000 nurse eggs *per* embryo in the North Atlantic deep-sea buccinid *Volutopsius norwegicus* (Gmelin 1791) (Thorson 1950). Often, within a species the nurse egg to embryo ratio varies from capsule to capsule within one clutch (Thorson 1950; Spight 1976a). For example Rivest (1983) found this ratio in the dire whelk *Lirabuccinum dirum* (Reeve 1846) to vary from 11 to 46 across capsules. Similar differences have been reported for other gastropods (Natarajan 1957; Spight 1976a). Within a capsule, however, there is usually little variation in the number of nurse eggs ingested by each embryo, with all embryos generally being equal in their ability to consume. Any differences observed are minimal, and juveniles hatching from each capsule are normally of a very similar size (Natarajan 1957; Spight 1976a; Rivest 1983; Chaparro and Paschke 1990; Chaparro et al. 1999; Lloyd and Gosselin 2007). Large size differences amongst capsulemates are unusual, but have been reported in some species of muricid gastropod (Gallardo 1979; González and Gallardo 1999; Cumplido et al. 2011). In gastropods, the number of eggs inside a capsule is usually positively related to capsule size. Within a species, larger capsules hold more eggs and more developing embryos (Gallardo 1979; Pechenik et al. 1984; Miloslavich and Dufresne 1994). The relationship between capsule size and number of eggs (including nurse eggs) has, however, previously been shown to be stronger than the relationship between capsule size and number of developing embryos (Spight 1976b). In some cases, the number of developing embryos within a capsule has been found to be independent of capsule volume. This suggests that embryos are distributed at random, while nurse eggs are regularly placed amongst capsules (Rivest 1983; Chaparro et al. 1999).

Intracapsular development and nurse egg and embryo partitioning has been investigated in several species of marine gastropod (Natarajan 1957; D'Asaro 1970; Spight 1976a; Rivest 1983; Cumplido et al. 2011). While some attempts have been made to examine encapsulated development in the common whelk *Buccinum undatum* (Linnaeus 1758) (Portmann 1925; Fretter and Graham 1985; Nasution 2003) it has not yet been fully described. Nasution (2003) gives the most in-depth account of development to date but his descriptions are incomplete and his reports of nurse egg consumption do not match my observations. Descriptions from Portmann (1925) better fit my observations but lack detail. There are also gaps in the current literature, and very limited knowledge exists on nurse egg partitioning and intracapsular embryo size ranges through development. Given the widespread distribution of *B. undatum*, its current commercial importance and its potential as a future candidate for aquaculture, it is important to understand fully the development in this species.

3.3. Aims and objectives

The specific aims of this chapter were to investigate development in the common whelk *B. undatum* at 6 °C. This temperature is within the local developmental thermal range for populations of this species from the Solent, UK.

The objectives of this chapter were to:

- Examine intracapsular development in *B. undatum* using a population from the Solent, at the southern end of the species distribution.

- Describe the ontogenetic stages in *B. undatum* and examine intracapsular ranges in embryo sizes.
- Establish number of eggs and number of developing veligers *per* capsule using populations from the northern and southern ends of the species distribution.
- Investigate differences in numbers of veligers and juveniles *per* capsule through development using the population from the southern end of the species distribution (the Solent).

3.4. Materials and methods

3.4.1. Embryonic development

Intracapsular development in *B. undatum*, was investigated using three egg masses from the Solent population, laboratory reared at the National Oceanography Centre, Southampton and laid in early January (Chapter 2, section 2.2; Fig. 2.1; Table 2.2). Each was maintained at 6 °C for the duration of development (Chapter 2, sections 2.3, 2.6). This was close to local water temperatures, which ranged 4.0 to 8.3 °C between January and March 2010 (Chapter 2; Fig. 2.3). Each week three capsules were randomly selected and dissected from each egg mass. The contents of each capsule were examined, ontogenetic stage was described and eggs or embryos were counted and as described previously (Chapter 2, section 2.5). From the trochophore stage and for the duration of nurse egg feeding, three capsules *per* egg mass were examined daily to determine the duration of short ontogenetic stages and the time taken to consume nurse eggs. Each egg mass was also examined non-invasively each week and the percentage of the mass at each developmental stage was estimated (Chapter 2, section 2.5; Fig. 2.2c-d). From this, embryonic development was described, including ontogenetic stages, developmental timing, change in embryo size, nurse egg partitioning and intracapsular size differences during development. During this stage of the investigation, sizes were used instead of weights because weights are very difficult to obtain from early developmental stages due to the small size of them.

3.4.2. Intracapsular contents through development

3.4.2.1. Relationship between capsule volume and number of embryos per capsule

In order to investigate the intracapsular contents of *B. undatum* egg masses, capsules were selected at random from all 35 egg masses from the Solent population trawled and stored in 4 % formalin. Each capsule examined was measured as previously described, prior to dissection (Chapter 2, section 2.5). To investigate the relationship between capsule volume and number of eggs or veligers within a capsule, from the Solent population, approximately 160 capsules at egg stage (i.e. prior to any development occurring; 15 egg masses; 10 to 11 capsules from each) and 160 capsules at veliger stage (18 egg masses, eight to nine capsules from each) were examined. Capsules ranging from 5.15 to 10.49 mm length (39.0 to 287.5 mm^3 volume) were compared (Chapter 2, section 2.5). Regression analyses were carried out to examine the relationship between capsule volume and number of eggs, and capsule volume and number of veligers.

3.4.2.2. Change in number of embryos per capsule through development

Change in number of embryos *per* capsule during development was investigated using capsules from the Solent population. One hundred capsules at veliger stage (12 egg masses, eight to nine capsules from each), and 100 capsules at pre-hatching juvenile stage (nine egg masses, 11 to 12 capsules from each) were examined (Chapter 2, section 2.5). For this comparison, capsules of a narrower size range (length 6 to 8 mm, volume 52.4 to 146.2 mm^3) were used (Chapter 2, section 2.5). Only veligers containing nurse eggs were included in the count; it was presumed veligers with no nurse eggs would not develop successfully. An analysis of covariance (ANCOVA) was carried to compare number of veligers *per* capsule to number of pre-hatching juveniles *per* capsule, using capsule size as a covariate.

3.4.2.3. Inter-population comparisons

In order to examine any inter-population differences in capsule content, number of eggs *per* capsule, and number of veliger *per* capsule were compared for egg masses from the

Solent and egg masses from Breiðafjörður (Chapter 2, section 2.2; Fig. 2.1). For each developmental stage (egg and veliger), capsules of a similar volume were used for analysis. Seven egg masses from the Breiðafjörður population were opportunistically sampled upon collection, and dissected as described previously (Chapter 2, section 2.5). In total, 30 capsules were sampled at the egg stage stage (three egg masses, 10 capsules from each) and 36 capsules were sampled at the veliger stage (four egg masses, nine

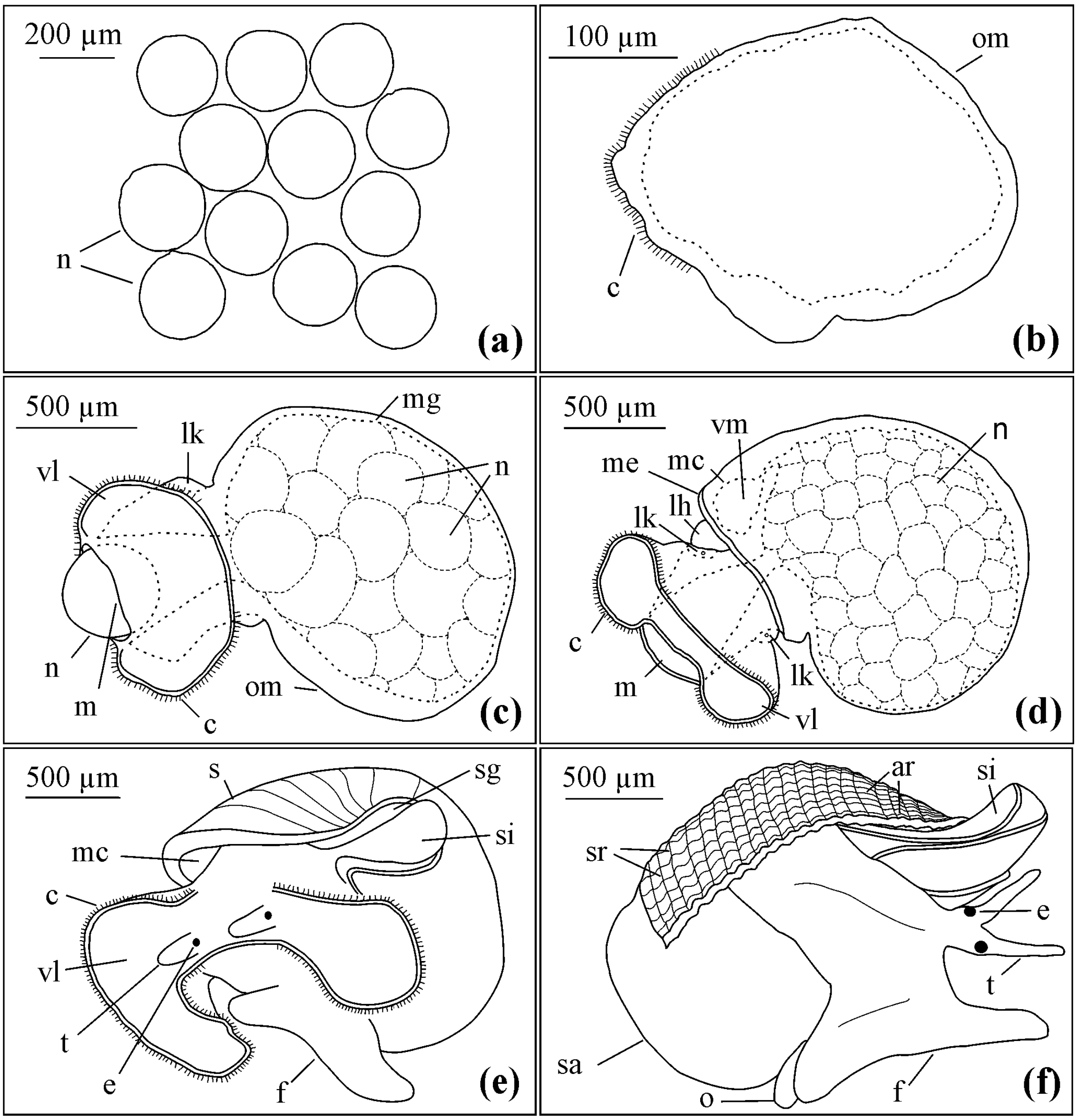

Figure 3.1. Intracapsular developmental stages of *Buccinum undatum*: sketches. (a) egg, **(b)** trochophore, **(c)** early veliger, **(d)** veliger, **(e)** pediveliger and **(f)** pre-hatching juvenile. *n* nurse egg or undeveloped embryo, *om* outer membrane, *c* cilia, *vl* velar lobe, *m* mouth, *mg* midgut, *me* mantle edge, *mc* mantle cavity, *vm* visceral mass, *lh* larval heart, *lk* larval kidney, *s* shell, *si*, siphon, *sg* siphonal groove, *t* tentacle, *e* eye, *f* foot, *o* operculum, *sa* shell apex, *sr* spiral ribs, *ar* axial ribs.

capsules from each). At the egg stage, capsule length ranged from 6.55 to 10.28 mm (85.6 to 267.7 mm^3 volume). At the veliger stage, capsule length ranged from 7.07 to 11.80 mm (83.7 to 243.6 mm^3 volume). These numbers were compared to number of eggs (from 104 capsules) and number of veliger (from 109 capsules) from the Solent population, from capsules which fell into the same size classes as the Breiðafjörður samples. ANCOVA was carried out to compare egg and veliger numbers between the Solent and Breiðafjörður populations. Capsule volume was used as a covariate. Regression analyses were carried out to examine the relationship between capsule volume and number of eggs, and capsule volume and number of veligers for the Breiðafjörður population.

3.5. Results

3.5.1. Ontogenetic stages

Seven ontogenetic stages were identified; egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile and hatching juvenile. These are described below.

Egg

Each capsule contains 475 to 2639 (mean 1094, S.D. ± 327.6) small spherical eggs with no definition. Eggs are cream or yellow in colour and have an average diameter of 234 µm. Within a capsule, egg diameter varies on average by 36 µm. Approximately 1 % of these eggs are developing embryos. The remaining are nurse eggs. At this stage both developing and nurse eggs are identical (Figs. 3.1a, 3.2a; Table 3.1). Egg capsules remain at this stage on average for 49 days.

Trochophore

After 42 to 70 days developing embryos become globular shaped with a non-circular translucent membrane around the darker embryo. A cilia band (prototroch) is present around approximately one third to half of the outer circumference of the membrane (Figs. 3.1b, 3.2b). Each trochophore is a little larger than an egg, with an average length of 321 µm. Each embryo remains at the trochophore stage for just 2 to 3 days (Table 3.1).

Table 3.1. Timing of intracapsular development in *Buccinum undatum* from the south coast of England at 6 °C. Mean size at each ontogenetic stage is displayed (mm). Means are determined as an average of n measurements; n dictates total number of individuals measured. n (capsules) dictates number of capsules individuals were measured from and that were examined at each stage. Where n/a is stated, value was inapplicable or not determined.

Ontogenetic stage	Mean time in days spent at each stage (individual)	Time at developmental stage in days (whole egg mass)	Mean size (mm ± SD)	Mean size variation within one capsule (mm)[a]	n	n (capsules)
Egg	49	0 to 56	0.23 (± 0.01)	0.04	3235	142
Trochophore	2	42 to 56	0.32 (± 0.02)	0.01	19	12
Early veliger	5	42 to 56	1.46 (± 0.15)	0.33	121	15
Veliger	18	42 to 77	1.65 (± 0.17)	0.27	97	17
Pediveliger	18	70 to 98	1.91 (± 0.32)	0.42	144	20
Pre-hatching juvenile	44	91 to 140	2.15 (± 0.29)	0.38	74	14
Hatching juvenile	n/a	133 to 140	2.43 (± 0.39)	n/a	102	n/a

[a]Only capsules with two or more individuals included.

Early veliger

As the early veliger stage is reached, the prototroch extends laterally to form paired velar lobes with marginal cilia around a central simple mouth. Velar lobes are used for collection of eggs and locomotory movement. Each early veliger is mobile but lacks obvious intentional direction. Behind each lobe and just in front of the main body of the early veliger, paired larval kidneys develop, slightly opaque in colour. Whole (generally nurse) eggs are manipulated into the mouth section using the cilia. These are engulfed and stored in the midgut (Portmann 1925), which forms a circular ball directly behind the mouth section, surrounded by a thin outer membrane. There is some asynchrony in the early development of the embryos from individual capsules. In total between two and 35 veligers develop *per* capsule (mean 11, S.D. ± 6.7). Each embryo consumes nurse eggs for three to seven days (at 6 °C). Total consumption by all embryos within a capsule occurs during the early veliger stage, over four to 10 days. Eggs are not damaged during consumption but are stored in the midgut, conserved for later nutritional use. Whole, undamaged nurse eggs can be seen inside each early veliger. Early veligers average 1.46 mm across their longest axis. Within one capsule embryo size may vary by as much as 0.85 mm. These size differences continue to be observed throughout development. Once all nurse eggs are consumed, early veligers, veligers and even pediveligers are occasionally found in a capsule, which have consumed no nurse eggs at all (Figs. 2.1d, 3.1c, 3.2c, 3.3a-b,d; Table 3.1). After the pediveliger stage, these ‘empty’ embryos were not observed, but occasional small, thin, empty shells were observed. The ‘empty’ embryos were therefore presumed not to survive to hatching.

Veliger

In the veliger the mantle edge thickens and a thin larval shell becomes visible around the midgut, creating a transparent layer. The midgut appears important in dictating the dimensions of this shell. The velar lobes become more separated and distinct and the larval kidneys continue to be seen, often with a central yellow spot. The central mouth section becomes more opaque, early foot development begins and no further nurse egg consumption is possible. The mantle edge and the visceral mass (white in colour) beneath it become obvious. A transparent pulsating membrane located dorso-laterally in front of the mantle edge becomes evident; this is often named the larval heart (Hughes 1990; Khanna and Yadav 2004). Nurse eggs stored beneath the mantle are still clearly individually discernible at this stage and even going into the pediveliger stage (Figs. 3.1d, 3.2d, 3.3b-d; Table 3.1). It is possible to break the mantle or shell on the back of the veliger or pediveliger and find nurse eggs still inside, which are not degraded and have not yet been digested. Embryos remain at the veliger stage for approximately 14 to 21 days. During this period development within a capsule becomes synchronised.

Pediveliger

At the pediveliger stage, the shell thickens and becomes increasingly apparent. The mantle cavity is initially visible beneath the mantle edge and the siphonal groove begins to form. The foot, eyes, tentacles and siphon appear. The velum and cilia, which are large at the beginning of this stage, begin to shrink back. They disappear by the end of the pediveliger stage. The larval kidneys and larval heart also disappear. Embryos remain at this stage for approximately 14 to 21 days (Figs. 3.1e, 3.2e-f, 3.3c; Table 3.1).

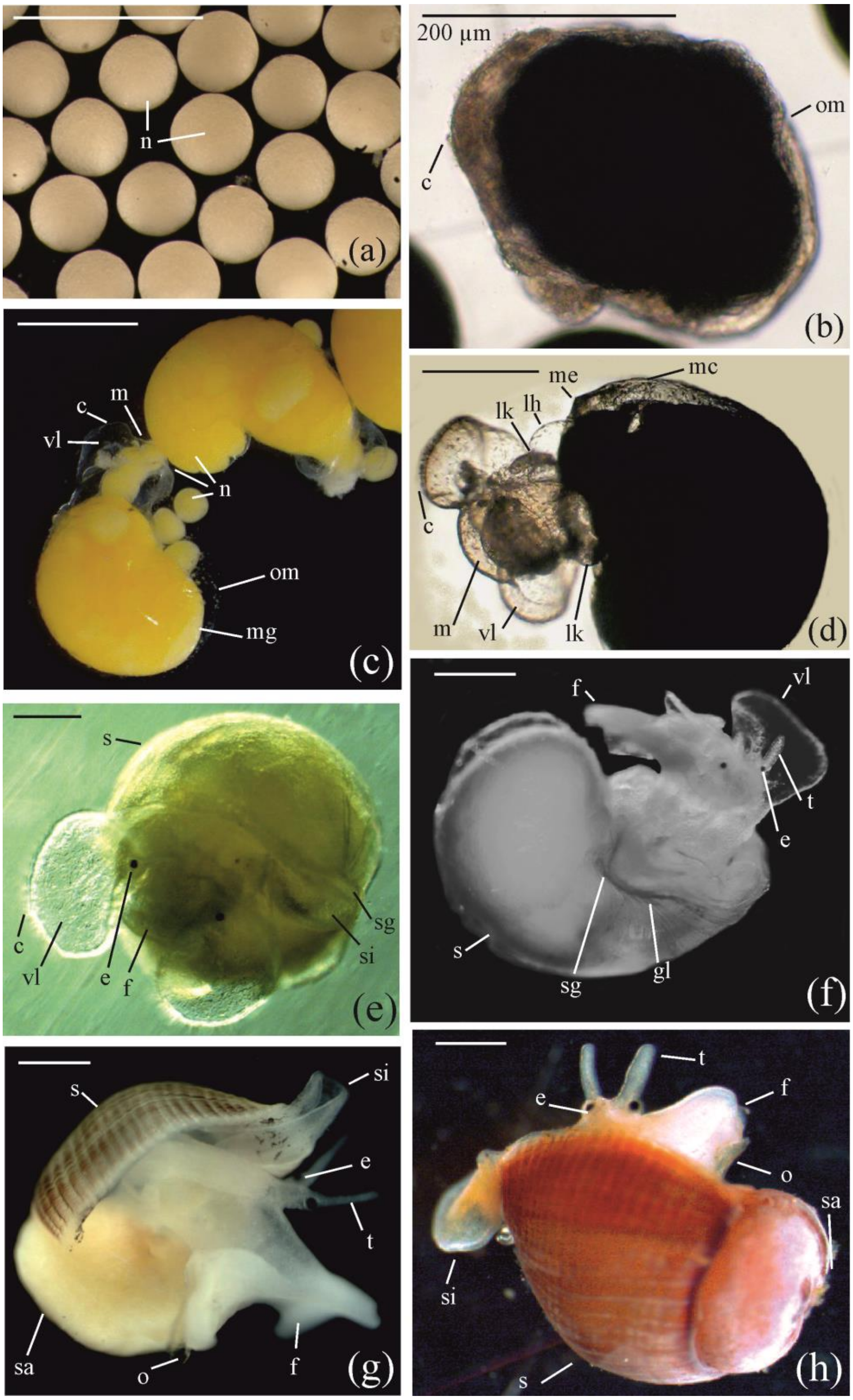

Figure 3.2. Intracapsular developmental stages of *B. undatum*: photos. (a) egg, (b) trochophore, (c) early veliger consuming nurse eggs, (d) veliger, (e) & (f) pediveliger, (g) pre-hatching juvenile and (h) hatching juvenile. *n* nurse egg or undeveloped embryo, *om* outer membrane, *c* cilia, *vl* velar lobe, *m* mouth, *mg* midgut, *me* mantle edge, *mc* mantle cavity, *lh* larval heart, *lk* larval kidney, *s* shell, *si*, siphon, *sg* siphonal groove, *t* tentacle, *e* eye, *f* foot, *o* operculum, *sa* shell apex. Where not otherwise stated , scale bars represent 500 µm.

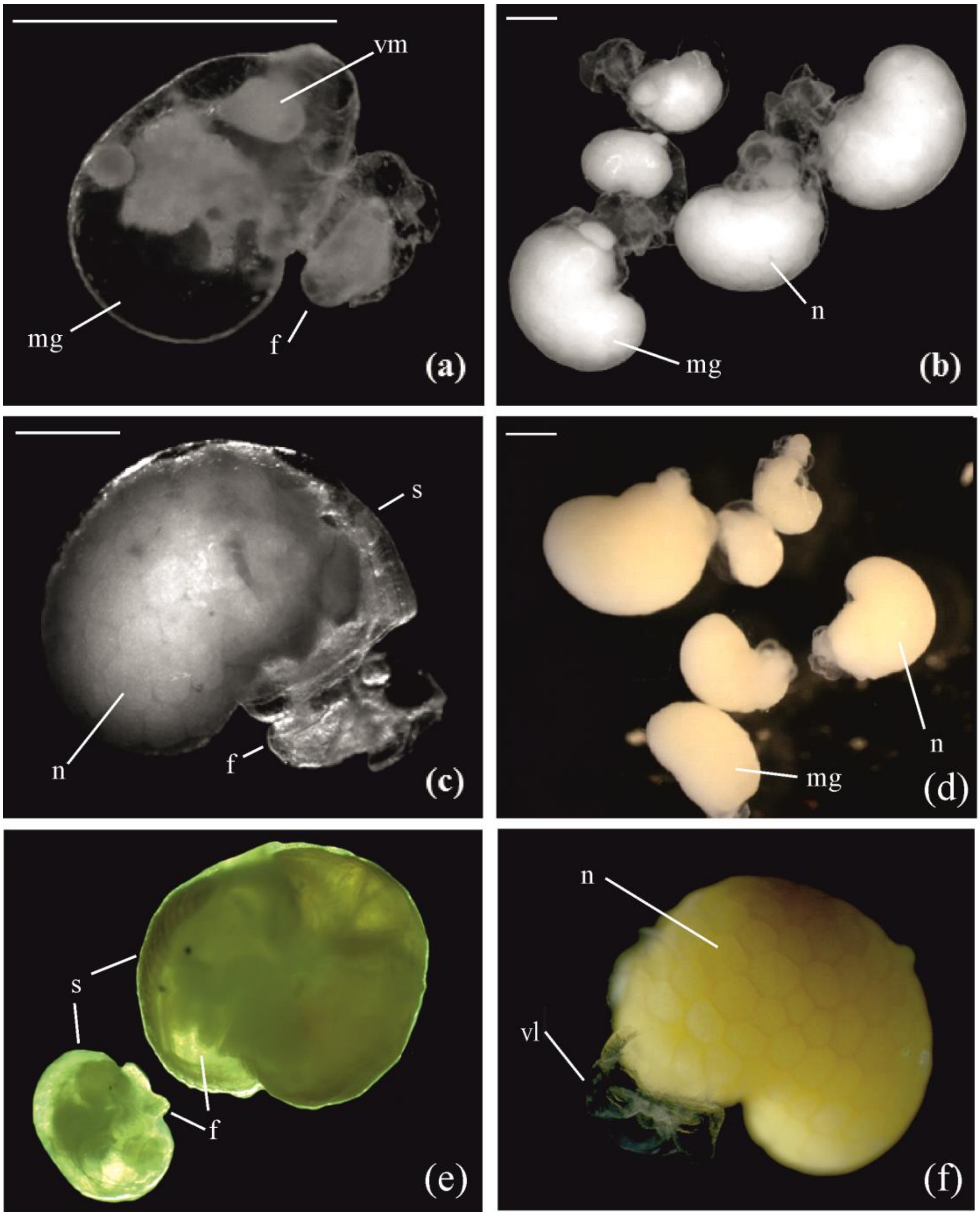

Figure 3.3. Photos of early development in *B. undatum*. **(a)** early pediveliger stage with empty midgut indicating few or no nurse eggs were consumed. **(b)** veligers of varying sizes developing alongside each other; within one capsule and following nurse egg consumption. **(c)** early pediveliger stage with individual nurse eggs still clearly discernible under the shell. **(d)** veligers of varying sizes developing alongside each other; within one capsule and following nurse egg consumption. **(e)** ‘normal’ healthy (top) and ‘empty’ (bottom) pediveligers. **(f)** veliger with individual nurse eggs clearly discernible under shell. *n* nurse egg, *vl* velar lobe, *mg* midgut, *vm* visceral mass, *s* shell, *sg* siphonal groove, *t* tentacle, *e* eye, *f* foot, *gl* growth lines. Scale bars represent 500 µm.

Pre-hatching juvenile

Shell growth continues and spiral and axial ribs begin to develop in the shell as the pre-hatching juvenile stage is reached. The shell thickens and colours brown (becomes pigmented). The first whorl becomes obvious and the shell shape elongates. Head, foot, tentacle and siphon features become more prominent and the operculum appears. The feeding proboscis also develops internally during this time. Pre-hatching juveniles complete development over a further 35 to 49 days before hatching commences. Pre-hatching juvenile size ranges from 1.57 to 3.06 mm. (Figs. 3.1f, 3.2g; Table 3.1).

Hatching juvenile

The features described for pre-hatching juveniles become more prominent. The juvenile emerges from the egg capsule through an opening created through radular scraping. They remain on the egg mass for a few days before moving off to feed. Overall hatching size ranged from 1.70 to 3.45 mm (Fig 3.2h; Table 3.1).

3.5.2. Embryonic development

Each egg mass took between 9 and 11 days to be laid, with complete intracapsular development taking 133 to 140 days (19 to 20 weeks) at 6 °C. Within each egg mass, development was asynchronous by up to 14 days throughout the developmental period. Within each capsule, development was initially asynchronous; both trochophore and early veliger stages, and early veliger and veliger stages were observed together in capsules. By late veliger stage development within a capsule was synchronous. Following an initial increase in embryo size as nurse egg consumption occurred, individual size (measured as change in length) increased at a steady rate throughout the remainder of the encapsulated period (Figs. 3.4, 3.5; Table 3.1). Within each capsule, large size differences were observed between embryos at all stages of development. Whole, undamaged nurse eggs were visible inside embryos throughout the veliger and pediveliger stages. Occasional early veligers, veligers and pediveligers were found which had not consumed any nurse eggs. Apart from the absence of nurse eggs and the smaller size, these embryos were completely normal in their development (Fig. 3.3a, e; Table 3.1).

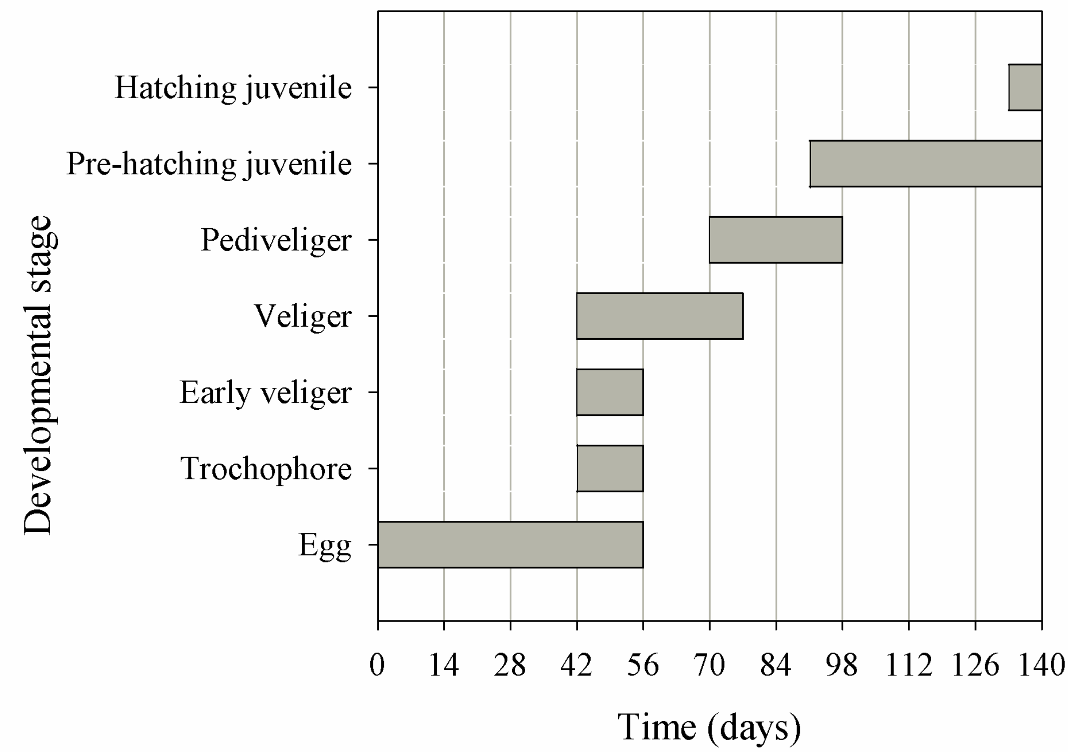

Figure 3.4. Developmental time (days) for *B. undatum* from Southampton Water (UK) at 6 °C. Times shown represent development across whole egg masses.

3.5.3. Intracapsular contents through development

3.5.3.1. Relationship between capsule volume and number of embryos per capsule

For the Solent population, number of eggs *per* capsule averaged 1094 and number of veligers *per* capsule averaged 11. Regression analysis showed there to be a positive relationship between capsule volume and number of eggs (y = 282 + 8.04 x; R^2 = 0.765; d.f. = 1; p $\leq$ 0.001), and capsule volume and number of veligers (y = 0.273 + 0.0927 x; R^2 = 0.562; d.f. = 1; p $\leq$ 0.001) for the Solent population. As a percentage of total eggs, on average 1 % develop into veligers (Fig. 3.6a-b).

3.5.3.2. Change in number of embryos per capsule through development

When examining capsules ranging from 6 to 8 mm in length (volume 52.4 to 146.2 mm^3), number of developing veligers *per* capsule ranged from 3 to 21 (mean 9, S.D. ± 3.4) and number of pre-hatching juveniles *per* capsule ranged from 2 to 20 (mean 9, S.D. ± 3.6). ANCOVA showed there to be no difference between the two groups (F = 1.28; d.f. = 1; p = 0.259). Both number of developing veliger and number of pre-hatching juveniles were positively related to capsule size (F = 8.96; d.f. = 1; $p \leq 0.005$).

3.5.3.3. Inter-population comparisons

When examining capsules ranging from 6.55 to 10.28 mm length (85.6 to 267.7 mm^3 volume), number of eggs *per* capsule ranged from 560 to 1441 (mean 1015, S.D. ± 241.9) in the Breiðafjörður population, and 765 to 2639 (mean 1229, S.D. ± 307.3) in the Solent population. ANCOVA showed number of eggs *per* capsule to be significantly lower in the Breiðafjörður population than in the Solent population (F = 433.73; d.f. = 1; $p \leq 0.001$). When examining capsules ranging from 7.07 to 11.80 mm length (83.7 to 243.6 mm^3 volume), number of veligers *per* capsule ranged from 9 to 38 (mean 16, S.D. ± 6.4) in the Breiðafjörður population, and 3 to 35 (mean 13, S.D. ± 6.6) in the Solent population. ANCOVA showed there to be no difference in number of veligers *per* capsule between the two populations (F = 1.28; d.f. = 1; p = 0.259). Regression analysis indicated both number of eggs (y = 280 + 3.81 x; R^2 = 0.553; d.f. = 1; $p \leq 0.001$) and number of veligers (y = 8.07 + 0.0522 x; R^2 = 0.134; d.f. = 1; $p \leq 0.05$) to be positively related to capsule volume for the Breiðafjörður population (Fig. 3.6a, b).

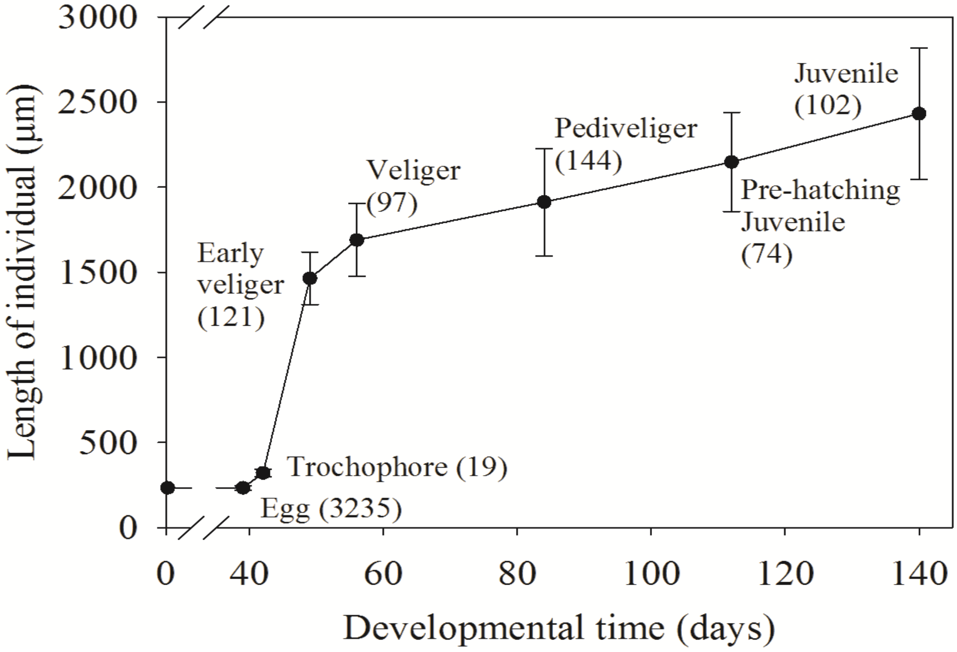

Figure 3.5. Change in size of individual *B. undatum* embryos during intracapsular development. Size displayed is average length of individual at each stage in µm (measured as length along longest axis). Nurse egg consumption occurs between trochophore and early

veliger stages. The average size displayed for early veliger is taken post nurse egg consumption. Error bars indicate ± 1 S.D. Bracketed numbers indicate number of individuals measured.

3.6. Discussion

3.6.1. Embryonic development and intracapsular contents data

The distribution of *B. undatum* extends from the southern coast of the UK, northwards up into the North Atlantic and Arctic oceans, across a temperature range of -1.5 to 22 °C (Bramblemet; CEFAS (Chapter 2; Section 2.2.1.1.); Martel et al 1986a). For the Solent population, annual temperatures vary seasonally, ranging approximately 4 to 22 °C, and egg laying and development normally occur in seawater temperatures ranging 4 to 10 °C. With temperatures maintained at 6 °C, the duration of intracapsular development (4.5 to 5 months) was similar to previous estimates of *B. undatum* development in British waters (Kideys et al. 1993; Valentinsson 2002). Longer and shorter periods have been reported across the species distribution (e.g. Martel et al. 1986a; Nasution 2003; Author, unpublished data). The observed differences in duration of development can be attributed to the known effects of temperature on metabolic rates in ectotherms (Evjemo et al. 2001; García-Guerrero et al. 2003).

In the present study, for the Solent population the number of eggs *per* capsule averaged 1094 and the number of developing veligers averaged 11. While egg numbers were similar to those indicated in previous studies, veliger numbers were similar to figures reported by Hancock (1967), but lower than other estimates (Portmann 1925; Martel et al. 1986a). Since number of veligers appears to be positively related to capsule volume across the species range (Gallardo 1979; Pechenik et al. 1984; Valentinsson 2002; Present study), it is likely that larger capsules were examined in the latter studies. It should be taken into consideration, however, that despite the evident similarities in intracapsular content of *B. undatum* egg masses from across the species range, the data presented in this study highlight that, when examined in detail, differences exist. When comparing the two study populations, although number of eggs *per* capsule appeared similar, the Breiðafjörður population had significantly less eggs *per* capsule than the Solent population despite having the same number of veligers developing.

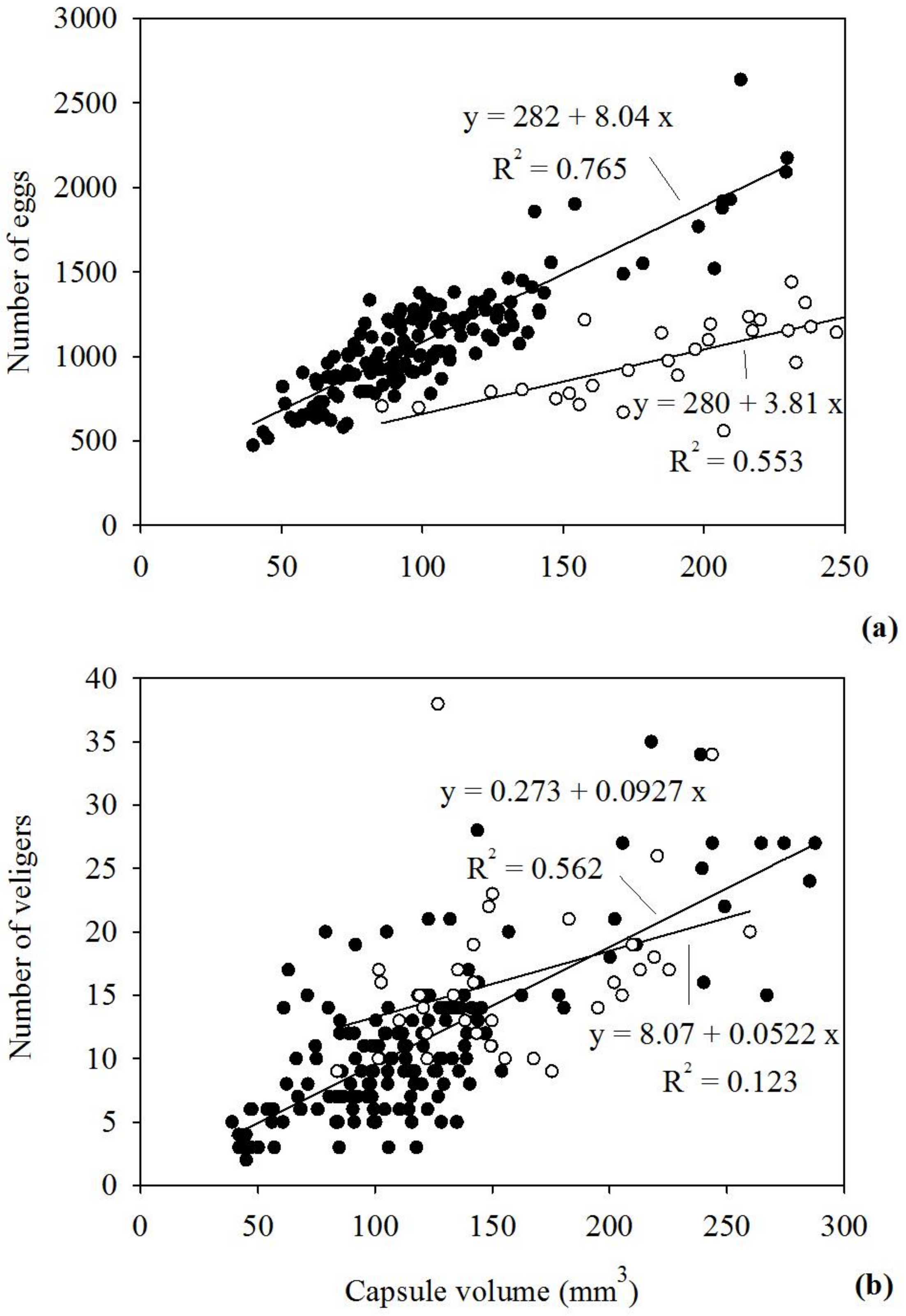

Figure 3.6. Relationship between capsule volume and (a) number of eggs, (b) number of veligers in egg masses of *B. undatum*. Closed circles denote the Solent population; open circles denote the Breiðafjörður population. All relationships are significant to $p \leq 0.05$. Equations and R^2 values are displayed.

The results for the Solent population indicate approximately 1 % of eggs developed, giving a ratio of 109 nurse eggs *per* embryo, almost identical to the 110 eggs *per* embryo reported by Portmann (1925). The percentage of eggs developing was also comparable to other previous estimates for *B. undatum*, which range from 0.2 % to 1.65 % (Martel et al. 1986a; Valentinsson 2002; Nasution 2003). Similar results have been reported for other buccinids including 1.1 to 2 % for *Buccinum isaotakii* (Kira 1959) (Ilano et al. 2004), 0.2 to 1.8 % for *Buccinum cyaneum* (Brugière 1792) (Miloslavich and Dufresne 1994) and 1 % for *Colus stimpsoni* (Mörch 1868) (West 1979).

Past studies provide conflicting views on the occurrence of intracapsular cannibalism in *B. undatum* (Table 3.2). Portmann (1925) indicated a reduction in number of individuals *per* capsule during development (from early veligers to veligers and pre-hatching juveniles), which was suggested to be due to cannibalism (Fretter and Graham 1985). Contrary to this, other studies have shown the number of developing embryos *per* capsule to remain constant during development, indicating no cannibalism (Hancock 1967; Martel et al. 1986a). The present results were in agreement with these latter studies. Similarly, no cannibalism during development was reported in the buccinids *B. cyaneum* (Miloslavich and Dufresne 1994) and *B. isaotakki* (Ilano et al. 2004), and only very rarely was it observed in the buccinid *L. dirum* Rivest (1983). It has, however, been reported in some other gastropods including *Crucibulum quiriquinae* (Lesson 1830) (Véliz et al. 2001), *Crepidula coquimbensis* (Brown and Olivares 1996) (Véliz et al. 2003; Brante et al. 2009) *Trophon geversianus* (Pallas 1774) (Cumplido et al. 2011) and a vermetid gastropod (Strathmann and Strathmann 2006).

Capsule size or volume has previously been shown to be a good indicator of number of eggs and veligers within a capsule. In the current study, these figures were both positively related to capsule volume. Number of eggs was more closely related to volume than number of veligers for both populations studied, suggesting eggs are more regularly distributed amongst capsules than are developing embryos. This pattern has been reported before for both *B. undatum* (Valentinsson 2002; Nasution et al. 2010) and other gastropods, including *B. cyaneum* (Miloslavich and Dufresne 1994), *B. isaotakki*(Ilano et al. 2004), *Hexaplex trunculus* (Linnaeus 1758) (Lahbib et al. 2010), *Acanthina monodon* (Pallas 1774) (Gallardo 1979), *Nucella lapillus* (Linnaeus 1758) (Pechenik et al. 1984) and *Nucella lamellosa* (Gmelin 1791) (Spight 1976b). Contrary

Table 3.2. Reproductive biology of *B. undatum* from present and previous studies. n/a indicates figures not available in study. av. denotes average.

Study	Location	Development temperature (°C)	Time to hatching (months)	Capsule size (length mm)	No. of eggs *per* capsule	Egg diameter (µm)	% of eggs that develop	No. of veligers *per* capsule	No. hatching juveniles *per* capsule	Length of shell at hatching (mm)
Portmann (1925)	Roscoff, France	5-9	n/a	n/a	50 – >2000	n/a	n/a	av. 30	av. 10	n/a
Hancock (1967)	Burnham on Crouch, UK	n/a	3 – 4	n/a	≤3000	n/a	n/a	13 to 14	n/a	n/a
Fretter and Graham (1985)	n/a	n/a	3 – 9	6 – 12	500 – 3000	200 – 300	n/a	av. 30	3 to 10	1 – 1.4
Martel et al. (1986a)	Gulf of St Lawrence, Canada	2-3	5 – 8	n/a	2700	n/a	1.10	av. 30	av. 30	3
Kideys et al. (1993)	Douglas, Isle of Man	n/a	3 – 5	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Valentinsson (2002)	Skagerrak, Sweden	4-8	3 – 4	7 – 9.5	700 – 2300	245 – 285	0.20 – 1.20	n/a	2 to 16	1.9 – 2.8
Nasution (2003)	Irish Sea, Northern Ireland	8-11	2.5 – 3	n/a	av. 2360[a]	340	0.47 – 1.65	n/a	n/a	Nearly 2[b]
Nasution et al. (2010)	Irish Sea, Northern Ireland	10	3	n/a	558 – 4196	n/a	0.47 – 1.65	n/a	n/a	2.1 – 3.1
Present study	**Southampton Water, UK**	**6**	**4.5 – 5**	**5 – 10.5**	**475 – 2639**	**200 – 260**	**1.01**	**av. 9 to 11**	**av. 9**	**1.70 – 3.45**
Present study	**Breiðafjörður, Iceland**	**3-4**	**n/a**	**6.5 - 12**	**560 - 1441**	**n/a**	**Approx. 1.51[c]**	**av. 16**	**n/a**	n/a

[a] range stated in journal was not possible. [b] no accurate value was stated in publication. [c] estimate based on capsules from a similar size class.

to this, number of eggs has been found to be related to, but number of veligers to be independent of capsule size in *L. dirum,* the calyptraeid *Crepipatella dilatata* (Lamarck 1822) (Chaparro et al. 1999) and the muricid *Nucella ostrina* (Gould 1852) (Lloyd and Gosselin 2007).

An initial rapid increase in embryo size was observed at the early veliger stage in the present investigation. This was followed by a relatively linear increase in size for the remainder of intracapsular development. Similar changes in size during development have been reported for *B. cyaneum* (Miloslavich and Dufresne 1994) and *B. isaotakki* (Ilano et al. 2004). For both, however, the initial increase was slower than was observed in this investigation. In *B. isaotakki* it is likely that this reflects the slower nurse egg consumption rate previously observed in this species (Ilano et al. 2004). Probably, nurse eggs are also taken up at a slower rate in *B. cyaneum.*

Previous hatching sizes for *B. undatum* have been reported ranging from 1.0 to 3.1 mm (e.g. Fretter and Graham 1985; Nasution et al. 2010). These are similar to hatching sizes observed in the present investigation, which averaged just below 2.5 mm in length.

3.6.2. Nurse egg partitioning

Life history theories suggest parental fitness is maximised by investing equally into all offspring (Smith and Fretwell 1974). Traditionally, resource partitioning (in the form of nurse eggs) during intracapsular development follows this trend. Embryos compete for nurse eggs, but within a capsule, competitiveness is normally equal. As a result, all embryos consume nurse eggs quite evenly. This does not mean hatchlings are always of a similar size; within one species, or even one clutch, the ratio of nurse eggs to developing embryo may vary greatly between capsules, resulting in large differences in offspring size. This is usually believed to be due to irregular distribution of embryos amongst capsules (Thorson 1950; Rivest 1983; Spight 1976a; Miloslavich and Dufresne 1994). Within a capsule, however, generally only small differences in offspring size are reported. For example Spight (1976a) examined two species of muricid gastropod (*Nucella emarginata* (Deshayes 1839) and *A. spirata*) and found that although embryo size varied considerably between capsules, within a capsule large differences were rare. Previous studies examining development in *B. undatum* have indicated similar results, and comparable observations have also been reported for the gastropods *L. dirum*

(Rivest 1983) and *C. dilatata* (Chaparro and Paschke 1990; Chaparro et al. 1999). In contrast, the present study found nurse egg partitioning to be quite different to that previously described for *B. undatum* or other buccinids. Large size differences were continually observed between embryos from any one capsule, and regularly individuals were found alongside a capsule mate four times their size (Fig. 3.3b,d). Although to my knowledge variations in nurse egg consumption have not previously been reported in other buccinids, such intracapsular differences have been described for a small number of gastropods, predominantly from the Muricidae. These include *A. monodon* (Gallardo 1979), *Chorus giganteus* (Lesson 1831) (González and Gallardo 1999) and *T. geversianus* (Cumplido et al. 2011). In *A. monodon* and *C. giganteus* intracapsular size differences continue to be evident at hatching, presumed to be related to earlier nurse egg consumption (Gallardo 1979; González and Gallardo 1999). In *T. geversianus* sibling cannibalism (which can also affect offspring size) occurs during later developmental stages and it is not clear whether hatching sizes vary (Cumplido et al. 2011).

It is widely assumed that offspring quality increases with size (e.g. Thorson 1950; Spight 1976a; Rivest 1983; Gosselin and Rehak 2007; Lloyd and Gosselin 2007; Przeslawski 2011). Larger hatchlings are less likely to be affected by factors such as physical stress, predation and starvation. While intracapsular size differences are generally believed to be due to competition (Gallardo 1979; González and Gallardo 1999), in the present investigation they are probably enhanced by a combination of asynchrony in development and short nurse egg consumption periods. Nurse egg feeding was found to be very rapid, with each early veliger consuming eggs for just three to seven days. This relates to 2 to 5 % of the developmental period. In comparison, in most gastropods nurse egg consumption occurs over a large proportion of intracapsular development (Table 3.3). Even the shortest uptake periods previously reported (8 to 20 % of the developmental period) (Rivest 1983) are still more than double the length of the consumption period observed by us. Within a capsule, the potential to take up nurse eggs is limited by the amount already consumed by earlier developers. Thus, while intracapsular asynchrony in early development is not uncommon (e.g. Vasconcelos et al. 2004; Fernández et al 2006; Lahbib et al. 2010), when it is combined with the short nurse egg consumption period seen in *B. undatum*, it follows that even a 24 h lag in initial embryonic development will put individuals at a

Table 3.3. Periods of development and nurse egg consumption times for different species of gastropods. All species included are direct developers.

Species	Temperature (°C)	Duration of intracapsular development (days)*	Duration of nurse egg consumption (days)*	Percentage of development over which nurse eggs are consumed	Authors
Buccinum isaotakii	2.5 to 10.2	200	40	20 %	Ilano et al. (2004)
Buccinum undatum	8 to 11	70	28	40 %	Nasution (2003)
Buccinum undatum	**6**	**133 to 140**	**3 to 7**	**2 – 5 %**	**Present study**
Crepipatella dilatata	17	18 to 26	Up to 26	100 %	Chaparro and Paschke (1990)
Hexaplex trunculus	22 to 24	49	35	71 %	Lahbib et al. (2010)
Lirabuccinum dirum	12	84 to 98	7 to 21	8 – 20 %	Rivest (1983)
Trophon geversianus	12 to 14	112	38	34 %	Cumplido et al. (2011)

*Some timings have been converted from weeks stated in original study.

distinct disadvantage. Rapid nurse egg consumption in *B. undatum* is consistent with findings by Portmann (1925), but contradictory to those of Nasution (2003).

Additionally, 6 °C is towards the lower end of the temperature range that the Solent population of *B. undatum* naturally develop in. Nurse egg consumption is even faster at warmer temperatures (Authors, unpublished data). This may lead to larger intracapsular size differences during development, and with predicted sea temperature elevations ((Hughes et al. 2010; MCCIP 2010), intracapsular size ranges may increase.

Normal veligers and pediveligers, which had not successfully consumed any nurse eggs, were occasionally found within a capsule in the present investigation (Fig 3.3a,e). It is likely that these individuals reached the feeding stage after all resources had been consumed. Since no further feeding occurs between nurse egg consumption and hatching, these embryos had no nutrition available to them for development and it was assumed they did not survive. This in itself is very unusual and even in the few reported cases of large intracapsular size differences between embryos (Gallardo 1979; González and Gallardo 1999; Cumplido et al. 2011), to my knowledge completely ‘empty’ embryos have not been observed.

In the current study it was noted that for several weeks following consumption, individual nurse eggs could still be observed through the thin veliger mantle and early shell (Fig. 3.3c). Throughout this period, if the mantle or shell was broken, whole eggs would spill out. This indicated that although eggs were rapidly consumed, they were not immediately utilised but instead were stored for later nutritional use. This phenomenon was also noted by Portmann (1925), who recognised that nurse eggs stayed intact inside *B. undatum* veligers for long periods of time. In comparison he found they disintegrated directly after consumption in *N. lapillus*. Nurse eggs have also been shown to be visible internally throughout the feeding period in *A. monodon* (Gallardo 1979), *L. dirum* (Rivest 1983) and *C. dilatata* (Chaparro and Paschke 1990). In each case, however, the literature suggests nurse eggs begin to be assimilated shortly following consumption. In other species such as *T. geversianus*, nurse eggs break down prior to consumption by embryos (Cumplido et al. 2011).

The range in size of embryos within a capsule and the occurrence of ‘empty’ embryos observed in this investigation indicates that a higher level of competition is occurring in *B. undatum* than is normally observed during intracapsular development in gastropods. While large intracapsular size differences have been observed in some muricid gastropods, to my knowledge competition for nurse eggs to the degree that some embryos are left with no nutrition for development has never previously been reported.

Chapter 4: Thermal tolerance during early ontogeny in the common whelk *Buccinum undatum* (Linnaeus 1758)

Published as: Smith K,E., Thatje, S., Hauton, C. (2013) Thermal tolerance during early ontogeny in the common whelk *Buccinum undatum* (Linnaeus 1785): bioenergetics, nurse egg partitioning and developmental success. Journal of Sea Research 79:32-39 (Appendix D)

4.1. Abstract

Temperature is arguably the primary factor affecting development in ectotherms, and as a result, may be a significant driving force behind setting species geographic limits. The shallow-water gastropod *Buccinum undatum* (Linnaeus 1758) is distributed widely throughout the North Atlantic, with an overall annual thermal range of below zero to above 22 °C. In UK waters it is a winter spawner; egg masses are laid and develop when sea temperatures are at their coolest (4 to 10 °C). Therefore, as water temperatures warm, development may be impaired in this species. Here, I examine the effects of temperature on the early ontogeny of *B. undatum* across the thermal range of 0 to 22 °C, using individuals from a population from the Solent, UK. Successful development was observed at temperatures ranging from 6 to 18 °C. Rates of development increased with temperature, but the proportion of each egg mass developing successfully decreased at the same time. With increasing temperature, the mean early veliger weight increased, but the number of early veligers developing *per* capsule decreased, suggesting a negative impact on the number of crawl-away juveniles produced *per* capsule. A similar pattern was observed when these data were compared to data collected from egg masses developing naturally at 3 to 4 °C, from Breiðafjörður, Iceland. Analysis of bioenergetics showed both carbon (C) and nitrogen (N) to increase with temperature in early veligers but not in hatching juveniles, indicating greater energy reserves are accumulated during early ontogeny to compensate for higher energetic demands during development at higher temperatures. The developmental plasticity observed in *B. undatum* suggests this species to be capable of adjusting to temperatures above those it currently experiences in nature. This may provide it with a thermal resilience to ocean warming at its current upper temperature distribution limit, reducing potential shifts in geographic distribution. This, however, may come at the cost of a reduced offspring number.

4.2. Introduction

Thermal tolerance plays a significant role throughout an organism’s life history. In marine invertebrates, temperatures outside a species tolerance range cause negative physiological effects (e.g. Pörtner et al. 2005; Somero 2010), impacting development, growth and survival throughout an individual's life. This thermal tolerance range may vary across a species' distribution, with population specific thermal ranges regularly being observed, generally varying with latitude. The thermal tolerance range of a species during development is often narrower than that which adults from the same population can tolerate. Within this, rates of development and growth scale with temperature, an effect which has now been recognised for over a century (Lillie and Knowlton 1897; Sewell and Young 1999; Weiss et al. 2009a). This pattern has been observed globally in a range of marine invertebrates and latitudinal trends can be observed indicating rates of growth and development in many shallow water organisms to largely increase from the poles to the tropics as seawater temperatures rise (Clarke 1983; Hoegh-Guldberg and Pearse 1995; Stanwell-Smith and Peck 1998). Outside a population's optimal developmental thermal range, developmental success is usually impaired (Lillie and Knowlton 1897; Johns 1981; Anger et al. 2003, 2004; Calcagno et al. 2005). For example, Anger et al. (2003) found the larval development in the lithodid crab *Paralomis granulosa* (Hombron and Jaquinot 1864) to be successful at temperatures ranging from 3 to 15 °C. Maximum survival, however, occurred between 6 and 9 °C; close to the local ambient temperatures of 5.4 to 9.8 °C.

As is now well documented, the results of global warming have led to increasing temperatures throughout the oceans (Barnett et al. 2005; IPCC 2007), rising, for example, by 0.2 – 0.8 °C *per* decade in UK and north-east Atlantic waters (Hughes et al. 2010; MCCIP 2010). Changing seawater temperatures are likely to negatively impact marine species, affecting developmental success and limiting distribution. In response to this, species range-shifts are predicted to occur globally, tracking isotherms characteristic of their current distribution (Ackerly et al. 2010; Burrows et al. 201l; Loarie et al. 2009). Such migrations have already been observed in a range of marine species, including crustaceans (Southward et al. 1995), gastropods (Zacherl et al. 2003) and fish (Dulvy et al. 2008; Nye et al. 2009; Perry et al. 2003). These are, however, and among other ecological factors, dependent on suitable habitat being available (Burrows et al. 2011).

The negative effects of temperature on larval and juvenile development is of particular concern and as a result a growing number of studies have recently contributed to this topic (e.g. Anger et al. 2004; Johns 1981; Sewell and Young 1999; Stanwell-Smith and

Peck 1998). To date, the majority of studies have examined species that exhibit fully or partially planktonic development (Anger et al. 2004; Johns 1981; Sewell and Young 1999; Stanwell-Smith and Peck 1998; Cancino et al. 2003; Lima and Pechenik 1985; Pechenik et al. 2003; Roller and Stickle 1989) and only rarely have the effects of temperature on development been described in a species with direct development (Fernandez et al. 2006). Such species have limited dispersal abilities, and typically migrate or radiate at a slower rate (Jabonski 1986; Thatje 2012). This suggests that species following a direct mode of development may be more 'at risk' from temperature change than those with planktonic development, if unable to respond to climate change *via* phenotypic plasticity or adaptation.

Buccinum undatum (Linnaeus 1758), is a cold-water spawner with encapsulated direct development. Since at the southern end of its distribution, development predominantly occurs during winter months when water temperatures are at their coolest, the distribution of this species may be impacted by increasing seawater temperatures, unless it is capable of developing under warmer temperatures. Here, I examine the full thermal scope for intracapsular development in *B. undatum* from its southernmost distribution range from the south coast of England. Reproductive trade-offs *per* capsule and bioenergetic changes in offspring development in response to the temperature are assessed, and discussed within a macroecological context of thermal adaptation.

4.3. Aims and objectives

The specific aims of this chapter were to investigate thermal tolerance during development in the common whelk *B. undatum* across the full thermal scope that this species is exposed to throughout its distribution.

The objectives of this chapter are as follows:

- Test the hypothesis that developmental rate will increase positively with temperature in *B. undatum* egg masses.

- Examine the full thermal scope for intracapsular development in *B. undatum* using the population from the Solent, at the southern end of the species distribution.
- Investigate changes in bioenergetic reserves during development across the thermal tolerance range.
- Establish reproductive trade-offs *per* capsule in response to temperature using populations from the edges if the species geographical distribution (the Solent and Breiðafjörður populations).

4.4. Materials and methods

4.4.1. *Buccinum undatum* from the Solent, UK

The effects of temperature on development were examined using egg masses from the Solent, collected between December 2009 and February 2010, and December 2010 and February 2011. Both laboratory reared and trawled egg masses were utilised in the investigation (Chapter 2, Section 2.2 and Table 2.2). Capsule size was limited to capsules with a volume of 100 to 150 mm^3 (Chapter 2, Section 2.5.1). A total of seven trawled and 14 laboratory reared egg masses were used in the investigation.

Upon collection, three capsules from each egg mass were dissected and their contents examined to confirm no development had occurred. The number of eggs was counted for each capsule used. Each mass was also examined non-invasively to check for any development (Chapter 2, Section 2.5.2; Chapter 3, Section 3.5.1). A one-way ANOVA was carried out to confirm ($F = 1.62$; d.f. 13; $p = 0.118$) there was no difference in number of eggs *per* capsule (and therefore level of maternal investment) between the egg masses used in the investigation (mean number of eggs *per* capsule 1175, S.D. ± 400). Egg masses were acclimated to one of seven temperatures as described previously (0, 2, 6, 10, 14, 18, 22 °C; one trawled and two laboratory reared egg masses maintained at each temperature; Chapter 2, Sections 2.3, 2.6).

Every week for the initial 14 weeks and every fortnight for the remaining developmental period, three capsules were randomly selected and dissected from each egg mass, the contents were examined and the developmental stage determined. From the trochophore stage and throughout nurse egg consumption, egg masses were examined daily to determine the duration of short ontogenetic stages. Each egg mass was also examined non-invasively every week and the percentage of the mass at each developmental stage was estimated. When early veliger development was completed (i.e. all nurse eggs had been consumed), a minimum of 10 capsules from each egg mass were opened, the content was counted and each embryo was stored for dry weight (Chapter 2, Section 2.5.2; Chapter 3, Section 3.5.1). At hatching, juveniles were sampled for dry weight and then de-calcified to determine total weight, shell weight, flesh weight and shell: flesh ratio (Chapter 2, Section 2.8.2). Any abnormal individuals were not sampled. Abnormal embryos included those with malformed heads, misshapen bodies or those lacking any mantle or shell development. Elemental analysis was then carried out on early veligers and on hatching juveniles (Chapter 2, section 2.8.3).

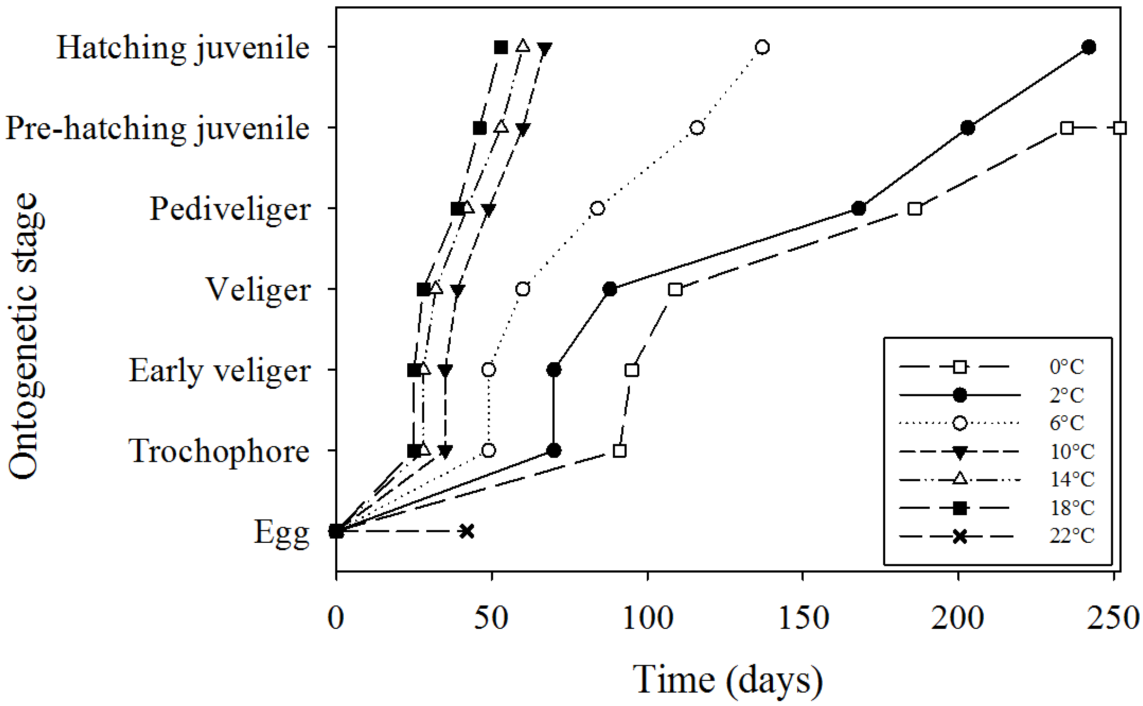

Figure 4.1. Mean developmental timing (days) for intracapsular development in *Buccinum undatum.* Egg masses are from the Solent, UK, and developed at temperatures ranging 0 to 22 °C.

Data was unmatched and did not have equal variance and therefore a non-parametric Kruskal-Wallis was used to analyse the effect of temperature on number of early veligers *per* capsule, early veliger and juvenile weights, and early veliger and juvenile elemental composition (Chapter 2, Section 2.9).

4.4.2. *Buccinum undatum* from Breiðafjörður, Iceland

Data from a sub-set of the capsules opportunistically sampled from the Breiðafjörður population, developed at approximately 3 to 4 °C (Chapter 2, Section 2.2.2) were examined in order to carry out an inter-population comparison. Number of early veligers *per* capsule (20 capsules) and early veliger dry weights (4 capsules, 8 samples *per* capsule), were sampled from capsules with a volume of 100 to 150 mm^3 (Chapter 2, Sections 2.5, 2.8.2).

This data was compared to that collected from egg masses developing at 6 and 10 °C, since these two temperatures are within the thermal range that *B. undatum* egg masses from the Solent currently naturally develop in (Chapter 2, Section 2.9). A one-way analysis of variance was carried out to examine the relationship between developmental temperature and number of early veligers *per* capsule or early veliger weight. *Post hoc,* analysis was carried out using Tukey's method. Prior to analysis, data on early veliger weights were subject to fourth root transformation to attain homoscedasticity (Levenes test, $p > 0.05$).

4.5. Results

4.5.1. Embryonic development

4.5.1.1. Duration of development and developmental success

Egg masses were observed for a total of 36 weeks (252 days). At all temperatures and within each capsule, development was initially asynchronous but was synchronised by the end of the veliger stage. Within an egg mass, some asynchrony in ontogenetic timing was observed between capsules throughout development. Between all egg masses maintained at the same temperature, the level of asynchrony and the overall developmental timing observed was equal (Fig 4.1; Table 4.1). At the highest temperature (22 °C) no development occurred; after 42 days all eggs had begun to

Table 4.1. Developmental periods in days for intracapsular development in *Buccinum undatum* from the Solent, UK, at temperatures ranging 0 to 22 °C. n/a indicates lack of development prevented average time from being determined.

	Time at developmental stage in days – whole egg mass (median number of days spent at stage)							
	Temperature	0 °C	2 °C	6 °C	10 °C	14 °C	18 °C	22 °C
Developmental stage	Egg	0 to 105 (91)	0 to 77 (70)	0 to 56 (49)	0 to 42 (33)	0 to 35 (28)	0 to 28 (24)	0 to 42[a] (n/a)
	Trochophore	63 to 112 (4)	56 to 84 (3)	42 to 56 (2)	28 to 42 (2)	21 to 35 (1-2)	21 to 28 (1-2)	n/a
	Early veliger	63 to 119 (16)	56 to 84 (12)	42 to 56 (5)	28 to 42 (4)	21 to 35 (3)	21 to 28 (2)	n/a
	Veliger	70 to 252 (n/a)	63 to 252 (n/a)	42 to 77 (18)	28 to 49 (7)	21 to 42 (6)	21 to 35 (5)	n/a
	Pediveliger	105 to 252 (n/a)	98 to 252 (n/a)	70 to 98 (18)	42 to 56 (7)	35 to 49 (7)	28 to 49 (6)	n/a
	Pre-hatching juvenile	217 to 252 (n/a)	154 to 252 (n/a)	91 to 140 (44)	49 to 70 (16)	42 to 63 (14)	35 to 56 (14)	n/a
	Hatching juvenile	n/a[b]	231 to 252[c]	133 to 140	63 to 70	56 to 63	49 to 56	n/a
Percentage of egg mass to successfully develop		0	0.2[c]	100	100	95	20	0

[a] All egg masses degraded after 42 days; [b] No hatching had occurred after 252 days; [c] A total of 11 juveniles hatched from approximately 500 capsules across 3 egg masses.

degrade and no further samples were collected. At temperatures ranging from 6 to 18 °C, intracapsular development was successful and took between 49 and 140 days. At the lowest two temperatures (0 and 2 °C) development was very slow and a high number of abnormal embryos were observed (61.6 % at 2 °C; 51.8 % at 0 °C) (Fig. 4.2a-d). At these two temperatures some individuals had reached pre-hatching juvenile stage in every capsule examined, but even juveniles deemed ‘normal’ generally possessed very thin, transparent shells, which were often broken with limited or no colouring (Fig 4.2e, f). Asynchrony in development was observed throughout the investigation within each capsule examined at 0 and 2 °C; pre-hatching juveniles, pediveligers and occasionally veligers were found together in individual capsules. After 36 weeks no hatching was observed at 0 °C and only 11 juveniles had successfully hatched at 2 °C, from an estimated 500 capsules developed at this temperature. Observations ceased at this point and individuals were deemed unviable. Of the temperatures at which successful development occurred (6 to 18 °C), rates of development were similar at 10 °C (63 to 70

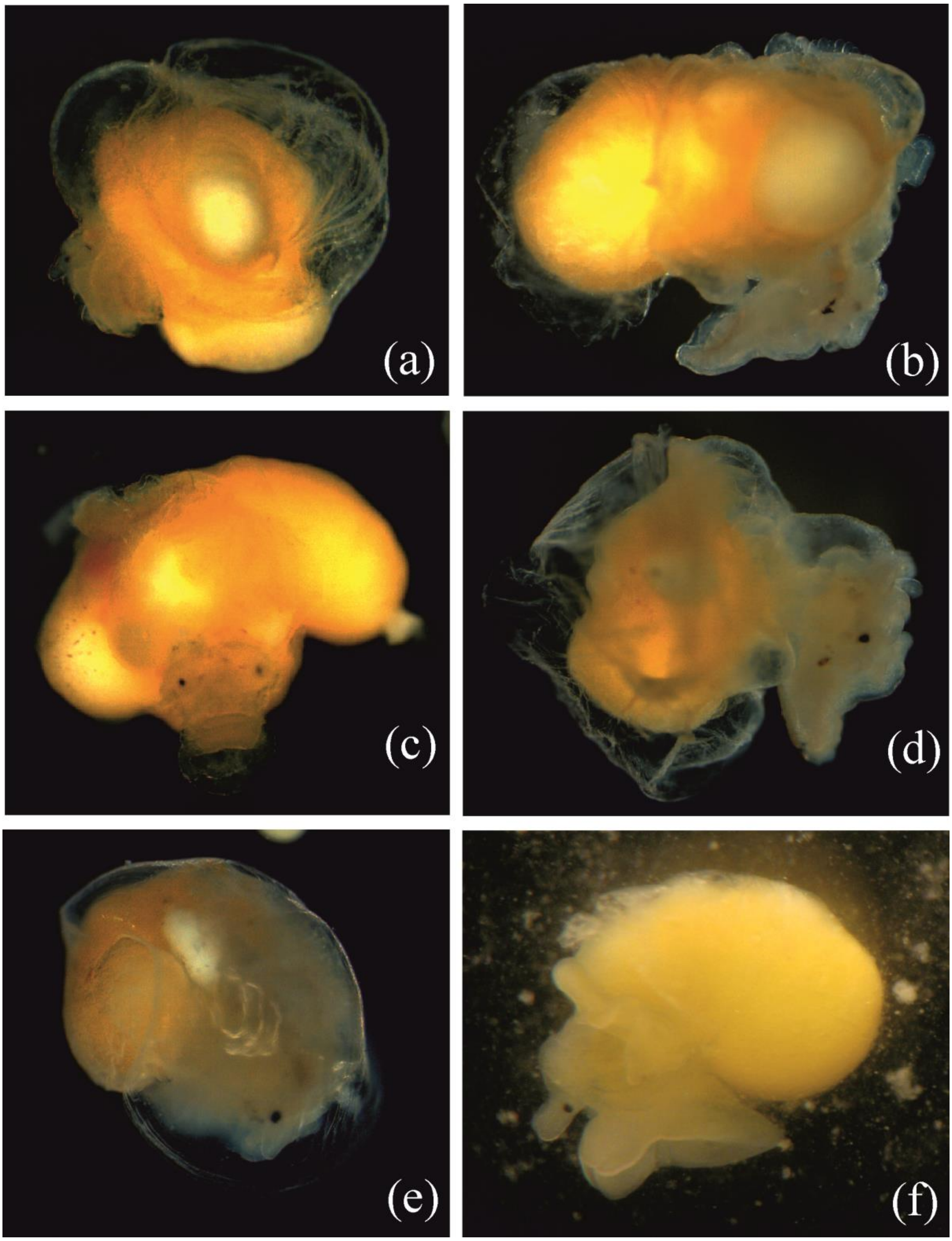

Figure 4.2. *Buccinum undatum* veligers and pre-hatching juveniles from the Solent, UK developed at temperatures of 0 to 2 °C. (a) to (d) ‘abnormal’ veligers of various ages. (e) and (f) ‘normal’ pre-hatching juveniles with little or no shell development.

days), 14 °C (56 to 63 days) and 18 °C (49 to 56 days) but took approximately twice as long at 6 °C (133 to 140 days). Across these temperatures, the percentage of egg mass, which successfully completed development, varied from 20 % at 18 °C to 100 % at 6 and 10 °C.

4.5.1.2. Nurse egg consumption

Nurse eggs were consumed during the early veliger stage at all temperatures at which development occurred. Consumption time (classified as the duration of the early veliger stage) within a capsule decreased with increasing temperature and ranged from 16 days on average at 0 °C to 2 days on average at 18 °C (Fig 4.1, Table 4.1). In capsules developing at temperatures ranging 6 to 14 °C, all nurse eggs were consumed by developing embryos in every capsule examined. All capsules examined developing at 18 °C, and occasional capsules examined developing at 0 or 2 °C, contained a number of unconsumed nurse eggs throughout the duration of development.

4.5.1.3. Embryo size

At all temperatures large size differences were observed between the embryos developing within any one capsule (See Chapter 3, Fig. 3.3b, d, e). At the early veliger stage, these differences were confirmed through examination of individual weight (see below). Early veligers that had not successfully consumed any nurse eggs were observed quite regularly after all nurse eggs had been consumed in a capsule. These ‘empty’ individuals were observed at the early veliger, veliger and occasionally the pediveliger stage, but no later in development (See Chapter 3, Fig. 3.3a, e). Although not quantified, frequency of ‘empty’ embryos appeared to increase with temperature.

4.5.2. Intracapsular content through early ontogeny

4.5.2.1. Buccinum undatum *from the Solent, UK.*

The number and weight of early veligers were examined across developmental temperatures ranging from 0 to 18 °C (Fig. 4.3a, b; Table 4.2). Weight of hatching juveniles was examined across developmental temperatures ranging 2 to 18 °C (Fig. 4.3c; Table 4.2). Kruskal-Wallis one way ANOVA showed number of early veligers *per* capsule to be significantly affected by developmental temperature (H = 28.09; d.f. = 5; p

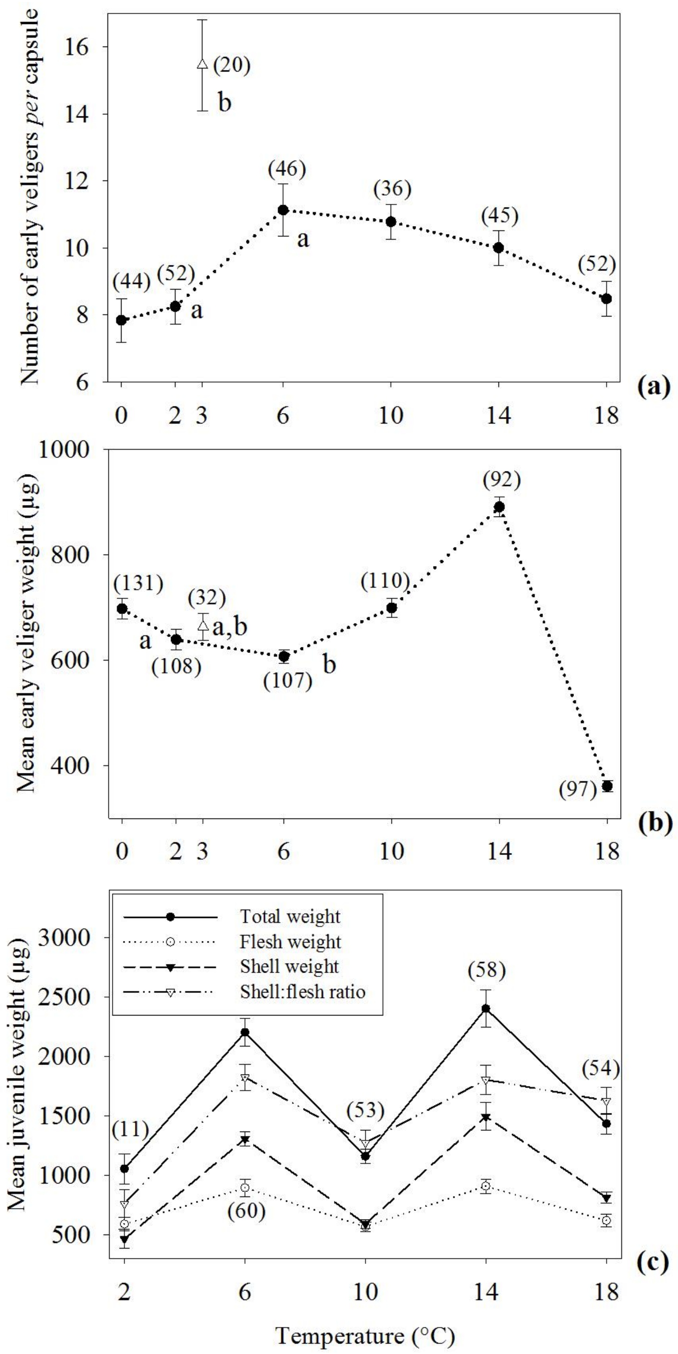

Figure 4.3. Weights and numbers of developing *B. undatum*. (a) Number of early veligers *per* capsule, (b) early veliger weight (post nurse egg consumption) and (c) juvenile weights. For (a) and (b), filled circled represent samples collected from the Solent, UK, and developed at temperatures ranging 0 to 18 °C. Open triangles represent samples collected from Breiðafjörður, Iceland, and developed at approximately 3 °C. For (c), symbols are displayed in the legend. Analysis by Kruskal-Wallis indicated number of early veligers *per* capsule, early veliger weight and juvenile total, flesh and shell weights and shell: flesh ratio to all be significantly affected by temperature ($p \leq 0.001$). One way ANOVA indicated number of early veligers *per* capsule and early veliger weight to vary significantly between egg masses from Breiðafjörður, Iceland and egg masses from the Solent, UK, developed at 6 or 10 °C. Different letters indicate data points which are significantly different as shown by *post hoc* analysis using Tukeys method. Error bars display standard error. Bracketed numbers indicate *n*.

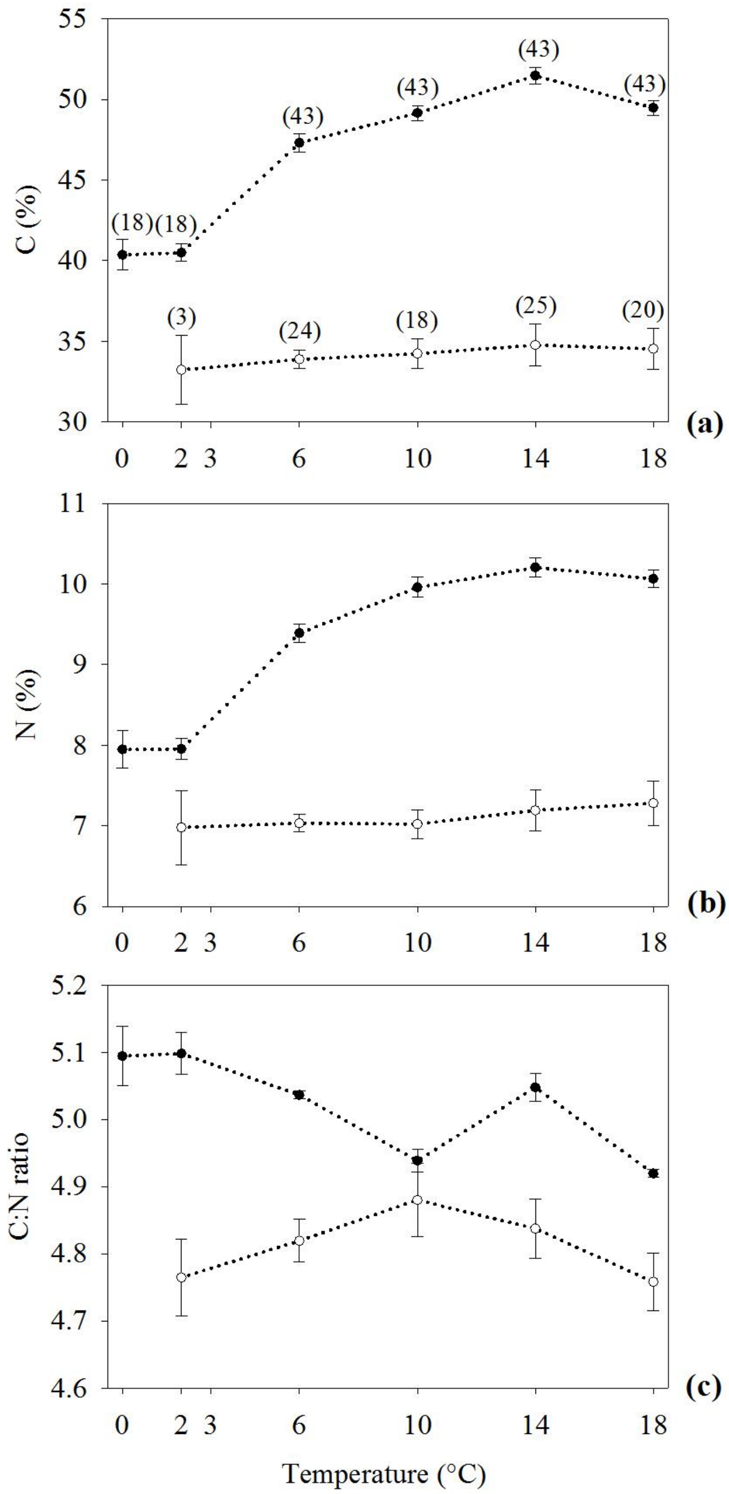

Figure 4.4. Changes in elemental composition in developing *B. undatum*. Changes in (a) C, (b) N and (c) C:N ratio between early veliger (closed circles) and hatching juvenile (open circles). Samples are from the Solent, UK, and developed at temperatures ranging 0 to 18 °C. Analysis by Kruskal-Wallis indicated carbon, nitrogen and C:N ratio to be significantly affected by temperature in early veligers ($p \leq 0.001$) but not in hatching juveniles. At each temperature, significant differences in carbon, nitrogen and C:N ratios were found between early veligers and juveniles ($p \leq 0.05$). Error bars display standard error. Bracketed numbers on plot (a) display *n* values and are identical for all 3 plots.

≤ 0.001). Numbers first increased from 0 to 6 °C and then decreased again from 6 to 18 °C. Early veliger weights were also significantly affected by temperature (H = 206.09; d.f. = 5; p ≤ 0.001), but an opposite pattern was observed (Fig. 4.3b; Table 4.2). Average weight decreased as temperature increased from 0 to 6 °C and then increased again as temperature increased from 6 to 14 °C, before decreasing at 18 °C. Within a capsule, early veliger weights varied between 75 – 603 µg at 0 °C (mean 334 µg), 74 – 759 µg at 2 °C (mean 326 µg), 473 – 1025 µg at 6 °C (mean 739 µg), 416 – 1240 µg at 10 °C (mean 913 µg), 332 – 1325 µg at 14 °C (mean 761 µg), and 74 – 809 µg at 18 °C (mean 354 µg). Across all individuals developing at each temperature (i.e. across all capsules), early veliger weights varied by between 900 and 1331 µg. In juveniles, temperature significantly affected total weight (H = 69.93; d.f. = 4; p ≤ 0.001), shell weight (H = 83.74; d.f. = 4; p ≤ 0.001), flesh weight (H = 26.96; d.f. = 4; p ≤ 0.001) and shell: flesh ratios (H = 25.89; d.f. = 4; p ≤ 0.001), but no correlation was observed between juvenile weight and temperature. At each temperature and across all individuals, hatching juvenile weight varied by between 1381 and 4661µg.

4.5.2.2. Buccinum undatum *from Breiðafjörður, Iceland.*

Number of early veligers *per* capsule averaged 15.45, and early veliger weight averaged 664 µg in the Breiðafjörður population (Fig 4.3a, b; Table 4.2). Within a capsule, early veliger weight varied by between 205 and 332 µg (mean 260 µg). Analysis by one-way ANOVA indicated both number of early veligers *per* capsule (F = 6.43; d.f. = 2; p ≤ 0.005) and early veliger weight (F = 3.44; d.f. = 2; p ≤ 0.05) to be significantly affected by temperature. *Post hoc* analysis showed number of early veligers *per* capsule in the Breiðafjörður population to be significantly higher than that reported for the Solent population developed at either 6 or 10 °C (p ≤ 0.05). *Post hoc* analysis showed there to be no difference in early veliger weight between the Breiðafjörður population and the Solent population at either 6 or 10 °C (p > 0.05). For the Solent population, early veliger weights varied significantly between egg masses developed at 6 and 10 °C (p ≤ 0.05).

4.5.3. Bioenergetic changes through early development

Elemental analysis was carried out on early veligers developed at temperatures ranging 0 to 18 °C and juveniles developed at temperatures ranging 2 to 18 °C (Fig 4.4). At 2 °C, due to the low number of hatchlings, only three juveniles were analysed in total.

Table 4.2. Number of embryos *per* capsule and embryo and juvenile weights for *B. undatum*. Samples from the Solent, UK, and Breiðafjörður, Iceland, developed at temperatures ranging 0 to 18 °C. Standard error (S.E.) is displayed.

	Location		UK						Iceland
	Temperature (°C)		0	2	6	10	14	18	3
Early veliger	Number of embryos *per* capsule	Range	2-18	2-20	2-28	5-18	5-20	2-16	10-38
		mean	7.84	8.36	11.13	10.78	10.00	8.48	15.45
		S.E.	0.65	0.52	0.77	0.51	0.51	0.51	1.41
		n	44	52	46	36	45	52	20
	Embryo weight (µg)	Range	123-1454	138-1426	16-1426	179-1431	389-1714	44-944	326-876
		mean	683.61	626.24	580.80	666.02	904.34	365.97	664.00
		S.E.	18.68	18.81	12.07	16.09	18.07	8.83	30.03
		n	131	108	107	110	92	97	32
Juvenile	Total weight (µg)	Range	n/a	413-1794	900-5517	442-2235	696-5357	422-2905	n/a
		mean	n/a	1053.64	2200.45	1159.26	2401.88	1432.852	
		S.E.	n/a	124.90	116.66	57.71	159.09	86.95	
	Flesh weight (µg)	Range	n/a	367-949	201-3223	163-1281	201-1891	181-1721	n/a
		mean	n/a	589.18	894.75	568.00	908.26	620.26	
		S.E.	n/a	54.35	72.78	38.92	60.68	53.63	
	Shell weight (µg)	Range	n/a	38-845	549-2393	88-1082	51-3731	85-1820	n/a
		mean	n/a	464.45	1305.7	258.63	1493.62	812.59	
		S.E.	n/a	80.82	62.41	35.86	116.87	47.17	
	Shell : flesh ratio	Range	n/a	0.10-1.34	0.50-3.86	0.12-3.16	0.08-3.94	0.25-4.23	n/a
		mean	n/a	0.76	1.82	1.27	1.80	1.62	
		S.E.	n/a	0.11	0.11	0.11	0.12	0.11	
		n	n/a	11	60	53	58	54	n/a

Throughout development the carbon mass fraction was higher than the nitrogen mass fraction. In early veligers a trend of increasing C and N with temperature was observed. Analysis by Kruskal-Wallis one-way ANOVA indicated percentages of C ($H = 96.06$; d.f. = 5; $p \leq 0.001$) and N ($H = 98.18$; d.f. = 5; $p \leq 0.001$) and C:N ratios ($H = 52.82$; d.f. = 5; $p \leq 0.001$) were all significantly affected by developmental temperature at the early veliger stage. Upon reaching the juvenile stage, no differences were observed between developmental temperatures in percentages of C ($H = 0.15$; d.f. = 4; $p = 0.997$) or N ($H = 0.15$; d.f. = 4; $p = 0.998$), or C:N ratios ($H = 2.64$; d.f. = 4; $p = 0.619$). Significant decreases in C, N and C:N ratio values were observed during development (from early veliger to hatching juvenile) at every temperature investigated. All changes were significant to $p \leq 0.001$ except for changes in C and C:N ratio at 2 °C (both significant to $p \leq 0.01$) and changes in N at 2 °C and C:N at 10 °C (both significant to $p \leq 0.05$). For C and N, percentage depletion increased as developmental temperature increased from 17.9 % (C) and 12.3 % (N) at 2 °C to 32.5 % (C) and 29.6 % (N) at 14

°C. Reported depletion of C and N, while still significant, was lower at 18 °C than at 14 °C. Rate of depletion in C:N ratio decreased between developmental temperatures of 2 and 10 °C, before increasing again as temperatures increased further.

4.6. Discussion

4.6.1. Embryonic development

4.6.1.1. Thermal tolerance during development

Within the distribution of a species, thermal tolerance ranges are often reported to vary between populations. Such ranges are ultimately dependent on temperature, and thus, shifts in thermal tolerance range may occur with latitude, or in association with ocean currents or other factors affecting local water temperatures. This illustrates a high level of thermal plasticity in response to local temperatures, indicating that population level differences in reproductive adaptations exist. (e.g. Storch et al., 2009; Thatje et al., 2005; Zippay and Hofmann, 2009). My results indicate this trend to be evident in *B. undatum*. In the present study, complete development was observed between 6 and 18 °C for a population at the southern end of the distribution, from the south coast of the UK. In comparison, populations of the common whelk from the Gulf of St Lawrence, Canada, where the Labrador current causes low annual temperatures, develop in water temperatures of 2 to 3 °C (Martel et al., 1986a), and in Breiðafjörður, Iceland, at the northern end of the species distribution, between 3 and 6 °C (Smith and Thatje, 2012; Authors, unpublished results). Similar fluctuations in thermal tolerance have been noted previously in gastropods (Zippay and Hofmann, 2009) and crustaceans (Storch et al., 2009; Thatje et al., 2005), with different populations having a narrow thermal tolerance range, which scaled with temperature and was specific to the latitude at which it was found. Within a single species population, previous studies have indicated thermal tolerance to often be lower during development than throughout adult life (Dawirs 1985; deRivera et al. 2007; Gosselin and Chia 1995; Weiss et al. 2009a). For example, hatched juveniles of the gastropod *Nucella emarginata* (Deshayes 1839) were negatively impacted by temperatures of 30 °C, while adults of the same species could easily withstand such temperatures (Gosselin and Chia 1995). In the present study, I found similar results with the thermal tolerance range identified during development (6 to 18 °C) being narrower than annual local water temperatures for the sampled population (4 to 22 °C). Such findings suggest that thermal tolerance during early ontogeny may be fundamental in setting the geographic limits of a species.

Developmental success (to hatching) was greatest within the natural developmental temperature range of the local populations (6 and 10 °C) and decreased at temperatures outside this. Similar scenarios have been observed on many previous occasions in marine ectotherms including temperate and sub-polar crustaceans (Anger et al. 2003; Johns 1981) and tropical and polar echinoderms (Sewell and Young 1999; Stanwell-Smith and Peck 1998). Interestingly, in each of the above studies, optimum success was observed at temperatures towards the middle or top of the thermal range investigated. In comparison, in the present investigation peak survivorship was at temperatures at the bottom of the thermal tolerance window for development and at the lower end of the habitat temperature limits for adults of this population. This may be related to the cold-water spawning observed in *B. undatum.* Several authors have linked spawning to falling temperatures, indicating low temperature to induce spawning in this species (Hancock1967; Smith and Thatje 2013; Valentinsson 2002). The preference for colder temperatures, evident in *B. undatum* is likely linked to the deep sea and cold-water origin of neogastropods (Hickman, 1984; Jablonski and Bottjer, 1991).

4.6.1.2. Developmental timing

The difference in developmental timing between 6 and 10 °C was large compared to other temperatures, with egg masses taking an additional 70 days to develop at 6 °C. In comparison, total time to hatching only varied by 7 days in duration between 10 and 14 °C, and 14 and 18 °C. Other studies have reported similar results, with small increases in temperature at the lower end of the thermal range causing much larger reductions in development time than similar changes at the upper end of the thermal range (Anger et al. 2003; Johns 1981). For example, Anger et al. (2003) reported that time from hatching to metamorphosis in the lithodid crab *P. granulosa* decreased from 116 days at 3 °C to 53, 40, 31 and 24 days at 6, 9, 12 and 15 °C, respectively. In *B. undatum*, egg masses laid in late December begin development in January and February as temperatures are reaching their lowest, whereas those laid in late February develop as temperatures are warming again. The difference in developmental timing between 6 and

10 °C suggests egg masses may hatch at approximately the same time (late spring, early summer), despite a two-month lag in laying time. This suggests there are ecological benefits to hatching at this time of year, probably related to obtaining optimum growth and survival. Amongst other things this may include factors such as temperature, food availability, and predatory pressures (Giese 1959; Pechenik 1999; Thorson, 1950).

4.6.2. Intracapsular content through early ontogeny

4.6.2.1. Number and size of early veligers

In the present investigation, the number of early veligers developing *per* capsule was used as a proxy for number of hatching juveniles, and therefore reproductive output. In *B. undatum*, number of embryos *per* capsule does not vary between early veliger development and juveniles hatching (Hancock, 1967; Martel et al., 1986a; Smith and Thatje, 2013). On rare occasions where an embryo does not complete development, soft and hard (shell) body parts remain obvious inside the capsule; although a scavenger when in adult form, developing *B. undatum* have been suggested to be unable to consume for the majority of intracapsular development (Smith and Thatje, 2013; Authors, unpublished results). The unconsumed nurse eggs observed in capsules throughout, at 0, 2 and 18 °C give support for this assumption. While change in number of embryos *per* capsule has never been investigated in *B. undatum* across a wide thermal range such as that examined in the present study, it would be expected to observe any deceased embryos during capsule dissection. In the present study, none were seen, indicating there to be no change in number of developing embryos through ontogeny at any temperature. We therefore considered number of early veligers *per* capsule to be a good proxy for reproductive output regardless of developmental temperature.

A general trend was observed for the total number of early veligers *per* capsule to decrease with increasing temperatures. In contrast, the occurrence of ‘empty’ embryos appeared to increase with temperature (although this was not remarked upon for the Breiðafjörður population). Since these embryos had not taken up any nutrition for development, and were not observed past the pediveliger stage, it was presumed they did not develop successfully. The simultaneous increase in empty embryos and decrease in number of developing embryos will potentially reduce the number of offspring completing development at higher temperatures and indicates that within each capsule, individuals developing later due to asynchrony in timings are at a greater disadvantage as temperatures increase. This suggests that despite the potential ecological benefits of rapid development to hatching during later, warmer months of the year, hatchling number and quality may be reduced under such conditions.

Both high and low temperatures have previously been shown to retard early development (Anger et al. 2004; Byrne et al. 2009; Fernandez et al. 2006; Gallardo and Cancino 2009; Sewell and Young 1999). As postulated above, it is likely that the present study population of *B. undatum* has adapted to develop optimally at local temperatures and any deviation above or below this is unfavourable. The observed trend of increasing early veliger weight in the Solent population, with increasing temperature can be explained by examining the number of embryos *per* capsule. Since for this population there was no difference in the number of nurse eggs *per* capsule, in capsules where a smaller number of embryos developed a higher number of nurse eggs was available for each developing embryo, thus leading to a greater mean embryo weight. As well as the number of nurse eggs consumed, embryo weight may also be affected by nurse egg size, and this factor should therefore be briefly considered. In *B. undatum,* nurse egg size is significantly related to capsule volume (see Chapter 2, Figure 2.4). However, since capsules of a narrow range of volumes were used in the present investigation, nurse egg size was expected to be homogenous across the samples and was therefore not considered a significant factor affecting embryo weight.

Interestingly, developmental data from egg masses collected from the Breiðafjörður population, fitted in with trends observed for the Solent population. Capsules collected from the Breiðafjörður population, of an equal size to those used in the present study and developed at approximately 3 to 4 °C, were found to have significantly more embryos developing *per* capsule than those observed for the Solent population, at temperatures ranging 6 to 18 °C. This data should, however, be interpreted with caution. While the previous investigation, examining a wider range of capsule sizes and using a larger data set, also found mean number of early veligers *per* capsule to be greater in egg masses collected from Breiðafjörður compared to those collected from the Solent, this result was not significant (see Chapter 3). Mean early veliger weight for the Breiðafjörður population was similar that reported in the present investigation for embryos from the Solent population developed at 6 and 10 °C. When a wider size range of capsules was examined, including more than double the number of samples, the same pattern was observed. This indicates such weights to be optimal for development and again highlights local adaptations, which may have occurred.

While the previous investigation did not report any difference in number of early veligers *per* capsule between the Breiðafjörður and the Solent populations, it did indicate number of eggs *per* capsule differ significantly. Patterns of increasing egg size with decreasing breeding temperature (Thorson's rule) have previously been observed both within, and between species (Laptikhovsky 2006). Since number of eggs *per* capsule is lower in the Breiðafjörður population than in the Solent population, but early veliger weight does not vary, despite a potential increase in veliger number, this suggests that in colder habitats, *B. undatum* may produce larger eggs in order to compensate for the low number.

4.6.2.2. Intracapsular nurse egg partitioning

During early development, embryos of the common whelk consume nurse eggs at a rapid rate, storing them in the mid-gut for later use (Portmann 1925; Smith and Thatje 2013). As an example, at 6 °C, nurse egg consumption within a capsule was completed over five days out of a 140-day intracapsular development period (Smith and Thatje 2013) and escalated as temperature increased. The intracapsular asynchrony observed during early development, however, means not all embryos begin nurse egg consumption at the same time, leading to considerable variations in both early veliger and hatching juvenile weights. The findings reported here give support for previous suggestions that a high level of competition occurs during development in *B. undatum* and indicate that a juvenile's predisposition for later life is highly dependent on how well it competes during these early days. Individuals that consume a higher number of nurse eggs inevitably hatch at a larger size.

4.6.3. Bioenergetics through early development

Following the initial positive relationship observed in early veligers between temperature and proportions of C and N, by hatching, proportions were comparable across all temperatures. Thus, although at higher temperatures more energy reserves are accumulated during early development, a greater metabolic loss is incurred, probably related to larger metabolic demands at higher temperatures. These results indicate that in *B. undatum* shifts in the energy budget occur across the species range during early development to allow for external temperature differences. This allows all juveniles to be at the same relative bioenergetic predisposition at hatching, regardless of temperature. Similar findings have previously been reported for the brine shrimp *Artemia salina* (Linnaeus 1758) over a four-day period (Evjemo et al. 2001) and the Australian red claw crayfish *Cherax quadricarinatus* (von Martens 1868) over a 20 to 37-day period (García-Guerrero et al. 2003). In both these studies bioenergetics continued to differ with temperature, regardless of developmental stage, demonstrating high plasticity in these species with embryos rapidly adapting to ambient temperatures. The decreases in C:N ratio observed in the present investigation, imply lipids to be used preferentially over proteins. The observed decrease was greatest at high and low temperatures, indicating lipid metabolism to be higher at the extremes of the thermal range. This suggests that while some adaptations have taken place, the cost of development continues to be higher at temperatures outside the species natural ambient range.

Given current rates of seawater warming (Hughes et al. 2010; MCCIP 2010) it is possible that temperatures of 14 °C will be observed during the current developmental period for southern populations within the next four decades. The developmental success observed for *B. undatum* at this temperature indicates the possibility that range shifts may not be observed in southern populations of this species. Increasing temperatures may, however, impair initial spawning, and are detrimental to total reproductive output. While development was successful at 14 °C, costs were incurred. Offspring numbers were reduced and each embryo required a greater amount of energetic reserves to reach the same relative condition at hatching. Ultimately, at the current upper thermal limit for development in *B. undatum* reproductive output may be impacted, negatively affecting population size at the southern extreme of the species distribution.

Chapter 5: The subtle intracapsular survival of the fittest: maternal investment, sibling conflict or environmental effects?

Published as: Smith, K.E., Thatje, S. (*in press*) The subtle intracapsular survival of the fittest: maternal investment, sibling conflict or environmental effects? Ecology http://dx.doi.org/10.1890/12-1701.1 (Appendix E)

5.1. Abstract

Developmental resource partitioning and the consequent offspring size variations are of fundamental importance for marine invertebrates, in both an ecological and evolutionary context. Typically, differences are attributed to maternal investment and the environmental factors determining this; additional variables, such as environmental factors affecting development, are rarely discussed. During intracapsular development for example, sibling conflict has the potential to affect resource partitioning. Here, I investigate encapsulated development in the marine gastropod *Buccinum undatum* (Linnaeus 1758). I examine the effects of maternal investment and temperature on intracapsular resource partitioning in this species. Reproductive output was positively influenced by maternal investment but additionally, temperature and sibling conflict significantly affected offspring size, number and quality *during* development. Increased temperature led to reduced offspring number, and a combination of high sibling competition and asynchronous early development resulted in a common occurrence of 'empty' embryos, which received no nutrition at all. The proportion of 'empty' embryos increased with both temperature and capsule size. Additionally, a novel example of a risk in sibling conflict was observed; embryos cannibalised by others during early development ingested nurse eggs from inside the consumer, killing it in a 'Trojan horse' scenario. My results highlight the complexity surrounding offspring performance. Encapsulation should be considered as significant in determining maternal output. Considering predicted increases in ocean temperatures, this may impact offspring quality and consequently species distribution and abundance.

5.2. Introduction

Offspring fitness is of fundamental importance in selection; it directly affects an individual's performance and chance of survival. Consequently, the success of a species depends on its ability to reproduce effectively. Selection favours those that produce the largest possible number of surviving young. Traditionally, quality is believed to increase with size, with larger offspring regularly (although not always) showing higher growth rates and survival in marine invertebrates (Rivest 1983; Moran and Emlet 2001; Marshall et al. 2006) and other taxa including birds (Rhymer 1988), insects, reptiles and plants (Kingsolver and Huey 2008). The increased maternal investment associated with producing larger, fitter young (Vance 1973) does, however, result in energetic trade-offs occurring (Vance 1973; Smith and Fretwell 1974). Most contemporary life history theory suggests 'optimal' offspring size may vary with environmental conditions, and to maximise fitness, mothers will adjust the size and number of offspring accordingly (McGinley et al. 1987; Bernardo 1996; Mousseau and Fox 1998). Amongst other variables, stress, habitat quality, temperature, maternal size and maternal nutritional status have all been identified as factors affecting the trade-off between size and number of offspring (Hadfield 1989; Marshall and Keough 2007; Crean and Marshall 2009; Kamel et al. 2010b).

Traditionally, maternal output is perceived to be greatest if maternal investment (i.e. the resources invested into offspring by the mother) is even amongst all offspring; consequently, the assumption is regularly made that the offspring produced by one female are of an equal size and fitness (Thorson 1950; Smith and Fretwell 1974; Bernardo 1996; Parker et al. 2002). In reality, however, intra-clutch variations in offspring size are often observed, and over the past decade have become a popular topic for debate. Within-brood variations in offspring size have now been recognised in diverse species, occurring both between co-habiting embryos exhibiting one developmental mode (e.g. Fox and Czesak 2000; Kudo 2001; Dziminski and Alford 2005; Marshall et al. 2008b), and between co-habiting embryos exhibiting different developmental modes (Poecilogony) (e.g. Gibson et al. 1999; Oyarzun and Strathmann 2011; Kesäniemi et al. 2012; Gibson and Carver 2013).

Bet-hedging, in which females inhabiting variable environments produce offspring of a range of sizes or developmental modes, is one of several explanations that have been suggested to explain within-brood variations in offspring size (Slatkin 1974; Philippi and Seger 1989; Koops et al. 2003; Marshall et al. 2008a; Crean and Marshall 2009). The reported differences are typically understood to be a maternal effect, occurring as a direct result of variations in the provisioning allocated to each embryo (Krug et al. 2012). Explanations for this often focus on the environmental factors affecting maternal provisioning (Bernardo 1996; Moran and Emlet 2001), and only rarely have other variables been considered (e.g. Lardies and Fernandez 2002; Fernandez et al. 2006; Parker et al. 2002; Kamel et al. 2010a, 2010b; Oyarzyn and Strathmann 2011). Assuming no poecilogony, species exhibiting intracapsular development often receive maternal provisioning during development in the form of nurse eggs, which are consumed for nutrition. Nutrients may alternatively be allocated at production to each embryo, or be obtained from intracapsular fluids or capsule walls (Ojeda and Chaparro 2004). Within each capsule, embryos are usually equal in their ability to consume, and therefore, intracapsular size variations are rarely observed (Spight 1976; Rivest 1983; Chaparro and Paschke 1990; Strathmann 1995). Within-brood variation in offspring size is instead achieved through irregular allocation of nurse eggs and embryos to capsules (Thorson 1950; Spight 1976; Rivest 1983). However, in a handful of species, size differences have been observed between the adelphophagic or direct developing embryos in one capsule (Portmann 1925; Gallardo 1979; González and Gallardo 1999; Strathmann and Strathmann 2006; Cumplido et al. 2011; Smith and Thatje 2013). Within a capsule, individuals, which consume more nutritional reserves, inevitably hatch at a larger size, and are typically understood to have a greater chance of survival. In these examples, the differences typically occur as a result of competition for intracapsular resources, or occasionally through cannibalism of partly developed siblings. Some maternal control may exist, for example some spionid polychaetes manually tear open egg capsules to limit cannibalism (e.g. Kamel et al. 2010b; Oyarzun and Strathmann 2011), but maternal effect may not always be the primary determinant of individual offspring fitness. An alternative source of variation is sibling conflict, although in marine invertebrates this is rarely considered a significant factor, and as a result has received little attention to date (e.g. Parker et al. 2002; Kamel et al. 2010a, 2010b).

Of particular interest is the intracapsular resource partitioning in *Buccinum undatum* (Linnaeus 1758), a common and commercially important North Atlantic gastropod. This species exhibits direct development, which is initially asynchronous, with no observed poecilogony. Nurse eggs are the primary nutritional reserve available throughout this period (Valentinsson 2002; Smith and Thatje 2013). To my knowledge, in other species exhibiting intracapsular development, all embryos obtain nutrition of some form, either incorporated into the egg during production, during intracapsular development, or during a later planktotrophic developmental stage (Gallardo 1979; González and Gallardo 1999; Kamel et al. 2010b; Cumplido et al. 2011). In contrast to most species, however, large intracapsular size differences have been observed between offspring, resulting in large size differences at hatching (Fig. 5.1a) (Portmann 1925; Smith and Thatje 2013). Additionally, common occurrences of ‘empty’ embryos have been

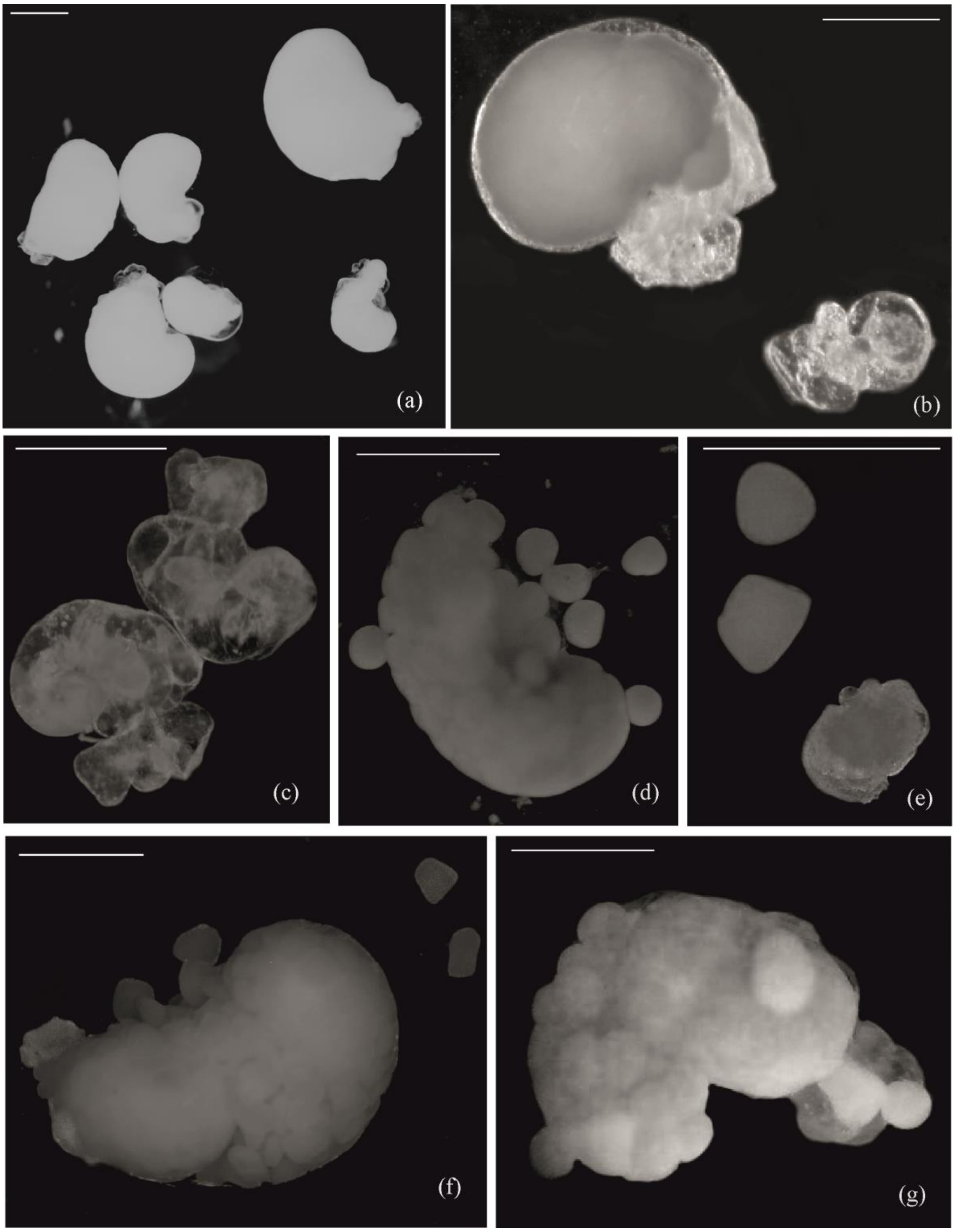

Figure 5.1. Images of *Buccinum undatum* during development. (a) embryos of varying sizes developing alongside each other; within one capsule and following nurse egg consumption. (b) a normal veliger next to an 'empty' veliger from the same capsule. The 'empty' veliger is visibly lacking nurse eggs inside its midgut. (c) late veliger stage with empty midguts indicating few or no nurse eggs were consumed. (d) a pierced embryo displaying intact nurse eggs spilling out. (e) contents of embryo midgut including nurse eggs and a trochophore (f) and (g) a 'victim' of cannibalism bursting out of the midgut of its consumer. Scale bars represent 500 µm.

reported (but not quantified) inside capsules (Fig. 5.1b, c), whereby some embryos do not consume any nurse eggs during development (Smith and Thatje 2013). These embryos often successfully complete early development, but do not survive to hatching, indicating an unusual example of sibling conflict. Lack of survival has been observed through the presence of small, thin empty shells, and the absence of live 'empty' embryos after the pediveliger stage (Smith and Thatje, 2013; authors, unpublished data). Sibling conflict may be reflected in the variety in number of nurse eggs consumed by different offspring and the occurrence of 'empty' embryos; embryos that did not succeed in acquiring any nutrition at all. Furthermore, evidence suggests the proportion of 'empty' embryos to be accentuated by increased temperature (Smith et al. 2013), suggesting environmental factors may play a significant role in modulating intracapsular resource partitioning, contributing to the variations resulting from sibling conflict. Considering this, predicted increases in seawater temperature (Hughes et al. 2010) could lead to a reduction in the number of individuals' successfully developing, potentially affecting species abundance. This rare example demonstrates a different effect of encapsulation, the unusual nature of which remains un-described.

Understanding the dynamics contributing to developmental resource partitioning in offspring is of great significance, in both the evolutionary and ecological context. Here, I contribute to discussion of the evolution of egg size and offspring size in marine invertebrates, with emphasis on the rarely considered effects of sibling conflict. I investigate the unusual intracapsular resource partitioning reported in *B. undatum.* I use this species to compare the consequences of maternal investment and environmental effects on sibling conflict during intracapsular development.

5.3. Aims and objectives

The objectives of this chapter are as follows:

- Examine the relationship between developmental temperature and intracapsular development in the common whelk *B. undatum*.
- Examine the relationships between maternal investment (capsule size) intracapsular development in the common whelk *B. undatum*.

5.4. Materials and methods

5.4.1. *Buccinum undatum* from the Solent, UK

In order to investigate nurse egg partitioning in *B. undatum,* egg masses from the Solent population were utilised, laboratory reared during January 2011. Because the number of nurse eggs and embryos *per* capsule are positively related to capsule size in *B. undatum,* (Valentinsson 2002; Smith and Thatje 2013), egg masses were divided into size classes depending on capsule volume (calculated according to Smith and Thatje 2013). Capsules with volumes of less than 100 mm^3 were classified as small, between 100 and 200 mm^3 were classified as medium and above 200 mm^3, as large. Egg masses were maintained in separate 1.8 L incubation tanks containing aerated, 1 µm filtered seawater. Each tank was acclimated stepwise, 1 °C temperature change every 24 h, to one of four temperatures (6, 10, 14, 18 °C), with capsules from every size class being developed at 10 °C and only medium capsules being developed at all other temperatures.

5.4.2. Resource partitioning

Development was assessed according to the findings of Chapter 3. Egg masses were inspected twice a week until early veligers became visible through the semi-transparent capsule walls. They were then examined daily until all nurse eggs had been consumed. Expected rates of nurse egg consumption were taken from chapter 4 (at temperatures ranging 6 to 18 °C, total nurse egg consumption in *B. undatum* took between approximately 2 and 5 days). If nurse egg consumption appeared to not have ended 24 h after the expected completion time for the experimental temperature, then when dissected, only capsules containing veligers which were too developed to continue nurse egg consumption were examined (see Chapter 3). Fifteen capsules were randomly dissected from every mass, the number of early veligers inside each was counted and the proportion of ‘empty’ embryos determined (classified as individuals who had consumed ≤ 1 nurse egg). Any nurse eggs, which had been consumed, were visible inside an embryo as yellow balls inside the translucent embryo body. Empty embryos could therefore be determined without dissection (Fig. 5.1b).

5.4.2.1. Nurse egg consumption

In order to assess nurse egg partitioning, the embryos from 10 of the 15 randomly selected capsules were individually dissected. Following initial consumption, in *B. undatum* nurse eggs are stored undamaged in the mid-gut prior to use, allowing number of eggs consumed to be easily distinguished. The outer membrane of the embryo was pierced, letting the consumed nurse eggs spill out (Fig. 5.1c), and allowing the number of nurse eggs to be counted. For each capsule, the range in number of eggs consumed within a capsule was also established, by subtracting the lowest number of eggs consumed by an embryo from the highest number of eggs consumed by an embryo.

5.4.2.2. Dry weight and elemental composition

The embryos from the remaining five capsules were sampled for dry weight. Only embryos containing more than one nurse egg were weighed, since ‘empty’ embryos were very fragile and were regularly damaged during sampling. A minimum of 30 embryos were weighed from each mass. If this number was not achieved from the 5 capsules already open, additional capsules were opened to obtain sample numbers. Elemental analysis was then carried out on 25 individuals from each experimental condition.

5.4.3. Maximum consumption

The maximum number of nurse eggs a veliger was capable of consuming was assessed from the content of 15 medium capsules randomly selected from an egg mass developed at 10 °C. As soon as early veligers became visible inside a capsule, the translucent marginally concave face of the capsule was removed, leaving the deeper, ribbed face. All but one of the early veligers was removed from the capsule. This embryo remained with the unconsumed nurse eggs in a covered petri dish filled with pre-incubated (10 °C; 1 µm, 12 h UV filtered) seawater until it had reached the veliger stage and nurse egg consumption was no longer possible. At this point the embryo was dissected as described previously, and the number of consumed eggs determined.

5.4.4. Statistical analysis

‘Empty’ embryos were included in counts for all data except dry weights and elemental (C, N), due to the small size, and therefore weight of these individuals.

A non-parametric Kruskal-Wallis one-way analysis of variance was used to compare number of embryos developing *per* capsule, number of nurse eggs consumed *per* embryo, embryo DW and proportion of ‘empty’ embryos to developmental temperature and capsule size. The number of nurse eggs consumed by embryos developing in small, medium and large capsules at 10 °C was also compared to the number of nurse eggs consumed by the maximum consumption embryos. A non-parametric test was used due to unbalanced and un-matched data sets for number of nurse eggs consumed *per* embryo and embryo DW. *Post hoc* analysis was carried out using Dunns test.

Since both C and N weights were determined using DW, an analysis of covariance was carried out (using DW as a covariate), to examine relationships between C and N, and developmental temperature or capsule size. Data transformation (1/x) was carried out prior to analysis of covariance.

Regression analysis was used on all 10 °C samples (all capsule sizes) to examine relationships between proportion of empty embryos, and number of embryos per capsule and capsule volume. Range in number of eggs consumed by embryos was also compared to number of embryos per capsule, capsule volume and total number of nurse eggs per capsule (determined by summing the number of nurse eggs consumed by all embryos in a capsule). Multiple and independent regression analysis was carried out to compare the dependent variables (proportion of empty embryos and range in number of eggs consumed) to the above factors.

5.4.5. *Buccinum undatum* from Breiðafjörður, Iceland

Data from a sub-set of the capsules opportunistically sampled from the Breiðafjörður population, developed at approximately 3 to 4 °C (see chapter 3) were examined in order to carry out an inter-population comparison. Number of early veligers *per* capsule (26 capsules) and early veliger dry weights (seven capsules, eight samples *per* capsule), were sampled from capsules with a volume of 100 to 200 mm^3. These data were used only as a comparison and no statistical analysis was carried out.

5.5. Results

5.5.1. Embryo midgut content

As discussed previously, during development, *B. undatum* embryos engulf nurse eggs and store them in the midgut, conserved whole for later nutritional use. When embryos were pierced to enable nurse eggs to be counted, on three separate occasions an early veliger was found inside, indicating it to have been consumed by the more developed embryo.

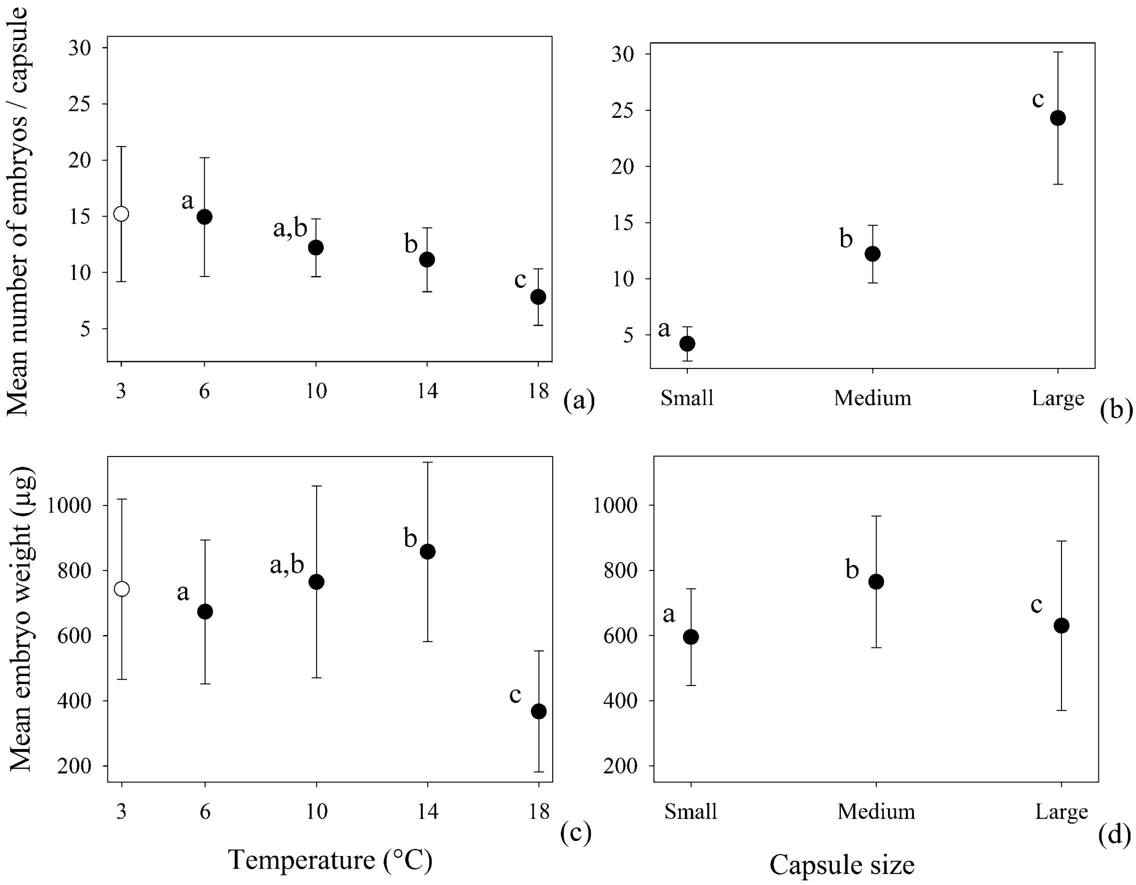

Figure 5.2. Number and weights of *B. undatum* embryos *per* capsule developing a) under different temperatures and b) in different sized capsules, and embryo weights from c) different temperatures and d) different sized capsules. Closed circles represent data collected from egg masses from the Solent, UK. Open circles represent data collected from Breiðafjörður, Iceland. For each plot (a-d), different letters indicate values that are significantly different as indicated using a non-parametric Kruskal-Wallis one-way ANOVA with *post hoc* analysis (Dunn's test). Analysis was carried out on Solent samples only. Error bars indicate standard deviation. 'Empty' embryos are included in embryo *per* capsule counts but not embryo weights.

Table 5.1. Number of embryos *per* capsule, dry weight (DW), contents of carbon (C), and nitrogen (N) (all in µg individual $^{-1}$) for *Buccinum undatum* embryos. Mean embryo weights are taken only from embryos which have consumed >1 nurse egg.

Capsule size	Developmental temperature (°C)	Number of embryos *per* capsule*		DW (µg)*		C (µg ind $^{-1}$)		N (µg ind $^{-1}$)		C:N ratio	
		$\bar{x}$	S.D. (*n*)	$\bar{x}$	S.D. (*n*)	$\bar{x}$	S.D. (*n*)	$\bar{x}$	S.D. (*n*)	$\bar{x}$	S.D. (*n*)
Small	10	4.2	1.52 (15)	595.2	148.23 (31)	317.2	57.98 (25)	65.3	12.09 (25)	4.9	0.04 (25)
Medium	6	14.9	5.28 (15)	673.1	203.72 (52)	354.5	86.93 (25)	69.1	17.04 (25)	5.1	0.06 (25)
	10	12.2	2.60 (15)	764.7	294.47 (56)	411.0	132.07 (25)	82.6	27.20 (25)	5.0	0.07 (25)
	14	11.1	2.85 (15)	857.2	274.87 (38)	474.4	147.44 (25)	93.8	30.18 (25)	5.1	0.11 (25)
	18	7.8	2.51 (15)	367.0	202.15 (30)	231.0	88.21 (25)	46.8	18.07 (25)	4.9	0.11 (25)
Large	10	24.3	5.89 (15)	629.7	259.84 (78)	359.9	139.05 (25)	73.4	29.45 (25)	4.9	0.08 (25)
	3 (Iceland data)	15.2	6.01 (26)	742.8	276.56 (56)	-	-	-	-	-	-

*Kruskal-Wallis one-way analysis of variance indicates data to be significantly affected by both developmental temperature and by capsule size ($p \leq 0.005$).

Table 5.2. Range and number of nurse eggs consumed by *B. undatum* embryos developed at temperatures ranging from 6 °C to 18 °C and in small, medium and large capsules. Number of embryos dissected (*n*) refers to all columns except the furthest right hand column. For the percentage of capsules containing embryos with ≤ 1 nurse egg, *n* = 15 for all conditions.

Capsule size	Developmental temperature (°C)	Number of embryos dissected (*n*)	Number of eggs consumed *per* embryo						
			Range across all capsules	Minimum range in one capsule		Maximum range in one capsule		Mean number of eggs consumed by one embryo in one capsule*	Percent of capsules containing embryos with ≤ 1 nurse egg*
				Number of embryos	Range (number of eggs consumed)	Number of embryos	Range (number of eggs consumed)		
Small	10	47	0-174	6	30 (48-78)	4	173 (1-174)	77.0	20%
Medium	6	170	1-239	15	38 (20-58)	14	138 (20-158)	102.3	13%
	10	116	0-182	10	67 (76-143)	9	182 (0-182)	112.6	40%
	14	124	0-260	10	60 (49-109)	16	243 (17-260)	148.9	67%
	18	78	0-92	4	22 (5-27)	12	92 (0-92)	42.4	87%
Large	10	257	0-187	35	86 (0-86)	27	186 (0-186)	62.22	87%

*Kruskal-Wallis one-way analysis of variance indicates results to be significantly affected by both developmental temperature and by capsule size (p ≤ 0.005).

5.5.2. Resource partitioning

In total, 170, 116, 124 and 78 embryos were dissected from medium size capsules at 6, 10, 14 and 18 °C respectively (10 capsules *per* temperature), and 47 and 257 embryos were dissected from small and large capsules, respectively (10 °C; 10 capsules *per* size class). All embryos from every capsule examined were alive. Weights were collected from 52, 56, 38 and 30 embryos from medium capsules at 6, 10, 14 and 18 °C respectively (five capsules *per* temperature), and 31 and 78 embryos from small and large capsules, respectively (10 °C; nine small capsules, five large capsules) and 56 embryos from the Iceland population. Mean number of embryos decreased with increasing temperature (across both populations) and increased with capsule size (Fig. 5.2a, b). In contrast, proportion of 'empty' embryos increased with both increasing temperature and capsule size. Mean number of nurse eggs *per* embryo, embryo DW, and C and N to increase between 6 and 14 °C, before decreasing at 18 °C. When comparing capsule size, each of these variables was greatest in medium capsules (Table 5.1, Fig. 5.2c, d). Mean embryo dry weight for the Iceland population was between that observed at 6 and 10 °C for the Solent population. In every capsule examined, of each size and at each developmental temperature, a large range was observed in number of eggs consumed by any one embryo (Table 5.2, Fig. 5.3, 5.4a,b). Across all capsules (excluding the maximum consumption experiment), individual embryos consumed between 0 and 260 nurse eggs. 'Empty' embryos were commonly observed at every temperature and in all capsule sizes. At 18 °C, every capsule opened contained additional nurse eggs, which had not been consumed by developing embryos.

Analysis by Kruskal-Wallis one-way ANOVA indicated number of embryos *per* capsule ($H = 23.48$; d.f. = 3; $p \leq 0.001$), number of nurse eggs *per* embryo ($H = 144.78$; d.f. = 3; $p \leq 0.001$), DW ($H = 37.61$; d.f. = 3; $p \leq 0.001$) and proportion of 'empty' embryos ($H = 25.59$; d.f. = 3; $p \leq 0.001$) to be significantly affected by temperature. Post hoc analysis showed all 18 °C data to be significantly different to all other temperatures ($p \leq 0.05$). For all variables except number of nurse eggs *per* embryo, 6 °C data also varied significantly to 14 °C data ($p \leq 0.05$). Analysis indicated number of embryos *per* capsule ($H = 38.91$; d.f. = 2; $p \leq 0.001$), number of nurse eggs *per* embryo ($H = 35.54$; d.f. = 2; $p \leq 0.001$), DW ($H = 10.87$; d.f. = 2; $p \leq 0.005$) and proportion of 'empty' embryos ($H = 15.40$; d.f. = 2; $p \leq 0.001$) to be significantly affected by capsule

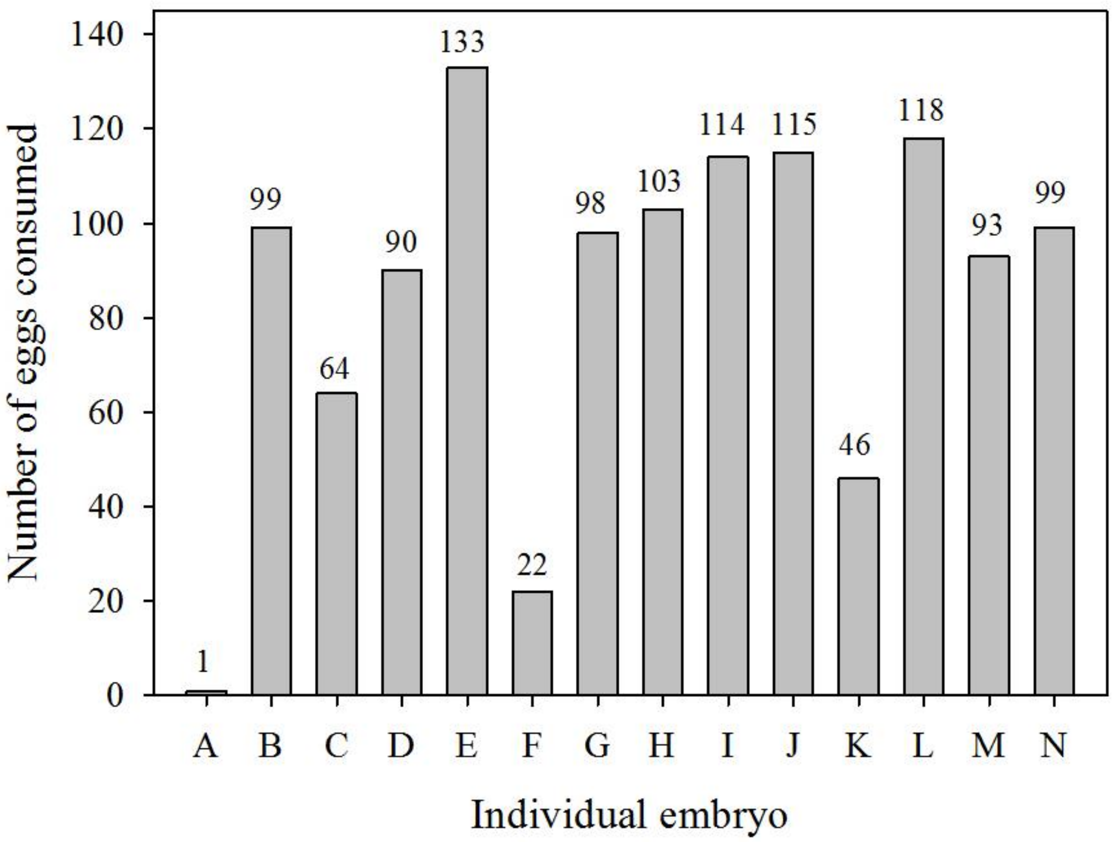

Figure 5.3. Typical nurse egg partitioning between embryos in one *B. undatum* capsule. Each bar indicates the number of nurse eggs consumed by an individual embryo. Embryos developed at 10 °C, medium sized capsule.

size. Post hoc analysis showed all data to vary significantly between all capsule sizes ($p \leq 0.05$). Number of embryos per capsule and proportion of 'empty' embryos increased with increasing capsule size. Number of nurse eggs per embryo and embryo DW increased from small capsules to medium capsules, then decreased again in large capsules.

Multiple regression analysis, which considered all independent variables in one test, indicated at least one independent variable (number of embryos per capsule, capsule volume or total number of nurse eggs per capsule) to significantly affect range in number of eggs consumed by embryos within a capsule ($F = 6.16$; d.f. 3; $p \leq 0.01$). Multiple regression analysis also indicated at least one independent variable (number of embryos per capsule or capsule volume) to significantly affect proportion of 'empty' embryos ($F = 8.24$; d.f. 2; $p \leq 0.001$). Independent regression analysis, comparing dependent variables to each independent variable separately, confirmed a positive relationship between number of embryos per capsule and range in number of nurse eggs consumed by embryos within a capsule (y = 77.1 + 2.46 x; R^2 = 0.333; d.f. = 1; p ≤ 0.001), capsule volume and range in number of nurse eggs consumed by embryos within a capsule (y = 69.0 + 0.308 x; R^2 = 0.382; d.f. = 1; p ≤ 0.001) and between total number of nurse eggs per capsule and range in number of nurse eggs consumed by embryos within a capsule (y = 69.0 + 0.308 x; R^2 = 0.382; d.f. = 1; p ≤ 0.001). Proportion of 'empty' embryos was also positively affected by number of embryos per capsule (y = 0.0101 + 0.00685 x; R^2 = 0.222; d.f. = 1; p ≤ 0.001) and capsule volume (y = - 0.0171 + 0.000855 x; R^2 = 0.282; d.f. = 1; p ≤ 0.001).

Analysis of covariance indicated C to be significantly affected by embryo DW (F = 2058.22; d.f. = 1; p ≤ 0.001) and temperature (F = 6.17; d.f. = 3; p ≤ 0.001), when using DW as a covariate. N was also significantly affected by embryo DW (F = 1978.08; d.f. = 1; p ≤ 0.001) and temperature (F =8.76; d.f. = 3; p ≤ 0.001). A significant effect for capsule size was not demonstrated for C (F = 2.86; d.f. = 2; p = 0.064) and no effect of capsule size was observed for N (F = 0.32; d.f. = 2; p = 0.729).

5.5.3. Maximum consumption

Embryos from the 'maximum consumption' experiment consumed between 245 and 325 nurse eggs (Fig. 5.4a, b). Analysis by Kruskal-Wallis one-way ANOVA indicated this to be significantly different from the number of eggs consumed by embryos developing naturally at 10 °C, in either small (H = 33.58; d.f. = 1; p ≤ 0.001), medium (H = 39.55; d.f. = 1; p ≤ 0.001) or large capsules (H = 42.43; d.f. = 1; p ≤ 0.001). All maximum consumption embryos consumed more nurse eggs than any individual developed naturally at any temperature, apart from one individual developed at 14 °C who consumed 260 eggs.

5.6. Discussion

Developmental resource partitioning in marine invertebrates is of fundamental importance in their life history. The resulting variations in offspring size may be of both ecological and evolutionary significance. One hypothesis for the fluctuation of maternal provisioning given to offspring from one clutch in a variable environment is bet-hedging (Slatkin 1974; Philippi and Seger 1989; Koops et al. 2003; Marshall et al. 2008a; Crean and Marshall 2009). Other hypotheses include selection for differing capabilities, as is observed with poecilogony, and unequal allocation of resources to offspring (e.g. Rivest 1983; Gibson et al. 1999; Oyarzun and Strathmann 2011). Explanations for these patterns often focus on maternal investment, and the influence that environmental factors have on maternal investment (Bernardo 1996; Moran and Emlet 2001), with less attention being paid to the effect of competition among siblings on size variation (e.g. Parker et al. 2002; Kamel et al. 2010a, 2010b). My results highlight the adverse effects that sibling rivalry can have on offspring size, number and quality, and additionally, emphasise that not only maternal investment, but also environmental factors to significantly affect resource partitioning during intracapsular development. *Buccinum undatum* provides an example of the varied potential outcomes of encapsulation and also an unusual and novel kind of sibling cannibalism.

The process of encapsulation allows for an elevated occurrence of sibling-to-sibling interaction, which presents a high potential for competition (Marshall and Keough 2007). Examples of species with variable resource partitioning within a capsule are rare, but have been reported in cases of poecilogony (Kamel et al. 2010b; Oyarzun and Strathmann 2011), sibling cannibalism or arrested development in siblings (Strathmann and Strathmann 2006; Kamel et al. 2010b; Oyarzun and Strathmann 2011), or through varied nurse egg consumption by embryos (Portmann 1925; Gallardo 1979; González and Gallardo 1999; Cumplido et al. 2011; Smith and Thatje 2013). In the Muricidae, in which this latter example is typically observed (e.g. Gallardo 1979; González and Gallardo 1999; Cumplido et al. 2011), intracapsular sizes are variable, but to my knowledge all offspring receive some nutrition. The differences seen have been attributed to competition, which occurs over the weeks or months of nurse egg consumption during development (e.g. Cumplido et al. 2011). In the buccinid *B. undatum*, intracapsular size differences are accentuated by the particularly rapid nurse egg consumption (typically 2 to 5 days) and the asynchrony in early development, observed in this species. Asynchronous development may result from the process of fertilisation and egg distribution during encapsulation. The resulting inequality in developmental stage and nurse egg consumption, can be hypothesised to be favoured by selection, or simply to be a by-product of reproduction in *B. undatum.* The earliest developers have a significant advantage over later developers, and the most delayed

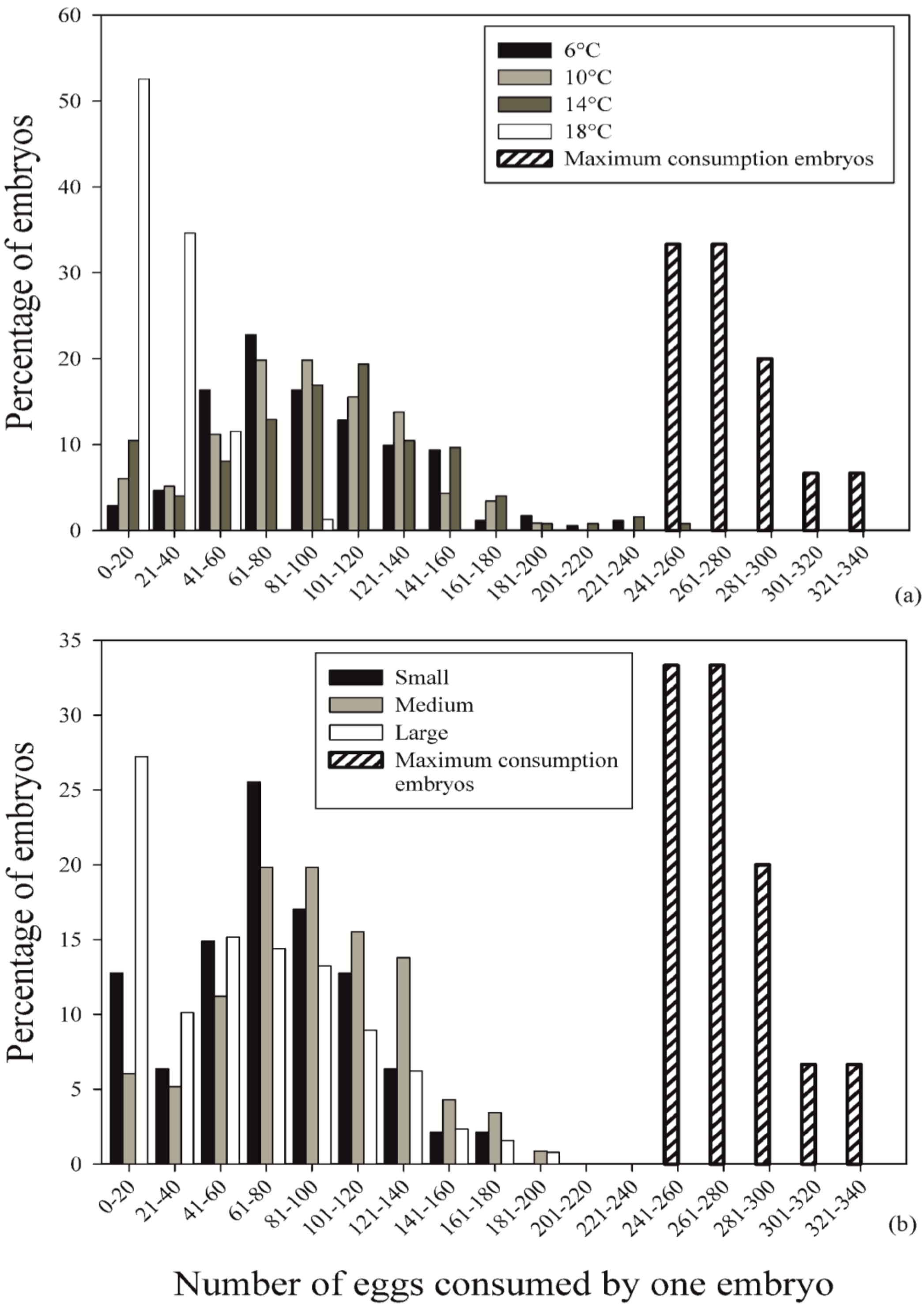

Figure 5.4. Proportion of *B. undatum* embryos consuming different quantities of nurse eggs. (a) Bars with solid colouring depict embryos developed at temperatures ranging from 6 °C to 18 °C. (b) Bars with solid colours depict embryos developed in small, medium or large capsules. (a) and (b) Bars with diagonal lines depict embryos from the maximum consumption experiment, which were allocated a surplus of nurse eggs to consume. Kruskal-Wallis one-way analysis of variance indicated number of nurse eggs consumed *per* embryo to be significantly affected by temperature and capsule size, and number of eggs consumed by maximum consumption embryos to be significantly greater than those consumed by embryos developing naturally at 10 °C from small, medium or large capsules ($p \leq 0.001$).

embryos regularly develop after all nurse eggs have been consumed (Smith and Thatje 2013). Although asynchronous development is not uncommon (e.g. Fernandez et al. 2006), nurse egg consumption is typically slower. Usually, asynchrony within a capsule is compensated for by the slower nurse egg consumption, leading to smaller intracapsular size variations than is observed in *B. undatum*. This selection for the fittest inhibits the number of embryo's successfully developing, as some out-compete others for the limited resources available.

Maternal provisioning to a capsule typically increases with both female and capsule size (Valentinsson et al 2002; Smith and Thatje 2013). In the present study, a higher total number of embryos developing *per* capsule was observed, but this was offset to a large degree by the increased proportion of 'empty' embryos observed in conjunction. The increased occurrence of 'empty' embryos and the lower mean weight observed in larger capsules suggests a lower nurse egg to embryo ratio. Consequently, embryos developing in medium capsules appeared to be better off; these individuals were on average larger and better provisioned than individuals in either large or small capsules. This may indicate that medium capsule sizes, and therefore intermediate numbers of embryos, are more beneficial on average for developing offspring. However, future work is necessary to test if this is an adaptive trend. Interestingly, the high number of eggs consumed by each embryo in the maximum consumption experiment indicated embryos to be capable of consuming many more nurse eggs than they naturally succeeded to do inside a capsule of any size. No 'large' embryos appear to develop naturally, in *B. undatum*; only 'medium' and 'small'.

Environmental factors play an important role in shaping life history. They affect not only maternal investment but also impact offspring growth and survival (Koops et al. 2003; Anger et al. 2004; Marshall and Keough 2007; Crean and Marshall 2009). Environmentally induced changes in offspring size or number occurring *during* embryonic development have, however, rarely been discussed in the context of life history theory (Lardies and Fernandez 2002; Kamel et al. 2010b; Oyarzun and Strathmann 2011). My results indicate a temperature-mediated shift in number of embryos developing, embryo weight and bioenergetic content, and proportion of empty embryos within a capsule. Comparisons can be drawn to the poecilogonic spionid polychaete *Boccardia proboscidea* (Hartman 1940). Cannibalism of small planktotrophic offspring by large adelphophagic (nurse egg consuming) offspring increases with duration of encapsulation, and consequently, the number of individuals hatching is reduced, but their average size increases. Time of hatching is dependent on environmental temperature and is decided by the mother, who tears open each capsule (Kamel et al. 2010b; Oyarzun and Strathmann 2011). In this example, and as is typically seen in marine invertebrates, a larger number of smaller offspring were produced under warmer condition. This observation is regularly attributed to maternal investment (Thorson 1950; Clarke 1992; Marshall and Keough 2007; Marshall et al. 2008b) and for *B. proboscidea*, is also ultimately controlled by the mother. In contrast, in *B. undatum* a larger number of smaller offspring are produced under *lower* temperature conditions, suggesting that in colder years a higher number of less energetically fit offspring will hatch. This resource allocation, although atypical, generally results in offspring receiving a higher level of reserves under warmer conditions, which could compensate for the greater bioenergetic demand necessary for development at higher temperatures, as has previously been reported in this species (Smith et al. 2013) and other marine invertebrates (Evjemo et al. 2001; García-Guerrero et al. 2003). This result is further supported by the greater number of offspring observed developing in the *B. undatum* egg masses from Iceland, where temperatures are naturally lower than in the UK. Similar patterns have also been observed in other gastropods (e.g. Fernandez et al. 2006; Collin 2012). Rapid nurse egg consumption and asynchrony in early development may represent forms of adaptation, which have evolved within *B. undatum* to allow for optimal survival under different thermal scenarios. Although more ‘empty’ embryos were observed under warmer conditions, considering the increased bioenergetic demand at high temperature, this may promote an overall increase in survivors. Temperature clearly plays an important role in intracapsular resource partitioning in *B. undatum.* It could be argued that asynchronous development is a maternal strategy to allow for differing numbers of successful offspring under varying developmental temperatures; this may explain patterns of inequality in other species (e.g. Fernandez et al. 2006). This would not, however, explain the increase in proportion of ‘empty’ embryos observed with increasing maternal investment, which appears to be of no maternal benefit.

Interestingly, increased developmental rates known to occur at higher temperatures eventually appear to limit nurse egg consumption. Head development, which begins at the veliger stage, prevents further nurse egg consumption (Smith and Thatje 2013). The data suggest that at 18 °C, the rate at which individuals develop is greater than the rate

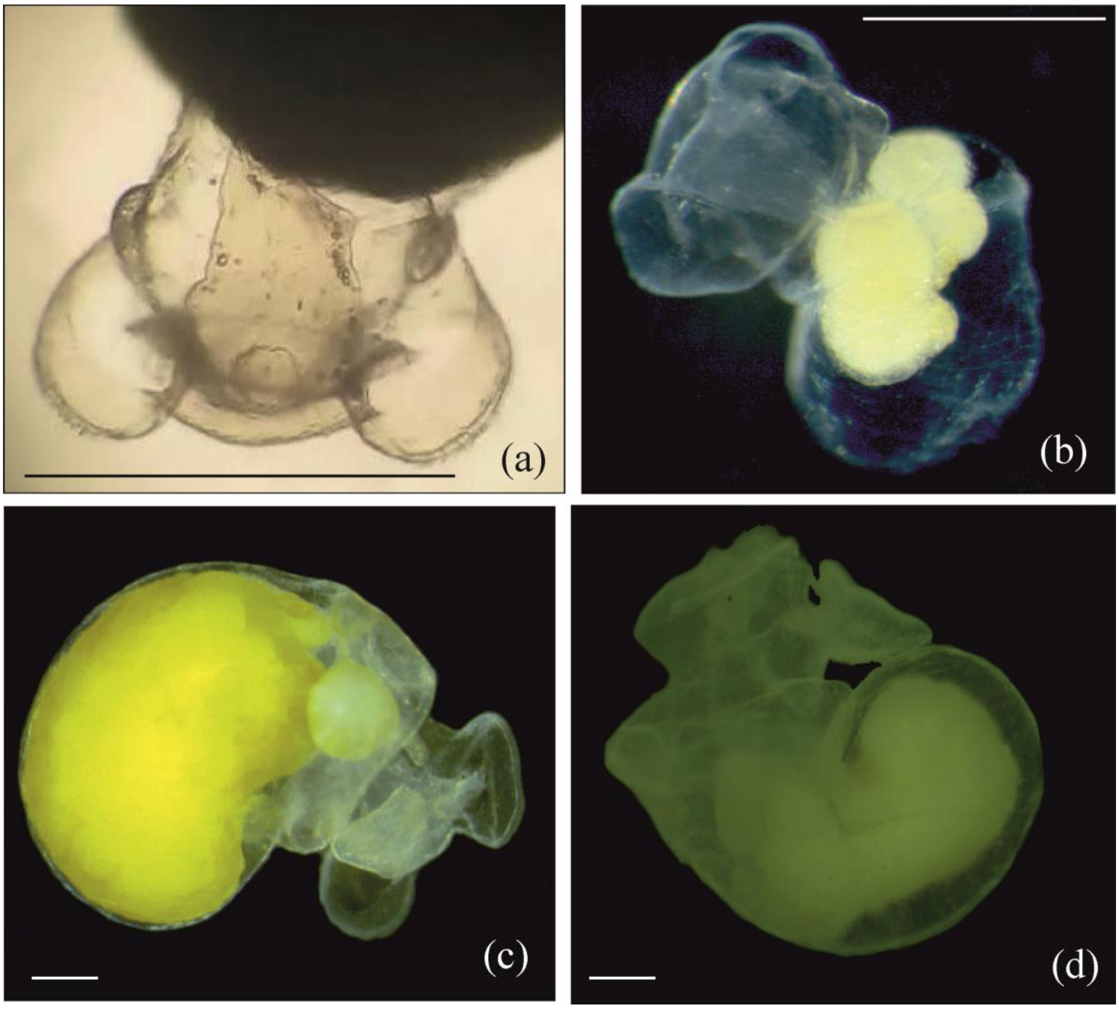

Figure 5.5. *Buccinum undatum* embryos at different developmental stages. (a) head of early veliger with simple mouth visible. (b) early veliger with few nurse eggs consumed. (c) pediveliger typical of 6, 10 or 14 °C, with full midgut. (d) pediveliger typical of 18 °C, with part full midgut. Scale bars represent 200 µm.

at which nurse eggs can be consumed. As a result, only a small proportion of eggs are consumed before embryos are no longer able to feed, meaning a limited amount of energy is available to them for development to the juvenile stage. The existence of 'empty' embryos at this temperature, despite the unconsumed nurse eggs, highlights just how rapid development is.

The common occurrence of 'empty' embryos reported here provides a unique example of the risks involved with encapsulation. In the rare examples of species exhibiting intracapsular poecilogony, a similar occurrence is observed, with seemingly 'empty' embryos developing alongside adelphophagic embryos (e.g. Kamel et al. 2010b; Gibson and Carver 2013). In these examples, however, the ‘empty’ embryos are planktotrophic, and feed post hatching. In contrast, in *B. undatum* the ‘empty’ embryos observed are direct developers and do not survive to hatching. During encapsulation, lack of survival of embryos has been noted in several species, including some vermetid gastropods (e.g. Strathmann and Strathmann 2006). In these examples, however, the deceased embryos are understood to be consumed by surviving embryos. In this study in contrast, embryos were not consumed by siblings; between the veliger and hatching juvenile stages, embryos appear unable to feed and even the occasional dead, nurse-egg containing embryo observed within a capsule was not ingested (Authors, unpublished data). Instead, ‘empty’ embryos appear to be an unfortunate consequence of development and are effectively wasted. They therefore represent an unusual loss of maternal investment, uncommon in encapsulated developments studied to date. Although rare, other examples of unexploited reproductive resources exist in marine invertebrates. For example, the gastropod *Lirabuccinum dirum* (Reeve 1846) occasionally lays capsules, which contain nurse eggs but no embryos (Rivest 1983). Additionally, capsules of *B. proboscidea* are sometimes opened before all nurse eggs have been ingested (Kamel et al. 2010b). In the latter example, however, mothers have been proposed to consume uneaten nurse eggs following the opening of capsules. Considering the low temperature origin of neogastropods (Jablonski and Bottjer 1991), and the reduction in number of ‘empty’ embryos with decreasing temperature seen here, I propose ‘empty’ embryos to be an evolutionary consequence of adaptation to warmer temperatures.

A curious feature to mention is the ‘accidental’ cannibalism observed in *B. undatum.* Early veligers appear to be relatively non-selective in what they consume, and occasionally, one was observed to have ingested another embryo, which had not yet begun development. Cannibalism is not uncommon during intracapsular development, and has been previously described in many species of gastropods and other marine invertebrates (e.g. Strathmann and Strathmann 2006; Kamel et al. 2010b). Generally, when cannibalism occurs, one individual will consume another in order to increase energy reserves for development. In *B. undatum,* however, the observed cannibalism does not have this result (Authors, unpublished data). Once ingested, embryos continued development inside the mid-gut of the consumer. They then took up nurse eggs from inside the mid-gut, and eventually burst out through the thin mantle surrounding the consumer (Fig. 5.1f, g). In this ‘Trojan horse’ scenario the victim became the ‘winner’ and the cannibal did not survive. This appears to be a defence mechanism against sibling cannibalism, and also presents a scenario where later development can be beneficial to offspring. Here, the cannibalised embryo gains a surplus of nurse eggs without having to compete for them. A disadvantage of this is the possibility that that particular egg is digested before commencing development.

This paper contributes a unique example of the diverse outcomes of encapsulated development, contributing to discussions of the trade-off between offspring size and number, in life history theory. It illustrates the adverse effects that both sibling rivalry and environmental factors can play in defining embryonic development, highlighting the importance of understanding the roles that different variables play during development as well as how they affect maternal investment. The combined effects of temperature and competition on intracapsular resource partitioning, and ultimately offspring quality, are of particular importance given the growing attention, which has been given to climate warming over recent decades (Hughes et al. 2010; MCCIP 2010). If current sea temperatures continue to rise at similar rates as those observed today (the English Channel and the southern North Sea, 0.8 °C increase *per* decade; Hughes et al. 2010), temperature increases of 4 to 8 degrees, as employed in this study, could become relevant in the next 50 to 100 years. Such increases in seawater temperature have the potential to cause population level changes in distribution and abundance. Initially, however, the developmental plasticity observed in this species may facilitate survival, providing a balance is struck between embryos being large enough to have sufficient reserves for development and plentiful enough to sustain a population.

Chapter 6: The secret to successful deep-sea invasion: does low temperature hold the key?

Published as: Smith, K.E., Thatje, S. (2012) The secret to successful deep-sea invasion: does low temperature hold the key? PLoS ONE 7 (12):e51219 (Appendix F)

6.1. Abstract

There is a general consensus that today's deep-sea biodiversity has largely resulted from recurrent invasions and speciations occurring through homogenous waters during periods of the Phanerozoic eon. Migrations likely continue today, primarily *via* isothermal water columns, such as those typical of Polar Regions, but the necessary ecological and physiological adaptations behind them are poorly understood. In an evolutionary context, understanding the adaptations, which allow for colonisation to high-pressure environments, may enable us to predict future events. In this investigation, I examine pressure tolerance during development, in the shallow-water neogastropod *Buccinum undatum* (Linnaeus 1758) using thermally acclimated egg masses from temperate and sub-polar regions across the species range. Fossil records indicate neogastropods to have a deep-water origin, suggesting shallow-water species may be likely candidates for re-emergence into the deep sea. My results show population level differences in physiological thresholds, which indicate low temperature acclimation to increase pressure tolerance. These findings imply this species is capable of deep-sea penetration through isothermal water columns prevailing at high latitudes. This study gives new insight into the fundamentals behind past and future colonisation events. Such knowledge is instrumental to understand better how changes in climate envelopes affect the distribution and radiation of species along latitudinal as well as bathymetric temperature gradients.

6.2. Introduction

Throughout their evolutionary history marine invertebrates have spread through the oceans by extension of physiological boundaries. Phylogenetic links can be found between species in every ocean and throughout latitudinal and bathymetric ranges (Palumbi 1994). It is generally accepted that past colonisations to new latitudes have taken place during geological periods of cooling or warming (e.g. temperature declines during the Pliocene and Pleistocene eras) or through gradual adaptation to warmer or colder temperatures (Zinsmeister and Feldmann 1984; Southward et al. 1995; Parmesan and Yohe 2003). Patterns indicate such shifts to predominantly occur from the tropics, towards higher latitudes, with poleward declines in species diversity being observed throughout the water column (Rex et al. 1993; Thomas and Gooday 1996; Culver and

Buzas 2000; Rex et al. 2000; Jablonski et al. 2006). Generally, invasions into, and emergences from, bathyal and abyssal depths are believed to primarily occur *via* isothermal water columns. Peak changes in diversity between shallow-water and deep-sea, appear in-line with certain geological periods when such isothermal waters were widespread (such as the Mesozoic and early Cenozoic eras), and more recently in high latitude areas through regions of deep-water formation (Wilson 1980; Sepkoski 1988; Young et al. 1997; Tyler and Young 1998; Thatje et al. 2005; Benitez-Villalobos et al. 2006; Mestre et al. 2009). Both fossil records and molecular phylogeny offer insight into such evolutionary paths. For example, Weddell Sea molluscs from the southern hemisphere can be tracked slowly north through fossil records from the Pliocene and Pleistocene periods (Zinsmeister and Feldmann 1984) and close phylogenetic relationships have been proposed between shallow-water and deep-sea caridean shrimp (Tokuda et al. 2006), mytilid mussels (Distel et al. 2000) and anomuran decapods (Hall and Thatje 2009).

Many factors (physical, chemical and biological) affect the dispersal and colonisation of species (Bohonak 1999), but for marine invertebrates, temperature and hydrostatic pressure create two substantial challenges. In shallow water temperature changes with latitude, and throughout the oceans both temperature and pressure change with depth. While both are known to affect biological systems throughout the whole animal, and are capable of causing major physiological disruptions, the impacts of these two variables have been found to be antagonistic, with pressure increases and temperature decreases often showing similar results (Somero 1992; Balny et al. 1997; Pradillon and Gaill 2007). The combined tolerance of a species to these two factors may therefore be vital in determining the shallow and deep limits in its vertical distribution (Thatje et al. 2010). While peak changes in faunal assemblage are believed to have primarily occurred during past geological periods, new colonisations likely continue to take place today (Southward et al. 1995; Tyler and Young 1998; Beaugrand et al. 2002; Parmesan and Yohe 2003; Perry et al. 2005). Current bathymetric migrations are being reported in areas with low water temperatures (Tyler and Young 1998; Perry et al. 2005), with recent studies indicating such events to be occurring in response to increasing surface water temperatures (Perry et al. 2005; Dulvy et al. 2008; Nye et al 2009). However, the growing amount of literature describing the combined effects of temperature and pressure on marine invertebrates typically indicates an increased sensitivity to pressure with decreasing temperature, with temperature being the dominant of the two factors. Work to date examining the combined effects of temperature and pressure on marine invertebrates typically focuses on echinoderms and crustaceans (Young et al. 1997; Tyler et al. 2000; Benitez-Villalobos 2006; Aquino-Souza et al. 2008; Thatje et al. 2010; Brown and Thatje 2011; Oliphant et al. 2011). Both adult and developmental stages have been investigated, but this includes only species with planktonic larvae. These studies generally exercise a small number of acute temperature and pressure treatments, with few studies examining the full physiological scope of an invertebrate with regard to both factors (Brown and Thatje 2011; Oliphant et al. 2011). Therefore, conclusions should not yet be drawn from the limited dataset available.

Understanding how the physiological scope of an organism is affected by thermal and hyperbaric changes may help us to realise the parameters that set its ecological boundaries. In turn this will aid us in predicting forthcoming bathymetric radiations, migrations and new evolutionary paths, which may occur within the oceans in response to the future effects of climate change (Tyler and Young 1998). In marine invertebrates, physiological tolerances often vary through ontogeny (e.g. Spicer 1994; Tyler and Young 1998; Mestre et al. 2009). A species combined tolerance throughout development is therefore key in dictating how successful future vertical migrations may be. In particular, it is important to understand where thresholds lie for species with non-planktonic development. Such species have limited dispersal capabilities, and migrations and radiations typically occur at a slower rate (Jablonski 1986).

Here, using *Buccinum undatum* (Linnaeus 1758), I examine the combined effects of temperature and pressure on early ontogeny. I use the novel approach of allowing thermal acclimation through development, and examining populations from different climates, across the species distribution range. Using metabolic rate as an indicator of physical performance, I investigate eco-physiological adaptations, which may indicate the potential for future deep-water colonisation.

6.3. Aims and objectives

The specific aims of this chapter were to investigate pressure tolerance in the common whelk *Buccinum undatum* at different developmental stages and across the full thermal tolerance range for development (Chapter 4).

The objectives of this chapter are as follows:

- Examine pressure tolerance of *B. undatum* veligers across the full thermal range, using individuals from populations from the southernmost and northernmost edges of the species geographic range (the Solent and Breiðafjörður populations).
- Examine pressure tolerance of *B. undatum* juveniles across the full thermal range, using individuals from populations from the southernmost and northernmost edges of the species geographic range (the Solent and Breiðafjörður populations).
- Test the hypothesis that *B. undatum* embryos will be less tolerant of high hydrostatic pressure at later developmental stages, when complexity is greater.
- Test the hypothesis that, thermal acclimation to lower temperatures will result in an increase in pressure tolerance in *B. undatum* embryos with decreasing temperature.

6.4. Materials and methods

The combined effects of temperature and pressure on early ontogeny were examined using egg masses collected by trawl from the Solent, between December 2009 and February 2010, and December 2010 and February 2011, and from Breiðafjörður, Iceland between April and May 2011 (Chapter 2, Section 2.2 and Table 2.2). Egg masses from the southern location (the Solent) were acclimated to 6, 10, 14 or 18 °C (classified *n* °C (S)), and from the northern location (Breiðafjörður) to 3 or 6 °C (classified *n* °C (N)). Three egg masses were maintained at each temperature, and for a minimum of 21 days prior further pressure experimentation (Chapter 2, Sections 2.3 and 2.6). Pressure experiments were carried out on two ontogenetic stages: veliger and hatching juvenile (Fig. 6.1; Chapter 3, Section 3.5.1; Smith and Thatje 2013). These stages were chosen as representative of the oldest and youngest ontogenetic stages, which could be manipulated without damage.

6.4.1. Effects of pressure on respiration rates

The effects of pressure on respiration rates were measured using three individuals placed into a 2.8 ml plastic vial containing pre-incubated 1 µm filtered seawater. For each egg mass a total of three experimental vials and an additional four blank vials (containing no animals) were prepared. The vials were filled and sealed as described, over a maximum period of 2 min (Chapter 2, Section 2.8.1). All seven vials were placed inside a cast-iron pressure vessel and pressurised to the selected experimental treatment (1, 100, 200, 300, 400 atm) using a Maximator model manual hydraulic pump (Chapter 2, Section 2.7.1). Pressures ranging 1 to 400 atm (equivalent to 0 to 4000 m water depth) were chosen to represent shallow water through to average ocean depths. The pressure vessel was then incubated at the experimental temperature for 4 h, before being rapidly depressurised (Chapter 2, Section 2.7.1). Pressure remained sustained for the entire experimental period.

Upon depressurisation the oxygen concentration of the water (% air saturation) was recorded for each vial. Each vial was gently agitated prior to the measurement being taken to ensure the oxygen level was constant throughout. The % air saturation was then

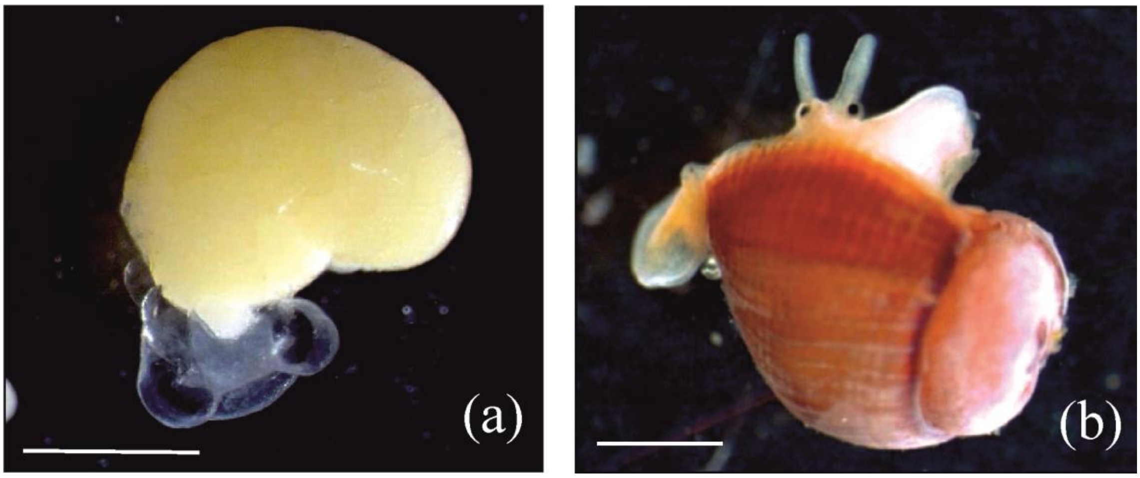

Figure 6.1. Intracapsular developmental stages of *Buccinum undatum*. (a) veliger and (b) juvenile. Scale bars represent 500 µm.

continuously logged for a minimum of one minute or until a constant level of air saturation was observed (Chapter 2, Section 2.8.1). Animals from each vial were sampled for dry weight (Chapter 2, Section 2.8.2).

Final respiration rates were calculated following an adaptation of methods described by Thatje et al. (2010) and Brown and Thatje (2011). Here, the difference in readings between blank and experimental vials was determined and oxygen consumption assessed *per* unit of dry weight. (Chapter 2, Section 2.8.1). In this method the concentration of oxygen in 100 % saturated seawater was calculated according to Benson and Krause (1984). This protocol was repeated for every temperature / pressure combination, for three egg masses at each temperature, and for veliger and hatching juveniles from each egg mass. This gave a total of nine experimental replicates for each developmental stage and every treatment. Oxygen consumptions recorded below, are stated as µmol O_2 mg^{-1} h^{-1} (± 1 S.D.).

6.4.2. Statistics

Data were analysed using General Linear Model ANOVA (two factors, crossed; temperature and pressure). *Post hoc* analysis was carried out using the Sidak simultaneous test. Prior to analysis all data were subject to square root transformation to attain homoscedasticity (Levenes test, $p > 0.05$). Since all egg masses were contributed to by many females, and egg capsules laid by one female could not be easily distinguished, the data collected from all the egg masses used in any one treatment were combined. Because no difference was found in C:N ratio between the Solent and Breiðafjörður egg masses (Chapter 2, Section 2.4), the results from the two populations were analysed together.

6.5. Results

6.5.1. Temperature and pressure interaction effects on respiration rates

Interaction effects of temperature and pressure were found to significantly impact oxygen consumption in hatching juveniles ($F = 4.42$; d.f. 20; $p \leq 0.001$), but not in veligers ($F = 0.56$; d.f. = 20; $p = 0.934$) (Table 6.1, fig. 6.2c, 6.3c). *Post hoc* analysis

Table 6.1. Results of General Linear Model ANOVA analysis. Testing the effects of temperature, pressure and temperature – pressure interactions on oxygen consumption in veliger and hatching juvenile *Buccinum undatum* from the Solent (UK) and Breiðafjörður (Iceland). Significance level is indicated by asterisks, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. *n* = number of replicates, df = degrees of freedom.

		Veliger		**Hatching juvenile**	
	n, df	**F statistic**	**p-value**	**F statistic**	**p-value**
Temperature	45, 5	30.14	≤ 0.001***	30.63	≤ 0.001***
Pressure	54, 4	0.94	0.440	105.01	≤ 0.001***
Temperature x pressure	9, 20	0.56	0.934	4.42	≤ 0.001***

($p \leq 0.05$) indicated there to be no difference in oxygen consumption in veligers between 1 atm, and any other pressure at any temperature (Table 6.2a). In hatching juveniles, *post hoc* analysis ($p \leq 0.05$) indicated that the effects of increasing pressure on oxygen consumption were reduced with decreasing temperature (Table 6.2b; Fig. 6.4). In veligers, the largest fluctuation in respiration rates across all pressures was observed at 6 °C (S), where mean oxygen consumption ranged from 0.012 (SD ± 0.004) µmol O_2 mg^{-1} h^{-1} to 0.017 (SD ± 0.009) µmol O_2 mg^{-1} h^{-1}. The smallest difference was at 3 °C (N) (range 0.004 (SD ± 0.002) to 0.005 (SD ± 0.002) µmol O_2 mg^{-1} h^{-1}). In hatching juveniles, the largest mean change in oxygen consumption across all pressures occurred at 14 °C (S) (range 0.002 (SD ± 0.001) to 0.012 (SD ± 0.005) µmol O_2 mg^{-1} h^{-1}) and the smallest was again at 3 °C (N) (range 0.002 (SD ± 0.001) to 0.004 (SD ± 0.001) µmol O_2 mg^{-1} h^{-1}).

6.5.2. Effects of temperature on respiration rates

Analysis indicated respiration rates in both veligers (F = 30.14; d.f. = 5; $p \leq 0.001$) and hatching juveniles (F = 30.63; d.f. = 5; $p \leq 0.001$) to be significantly affected by temperature (Table 6.1, fig. 6.2a, 6.3a). In veligers, at 1 atm of oxygen consumptionincreased as temperature increased in samples from Breiðafjörður, but decreasing with increasing temperature in samples from the Solent. In juveniles, the trend was for oxygen consumption to decrease as temperature dropped, across both populations. *Post hoc* analysis ($p \leq 0.05$) indicated several groupings of similar temperatures for both veligers and juveniles, when pressures were averaged for each temperature (Fig 6.2a, 6.3a).

6.5.3. Effects of pressure on respiration rates

Analysis indicated pressure to significantly affect respiration rates in all hatching juveniles ($F = 105.01$; d.f. = 4; $p \leq 0.001$), but not in veligers ($F = 0.94$; d.f. = 4; $p = 0.440$) (Table 6.1, fig. 6.2b, 6.3b). A trend was observed of oxygen consumption to decrease with increasing pressure in juveniles from both Breiðafjörður and the Solent. When all temperatures were averaged for each pressure, *post hoc* analysis ($p \leq 0.05$) indicated hatching juveniles to be significantly affected by pressures of 200 atm or more (when compared to 1 atm). *Post hoc* analysis indicated there to be no difference in oxygen consumption in veligers between any pressures ($p \leq 0.05$).

6.6. Discussion

6.6.1. Cold water preference in Buccinidae?

Phylogenetically, neogastropods are believed to have a cold, deep-water origin, first appearing in fossil record on the ‘deep’ outer shelf during the early cretaceous period (Sepkoski 1988; Jablonski and Bottjer 1991; Jablonski 2005a). Since then, they have evolved over millions of years to inhabit every corner of the oceans, across the full bathymetric and latitudinal range (Martell et al. 2002). In particular within the Buccinidae, a distinct cold-water preference remains evident today. Species abundance is greatest in sub-polar and temperate areas (http://iobis.org/mapper/), and many species (including *B. undatum*) with ranges covering temperate or sub-tropical climates, show preference for cold water in their breeding cycle, with spawning and development occurring when annual water temperatures are at their lowest (D’Asaro 1970; Fretter and Graham 1985). Recent work on thermal acclimation indicates that although limits of a species thermal tolerance range can be shifted during colonisations of new environments, optimal performance rarely acclimates to changes in environmental temperature (Angiletta 2009; Ravaux et al. 2012). It is therefore likely that the historical low-temperature preference observed during breeding in this group may remain

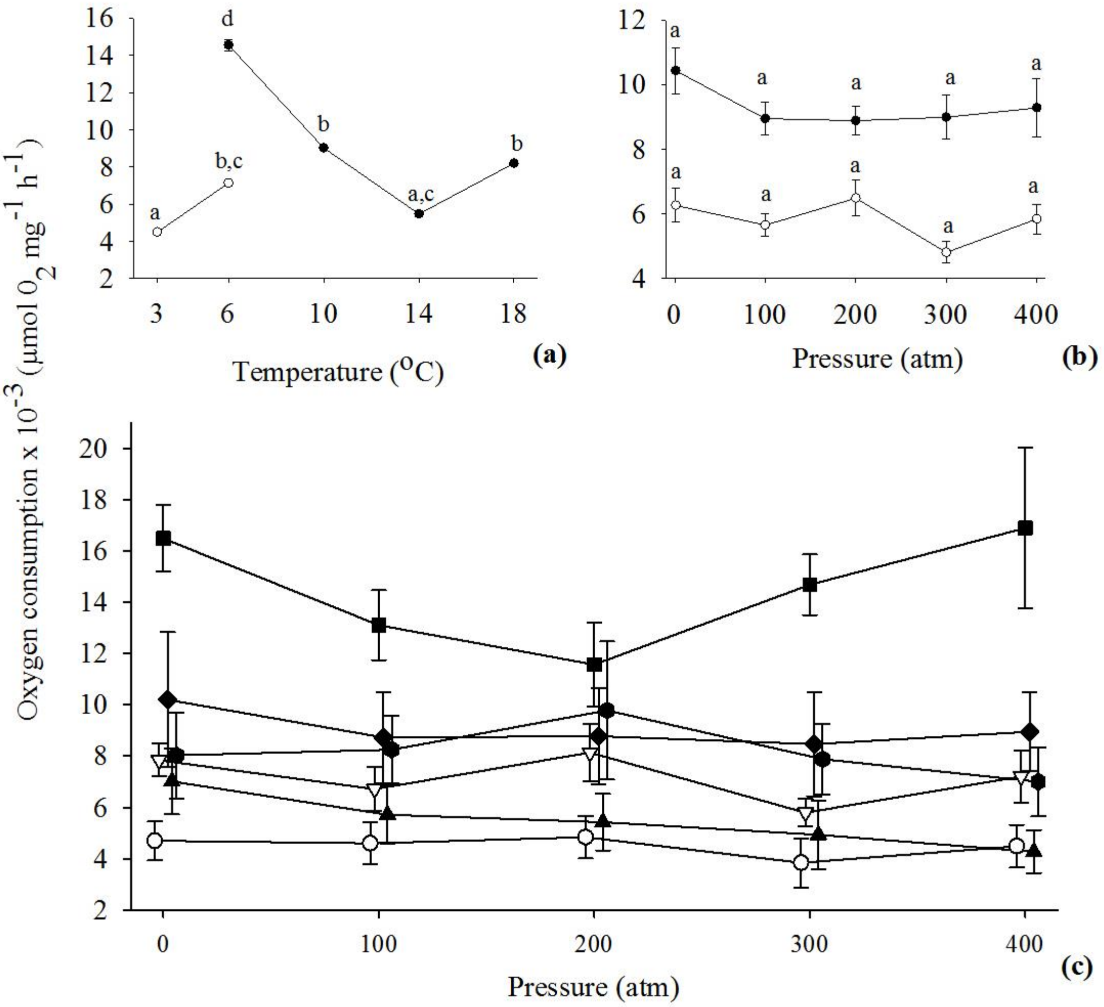

Figure 6.2. Oxygen consumption of veliger *B. undatum* from the Solent (UK) and Breiðafjörður (Iceland). (a) Effects of temperature on oxygen consumption. Data for each temperature are averaged from five pressures (1, 100, 200, 300, 400 atm). Open circles, Breiðafjörður data; closed circles, Solent data. General Linear Model ANOVA indicated oxygen consumption to be significantly affected by temperature ($p \leq 0.001$); different letters indicate values that are significantly different ($p \leq 0.05$). For each data point $n = 45$. (b) Effects of hydrostatic pressure on oxygen consumption. Data for each pressure are averaged across temperatures (6, 10, 14, 18 °C for Solent samples; 3, 6 °C for Breiðafjörður samples). Open circles, Breiðafjörður data; closed circles, Solent data. Oxygen consumption was not affected by pressure; different letters indicate values that are significantly different ($p \leq 0.05$). For each data point $n = 54$. (c) Change in oxygen consumption with pressure, at 6 temperatures. Open circles, 3 °C Breiðafjörður; open triangles, 6 °C Breiðafjörður; closed squares, 6 °C Solent; closed diamonds, 10 °C Solent; closed triangles, 14 °C Solent; closed circles, 18 °C Solent. Oxygen consumption was not affected by temperature – pressure interactions; see table 6.2 for *post hoc* analysis (Sidak simultaneous test). Error bars display standard error. For each point, $n = 9$.

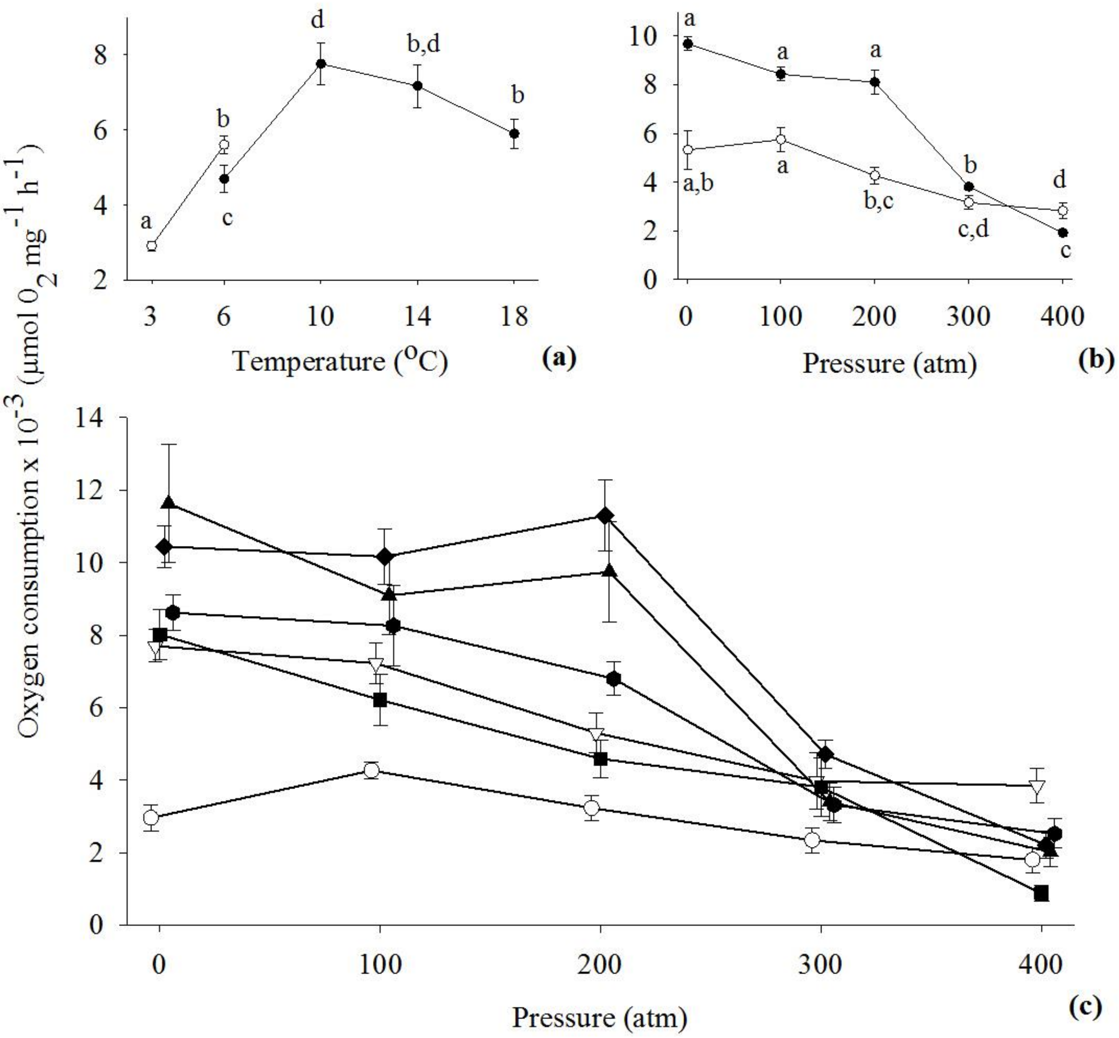

Figure 6.3. Oxygen consumption of hatching juvenile *B. undatum* from the Solent (UK) and Breiðafjörður (Iceland). (a) Effects of temperature on oxygen consumption. Data for each temperature are averaged from five pressures (1, 100, 200, 300, 400 atm). Open circles, Breiðafjörður data; closed circles, Solent data. General Linear Model ANOVA indicated oxygen consumption to be significantly affected by temperature ($p \leq 0.001$); different letters indicate values that are significantly different ($p \leq 0.05$). For each data point $n = 45$. (b) Effects of hydrostatic pressure on oxygen consumption. Data for each pressure are averaged across temperatures (6, 10, 14, 18 °C for Solent samples; 3, 6 °C for Breiðafjörður samples). Open circles, Breiðafjörður data; closed circles, Solent data. Oxygen consumption was significantly affected by pressure ($p \leq 0.001$); different letters indicate values that are significantly different ($p \leq 0.05$). For each data point $n = 54$. (c) Change in oxygen consumption with pressure, at 6 temperatures. Open circles, 3 °C Breiðafjörður; open triangles, 6 °C Breiðafjörður; closed squares, 6 °C Solent; closed diamonds, 10 °C Solent; closed triangles, 14 °C Solent; closed circles, 18 °C Solent. Oxygen consumption was significantly affected by temperature - pressure interactions ($p \leq 0.001$); see table 6.2 for *post hoc* analysis (Sidak simultaneous test). For each data point, $n = 9$. Data were analysed using General Linear Model ANOVA. Error bars display standard error.

beneficial for optimal performance. Amongst others, cold-water spawning and development has been observed in species from the Buccinidae, Conidae, Fasciolariidae and Muricidae (D'Asaro 1970; Fretter and Graham 1985). My results indicate that in *B. undatum* this historical cold-water preference is particularly prevalent during early development. In juveniles, oxygen consumption was related to temperature (Fig. 6.3), as has been observed on many previous occasions for invertebrates (Clarke 1993; Childress 1995; Allan et al. 2006; Thatje et al. 2010; Brown et al. 2011; Cancino et al. 2011; Oliphant et al. 2011). In veligers, in comparison, oxygen consumption increased with temperature until it exceeded 6 °C, at which point it reduced (Fig. 6.2), indicating thermal optima, and potentially also thermal maxima, to be lower at this developmental stage than for juveniles. These results are also supported by observations which indicate *B. undatum* to have a narrower thermal tolerance range during development than during adult life (Author, unpublished results). For each population, the highest oxygen consumption readings were taken at 6 °C , indicating thermal optima may be relatively constant across the distribution range regardless of habitat temperature. The thermal optima I observed, also coincided with the minimum, and most successful, temperature at which complete intracapsular development occurs in *B. undatum* at the southern end of their distribution (Smith et al. 2013). Northern populations, in comparison, and those which are historically adapted to sub-polar or polar climates, are also able to develop at temperatures below this (Martel et al. 1986a; Valentinsson 2002).

6.6.2. Thermal and hyperbaric effects on physiological thresholds and the importance of complexity

Temperature and pressure have both frequently been argued to be major factors affecting the physiology of invertebrates, thus playing a pivotal role in their distribution patterns (Somero 1992; Clarke 2003; Pradillon and Gaill 2007; Angiletta 2009; Thatje et al. 2010). Growth, survival and developmental success may all be affected by these factors, and internally all membrane based processes have the potential to be disrupted by them (Somero 1992; Hazel 1995; Anger et al. 2003; Pradillon and Gaill 2007). Organism complexity plays a key role in determining the intensity of such impacts, in particular in response to pressure. Quite simply, as an organism develops its complexity increases. This appears to make it more susceptible to the negative effects of pressure, often narrowing its tolerance window. Studies carried out on echinoderms indicate that when individuals are in their most complex form (i.e. adult) pressure tolerance is much lower than during development (Tyler and Young 1998). The effects of both temperature and pressure have also been shown to vary during development in invertebrates (Tyler et al. 2000; Mestre et al. 2009; Weiss et al. 2009a; 2009b).

While both temperature and pressure create substantial physiological obstacles for marine invertebrates, when comparing the effects of the two variables, temperature is commonly observed to be the dominant factor. Changes in temperature continuously affect invertebrates (e.g. Clarke 2003; Angilletta 2009; Somero 2010), but past authors have demonstrated the effects of pressure on shallow-water invertebrates (crustaceans and echinoderms) to only become significant when equivalent to 2000 m or more (Aquino-Souza et al. 2008; Thatje et al. 2010; Oliphant et al. 2011). The results of this study support these past investigations; temperature significantly affects veligers and juveniles, whereas pressure only affects juveniles, and only when greater than 200 atm. These findings also show a similar ontogenetic shift in pressure tolerance as have been previously reported (Tyler et al. 2000; Mestre et al. 2009), with juveniles being more susceptible than veligers.

6.6.3. Polar climates: the optimum environment for deep-sea invasion?

Several theories exist regarding the evolutionary expansion of ocean biodiversity. It is generally accepted that recurrent bathymetric colonisations and speciation events have occurred, primarily *via* isothermal water columns. Ocean temperature profiles have, however, varied across geological periods, and with this, shifts in patterns of biodiversity. For example, well established patterns indicate latitudinal expansions in both shallow-water and deep-sea species diversity, with overall decreases from the tropics to the poles (Rex et al. 1993, 2000; Jablonski et al. 2006). Such gradients are believed to have commenced in line with global cooling events such as that in the early Cenozoic, when ocean waters ceased to be homogenous, potentially limiting bathymetric migrations, and have been linked to the onset of seasonally fluctuating food supplies (Thomas and Gooday 1996; Culver and Buzas 2000). Fossil records also show increases in speciation and hence, biodiversity following glacial retreat (Kiel and Nielsen 2010; Albaina et al. 2012). Theories suggest opportunistic taxa may go through rapid adaptive radiation in order to fill empty niches (Kiel and Nielsen 2010). Such opportunistic taxa include species, which have been hypothesised to survive glacial advances through migration to deeper waters (Albaina et al. 2012). Conflicting views exist regarding the expansion of extant fauna of deep-sea chemosynthetic environments. While previous studies have suggested deep-sea vents and seeps to have been colonised by fauna from shallow-water seeps, which exhibit adaptations beneficial for such environments (Jacobs and Lindberg 1998), recent works instead suggest invasions primarily occur latitudinally from adjacent deep-sea environments, indicating isothermal water columns to contribute minimally in facilitating such invasions (Pedersen et al. 2010; Kiel et al. 2012).

Here, I focus on the widely accepted hypothesis of bathymetric migrations occurring *via* isothermal water columns (Wilson 1980; Sepkoski 1988; Young et al. 1997; Tyler and Young 1998; Thatje et al. 2005; Benitez-Villalobos et al. 2006; Mestre et al. 2009). While during past geological periods (e.g. the late Mesozoic and early Cenozoic), ocean waters were warm and relatively homogenous throughout, today ocean temperatures average ~4 °C below 1000 m (Wilson 1980; Sepkoski 1988; Young et al. 1997; Tyler and Young 1998; Raupach et al. 2009). Regions where surface waters are of a similar temperature to this make up the largest areas of isothermal waters. If I consider bathymetric colonisations and speciation events to primarily occur *via* such waters, these areas are therefore the most likely locations for modern bathymetric migrations.

Since the effects of temperature and pressure go hand in hand, these two variables should be evaluated collectively when considering the potential for deep-water colonisation. The results from the present investigation imply *B. undatum* to theoretically be capable of surviving the combined thermal and hyperbaric conditions characteristic of the deep sea. While ontogenetic shifts were evident in both pressure and temperature thresholds, contrary to past studies (e.g. Tyler et al. 2000; Benitez-Villalobos 2006; Thatje et al. 2010; Brown and Thatje 2011; Oliphant et al. 2011), my results indicate pressure sensitivity to decrease at low temperature for the populations examined, with individuals being capable throughout development of withstanding pressures equivalent to at least 4000 m at the lowest experimental temperature of 3 °C (Fig. 6.4).

Table 6.2. *Post hoc* analysis (Sidak simultaneous test) of the effects of pressure on oxygen consumption (when compared to 1 atm) across 6 temperatures (3 to 18 °C). (a) veliger and (b) hatching juvenile *B. undatum* from the Solent (UK) and Breiðafjörður (Iceland). Significance level is indicated by asterisks, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. $n = 9$. T = test statistic.

(a) *Veliger*

		Pressure (atm)							
		100		200		300		400	
	Temperature (°C)	**T**	**p-value**	**T**	**p-value**	**T**	**p-value**	**T**	**p-value**
The Solent, UK	**18**	0.167	1.000	0.588	1.000	-0.036	1.000	-0.571	1.000
	14	-0.768	1.000	-0.924	1.000	-1.344	1.000	-1.631	1.000
	10	-0.512	1.000	-0.517	1.000	-0.731	1.000	-0.177	1.000
	6	-1.341	1.000	-2.004	1.000	-0.652	1.000	0.246	1.000
Breiðafjörður, Iceland	**6**	-0.674	1.000	0.053	1.000	-1.117	1.000	-0.459	1.000
	3	-0.052	1.000	0.140	1.000	-0.765	1.000	-0.210	1.000

(b) *Juveniles*

		Pressure (atm)							
		100		200		300		400	
	Temperature (°C)	**T**	**p-value**	**T**	**p-value**	**T**	**p-value**	**T**	**p-value**
The Solent, UK	**18**	-0.590	1.000	-1.620	1.000	-5.570	<0.001***	-6.860	<0.001***
	14	-1.800	1.000	-1.310	1.000	-7.490	<0.001***	-9.700	<0.001***
	10	-0.250	1.000	0.540	1.000	-5.220	<0.001***	-8.840	<0.001***
	6	-1.746	1.000	-3.426	1.000	-4.706	0.002**	-9.530	<0.001***
Breiðafjörður, Iceland	**6**	-0.459	1.000	-2.375	1.000	-4.080	0.026*	-4.075	0.027*
	3	1.786	1.000	0.379	1.000	-0.995	1.000	-1.970	1.000

Previous studies indicate theoretical depth penetration by shallow-water crustaceans and echinoderms to be possible at temperatures close to the upper limits of, or above, those experienced in their natural environment (e.g. Young et al. 1997; Tyler et al. 2000; Benitez-Villalobos 2006; Brown and Thatje 2011; Oliphant et al. 2011). The antagonistic effects of temperature and pressure imply that increases in one, may compensate to some degree for increases in the other. For example, membrane fluidity is affected by both variables but while high pressure decreases fluidity, high temperature increases it. If both variables increase simultaneously therefore, membrane fluidity may be minimally affected (for reviews see Somero 1992; Pradillon and Gaill 2007). Environmental changes, however, rarely follow this pattern, and adaptations often occur as a result of changes in just one variable. Since similar adaptations develop as a result of increased pressure or decreased temperature, organisms which have already adapted to low temperatures, for example, may exhibit adaptations which aid tolerance of high pressure. Growth at low temperatures has been shown to increase pressure resistance in the bacterium *Escherichia coli* (Castellani and Chalmers 1919) (Casadei and Mackey 1997; Casadei et al. 2002). Similarly, studies examining the effects of pressure on shallow-water echinoderm embryos indicate pressure tolerance at native temperatures to be greater in polar species (Tyler et al. 2000) than in temperate species (Aquino-Souza et al. 2008). I suggest that in other marine invertebrates a similar phenomenon occurs, with cold-adapted populations being less affected by increases in pressure than warm-adapted populations. Under this scenario, as ocean surface waters continue to warm, *B. undatum* and similar cold-adapted species may migrate along isotherms, taking refuge in deeper waters which appear optimal for their physiology and using this as a mechanism to survive. Such migrations have already been reported in a range of cold water marine fish, in response to warming surface waters (Perry et al. 2005; Dulvy et al. 2008; Nye et al 2009). Similar migrations have also been suggested in response to decreasing temperatures; evidence of glacial refugia indicates some species to have previously seeked refuge in deeper waters in order to avoid advancing ice sheets (Thatje et al. 2005; Albaina et al. 2012).The relationship between depth range and latitude often observed in marine invertebrates gives support for this, with several species showing patterns of increasing depth limits at high latitudes (Macpherson 2003). While *B. undatum* is known to exist in waters shallower than 250 m (Rosenberg 2009), to my knowledge, records detailing variations in depth distribution across the species range are incomplete and further study is needed to determine whether this species follows the same pattern.

While theoretically, this study shows *B. undatum* to be capable of cold, deep-water penetration, the shallow-water distribution of this species suggests factors other than temperature and pressure may currently limit its distribution. Species range limits are

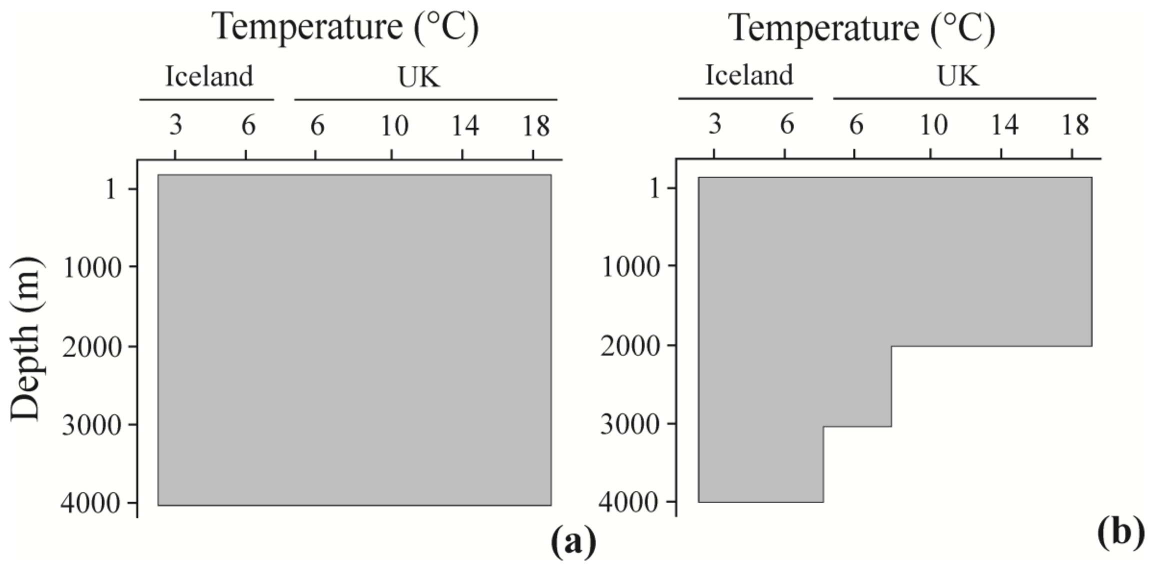

Figure 6.4. Theoretical depth penetration in (a) veligers and (b) juveniles of *B. undatum* from the Solent (UK) and Breiðafjörður (Iceland), developed at temperatures ranging 3 to 18 °C. Defined depth limits are based on the results of the *post hoc* analysis (Sidak simultaneous test); results in table 6.2. Results are significant to $p \leq 0.001$.

set by a combination of many physical, chemical and biological factors (Benitez-Villalobos et al. 2006; Howell et al. 2002; Gaston et al. 2009; Bozinovic et al. 2011). Such factors include food availability, predation, competition, habitat and water chemistry (Benitez-Villalobos et al. 2006; Howell et al. 2002). Alternatively, the thermal and hyperbaric tolerance observed throughout early ontogeny in this species may not be representative of that which it can tolerate throughout the duration of its entire life history; an important aspect that remains subject to future study, including the challenge of long-term maintenance of invertebrates under high hydrostatic pressures.

My study provides evidence that if thermally acclimated throughout development, high-pressure tolerance is possible at the low temperatures typical of deep-sea environments. Although questions remain regarding the potential for complete egg mass development and successful growth to adult life under high pressure, or the impact of additional factors such as species interactions, my findings suggest populations of *B. undatum* from the northern end of the distribution to be theoretically capable of submergence into deep water through cold isothermal water columns typical of those found in Polar Regions. Cold-acclimated juveniles from the southern end of the distribution also showed greater tolerance to pressure (to 400 atm), relative to warm-acclimated individuals from the same population (to 200 atm).

Thermal acclimation, and hence, phenotypic plasticity is an important mechanism affecting physiological scope, and past studies have indicated shifts in thermal range as a result of acclimation, which in turn affects performance at a given temperature (Zippay and Hofmann 2010; Ravaux et al. 2012). The present study increases knowledge of the physiological effects of temperature and pressure on invertebrates and highlights the importance of thermal acclimation in experimentation, giving fresh insight into how evolutionary colonisations *via* polar isothermal water columns may have been achieved.

Chapter 7: The metabolic cost of development under pressure

7.1. Abstract

Bathymetric trends in physiological adaptations observed in marine invertebrates include shifts in metabolic rate and egg size. Such shifts are understood to be set predominantly by temperature, which typically decreases with depth; hydrostatic pressure is rarely considered significant. However, invertebrate egg size has been observed to increase with depth and in the absence of a thermal gradient. These observations suggest hydrostatic pressure may also be important in determining the allocation of resources to offspring. Here, I present data on the effects of sustained exposure to pressure on the encapsulated development of the shallow-water marine gastropod *Buccinum undatum* (Linnaeus 1758). The Buccinidae family includes a wide range of shallow and deep-water species, distributed globally. I test the hypothesis that an increase in pressure will lead to an increase in energetic expenditure during development. My results indicate that high hydrostatic pressure significantly increases the metabolic cost associated with development. An increase in use of bioenergetic reserves, and a simultaneous lag in developmental rate, was observed with increasing pressure. This demonstrates a subtle, but critical, effect of pressure on development. I suggest hydrostatic pressure is a significant contributor to establishing depth range limits in shallow-water invertebrates. This may provide an important and previously underestimated evolutionary bottleneck, influencing bathymetric range extensions of shallow-water fauna into the deep ocean.

7.2. Introduction

Metabolism is crucial in controlling both the energetic and oxygen requirements necessary to sustain life. Consequently, adjustments to metabolic rates are widely regarded as among the most important of evolutionary adaptations. They typically vary between habitats, with closely related species often exhibiting different rates. In marine invertebrates, a reduction in metabolic rate with increasing bathymetric depth has been observed, indicating a decrease in energetic requirement. Such shifts have been identified in crustaceans (Mickel and Childress 1982; Childress et al. 1990; Company and Sardà 1998) and molluscs (Seibel et al. 1997; Seibel and Childress 2000), predominantly reported to occur as a function of temperature (Childress et al. 1990;

Childress 1995) or locomotory capacity (Ikeda 1988; Childress et al. 1990; Company and Sardà 1998; Seibel and Drazen 2007).

Bathymetric trends of increasing egg size (or energetic investment *per* embryo when considering encapsulation) with depth are also found within the oceans. Such patterns have been reported in a range of taxa including crustaceans (King and Butler 1985; King 1987; Van Dover and Williams 1991; Mauchline 1995; Young 2003; Morley et al. 2006), echinoderms (Young et al. 1997) and gastropods (Thorson 1950; Gage and Tyler 1991). Egg size can be considered a function of energetic allocation for development and is, in most but not all cases, related to lipid content (Anger et al. 2002). Larger eggs have more lipid reserves, allowing offspring a greater amount of energy for development and indicating a tendency for lecithotrophy (Herring 1974a; Strathmann 1985; Anger 2001; Anger et al. 2003; Rosa et al. 2007). An increase in egg size is usually attributed to the low temperature and reduced productivity (and therefore food availability) typical of the deep sea. Selecting for larger eggs facilitates a long larval duration, and the incorporated lipid reserves help sustain development through periods of starvation (e.g. King and Butler 1985). Reduced metabolic rate may also mediate these challenges by controlling the expenditure of energetic reserves. Irrespective, the increased maternal cost associated with producing larger eggs suggests the resulting higher energetic reserves are necessary for successful development in the deep sea.

The assumption that egg size is dependent only on temperature and food availability would predict size to remain constant at depths below approximately 1000 m. Food availability is greatest in surface waters and below 1000 m temperatures are relatively homogenous throughout the oceans (Gage and Tyler 1991). In contrast to expectations, however, the size of lecithotrophic eggs of high-latitude lithodid crabs have been shown to increase with depth despite a relatively isothermal water column (Morley et al. 2006), and egg size in galatheid crabs has been found to increase continuously from surface waters to 4000 m depth (Van Dover and Williams 1991). These findings imply the bathymetric increase in allocation of lipids to offspring, persists from shallow water down to the abyss (Thatje and Mestre 2010). Predictions which consider only temperature and productivity effects, would also anticipate species from hydrothermal vent environments to produce smaller eggs than related non-vent species. Vents are characteristically warm, productive habitats when compared to most of the deep sea

(Gage and Tyler 1991). The limited data available, however, indicate closely related species to produce eggs of a similar size in both vent and non-vent environments (Herring 1974b; Ramirez-Llodra et al. 2000). These observations suggest variables other than temperature and productivity may contribute to bathymetric adaptations observed in egg size. Hydrostatic pressure is the only variable in the ocean to change continuously with depth and over long evolutionary time periods, it has also remained the most constant environmental parameter in the oceans. Pressure may therefore be important in determining the allocation of energetic reserves to offspring. Despite this, it is rarely considered important in influencing bathymetric trends (but see Seibel and Drazen 2007).

The physiological impacts of pressure on marine invertebrates may be of significant ecological and evolutionary consequence. Understanding the effects of this may increase knowledge of the dynamics contributing to both modern and historical adaptations and range shifts. Acute exposures to high pressure, not exceeding 50 h duration, have indicated a reduction in developmental rate (Benitez-Villalobos et al. 2006; Aquino-Souza et al. 2008; Mestre et al. 2009). The effects of pressure on development in marine invertebrates have never been examined beyond this. Using the shallow-water gastropod *Buccinum undatum* (Linnaeus 1758) as a model species, I investigate the effects of sustained pressure over periods of 16 to 20 days throughout early (lecithotrophic) ontogeny. I test the hypothesis that an increase in pressure will lead to an increase in energetic expenditure during development.

7.3. Aims and objectives

The overall aim of this chapter was to investigate the effects of sustained exposure to high pressure on development of the common whelk *B. undatum*, at an ecologically relevant temperature.

The objectives of this chapter are as follows:

- Examine sustained tolerance to high pressure of *B. undatum* during development, using egg masses from the Solent population.

- Test the hypothesis that an increase in energetic expenditure during development will be observed with increasing pressure in *B. undatum* embryos.

7.4. Materials and methods

Here, I examine the effect of pressure on the initial stages of development and nurse egg consumption in *B. undatum*, to the point at which only developing embryos are present within a capsule (see Chapter 3, Section 3.5.1). Experiments were carried out across pressures ranging from 1 to 300 atm (equivalent of 1 to 3000 m water depth) at 10 °C. This temperature is within the natural developmental range of this species and is also relevant as the approximate water temperature at 1000 m water depth in the North East Atlantic, at the southern end of the species distribution (Locarini et al. 2010). The known bathymetric range of *B. undatum* is limited to approximately 250 m water depth (Rosenberg 2009), but the short-term (4 h) pressure tolerance of veliger has been established as equivalent to 4000 m water depth (Chapter 6).

7.4.1. Egg mass collection and maintenance

This investigation was carried out using egg masses collected by trawl from the Solent during January and February 2011 and 2012 (Chapter 2, Section 2.2 and Table 2.2). Upon collection, three capsules from each egg mass were dissected and their contents examined to confirm no development had occurred. Each mass was also examined non-invasively to check for any development (Chapter 2, Section 2.5.2; Chapter 3, Section 3.5.1). Only egg masses without discernible embryonic development (i.e. at the egg stage) were used in the investigation.

7.4.2. Pressure tolerance during development

Each egg mass was carefully dissected into halves down the centre, from the point at which it was attached to hard substrate (Fig. 7.1). Since egg masses are laid from the point of attachment upwards, this ensured that both halves contained egg capsules of an equal age. Each egg mass half was then exposed to either the control or the experimental treatment. In total, 3 egg masses were used for each treatment.

Experimental treatments were carried out using the IPOCAMP pressurised incubator (Chapter 2, Section 2.7.2 and Fig. 2.7).

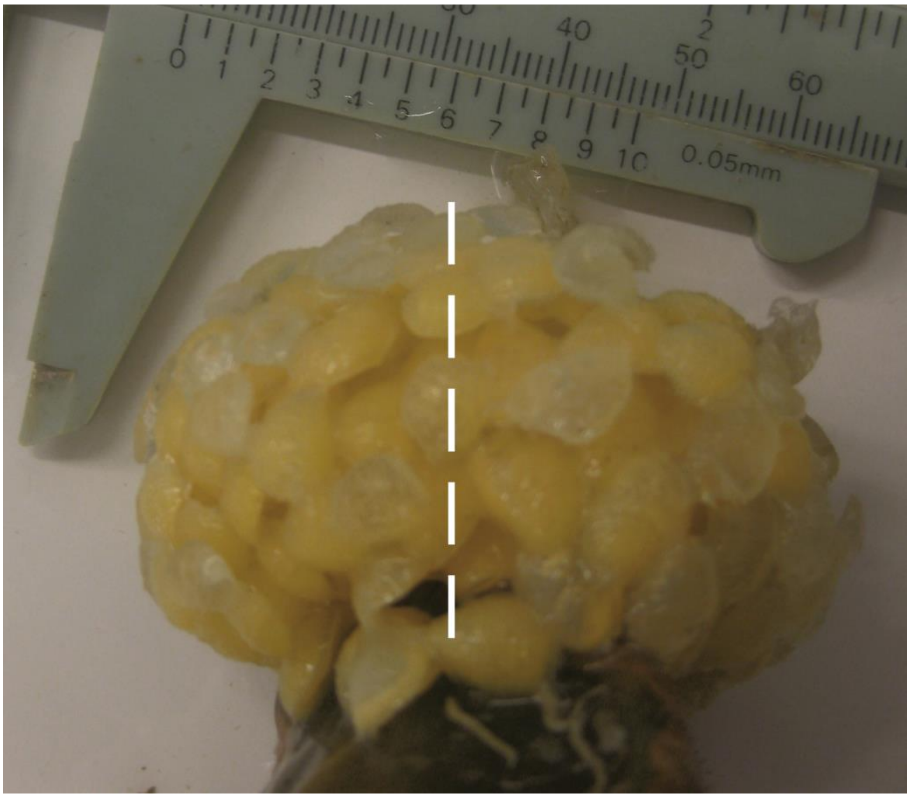

Figure 7.1. *Buccinum undatum* egg mass attached to a rock, illustrating the point at which it was dissected into two halves in order to ensure each half contained eggs of an equal age. White line indicates dissection point.

Control treatments were maintained independently at 10 °C (Chapter 2, Section 2.3). Each control egg mass half was examined non-invasively each day in order to establish development (Chapter 2, Section 2.5.2). Egg capsules were maintained until all nurse eggs had been consumed and only developing embryos remained. Once all three control egg masses had reached this developmental stage, the treatment was ended. The 1 atm experimental treatment was carried out to establish that incubation in the IPOCAMP had no direct effect on development. Since exact age of each egg mass was unknown, and based only on developmental stage, experimental duration varied, from 20 days at 1 atm, to 18 days at 100 and 200 atm, to 14 days at 300 atm.

Pressure was released from the IPOCAMP at the same rate at which it had been pressurised (10 atm pressure decrease every 10 min) (Chapter 2, Section 2.7.2). Following depressurisation, the control and experimental halves of each egg mass were compared. Ten randomly selected capsules were dissected from each half, and their contents examined. For each capsule, the number of embryos (normal and abnormal) was counted, and their developmental stage was assessed. From this, the proportion of embryos at each stage was calculated. The developmental age of each egg mass was then estimated relative to known developmental rates for *B. undatum* at 1 atm, 10 °C (Chapter 4, Fig. 4.1 and Table 4.1).

7.4.3. Dry weight and elemental composition

From the 10 dissected capsules, 20 embryos at the veliger stage of development were individually sampled at random to determine dry weight (Chapter 2, Section 2.8.2). Since weight and bioenergetics content change with development (Chapter 4), only embryos at the veliger stage of development were used to compare these variables (Chapter 3, Section 3.5.1). A total of 60 embryos were sampled from each control treatment and each experimental treatment.

In order to examine embryo bioenergetics, elemental analysis was carried out on 10 control treatment and 10 experimental treatment veligers from each high pressure condition (Chapter 2, Section 2.8.3). Samples were selected randomly from the freeze-dried individuals. Percentages of carbon (C) and nitrogen (N) were determined during analysis. The C and N biomass for each individual was then calculated using from the C:N ratio and the DW.

7.4.4. Data analysis

Between egg masses, age varied to some degree and capsule size was not standardised due to availability of material; the latter factor can impact number and weight of offspring (Chapter 3). For each experimental treatment, egg masses were therefore only directly comparable to the related control (1 atm) treatment. Each comparison of control and experimental treatment was therefore analysed independently.

A one-way analysis of variance (ANOVA) was used to compare number of embryos *per* capsule, embryo dry weight, C biomass, N biomass and C:N ratio, of control and experimental treatments. For number of embryos *per* capsule $n = 30$ for each ANOVA. For embryo dry weight $n = 60$ for each ANOVA. For C biomass, N biomass and C:N ratio $n = 10$ for each ANOVA.

Table 7.1. Percentage of *Buccinum undatum* embryos which have developed to or beyond independent developmental stages, at 10 °C under different pressure treatments. Under each experimental condition the two pressures recorded are directly comparable. Each estimate is based on three egg masses developed in parallel.

Experimental Condition	Pressure (atm)	Percentage of embryos reaching developmental stage			
		Trochophore	Early veliger	Veliger	Pediveliger
1 atm	1	100	100	96.7	61.7
	1	100	100	86.7	56.7
100 atm	1	100	100	71.9	25.4
	100	100	100	56.7	11.7
200 atm	1	100	100	98.3	56.4
	200	100	100	79.6	5.0
300 atm	1	100	100	71.7	48.2
	300	1.7	0.0	0.0	0.0

7.5. Results

Due to the low developmental success observed in 300 atm experimental egg masses, statistical analyses were carried out on data from 1, 100 and 200 atm samples only.

7.5.1. Developmental success

Embryos developed successfully in the IPOCAMP at pressures of 1, 100 and 200 atm. At 300 atm, no development was observed except for in two individuals found inside one egg capsule, both of which had reached the trochophore stage. All control treatment egg masses (1, 100, 200 and 300 atm) developed at a similar and expected rate (Fig. 7.2, Table 7.1), based on previously established developmental rates (Chapter 4, Table 4.1). Analysis by ANOVA indicated there was no difference in the number of embryos developing *per* capsule in each condition (control or experimental) at 1, 100 or 200 atm (Fig. 7.4; Table 7.2).

7.5.2. Rate of development

In all egg masses developed at 1, 100 and 200 atm (control and experimental), and in all control egg masses for 300 atm treatment at least some individuals reached the pediveliger stage (see Smith and Thatje 2013). Within each treatment, all experimental egg masses developed at a similar rate. An increasing lag in development was, however, observed with increasing pressure (Fig. 7.2 and 7.3; Table 7.1). Using developmental timings from Smith et al. (2013), development was estimated to be delayed by approximately 3 days in 100 atm experimental treatments, relative to expected 1 atm developmental rates. In 100 atm experimental treatments, 15 % less embryos reached the veliger stage and 13 % less reached the pediveliger stage. At 200 atm, an approximate 6 day lag in development was observed; 18 % less embryos reached the veliger stage and 51 % less reached the pediveliger stage (Table 7.1).

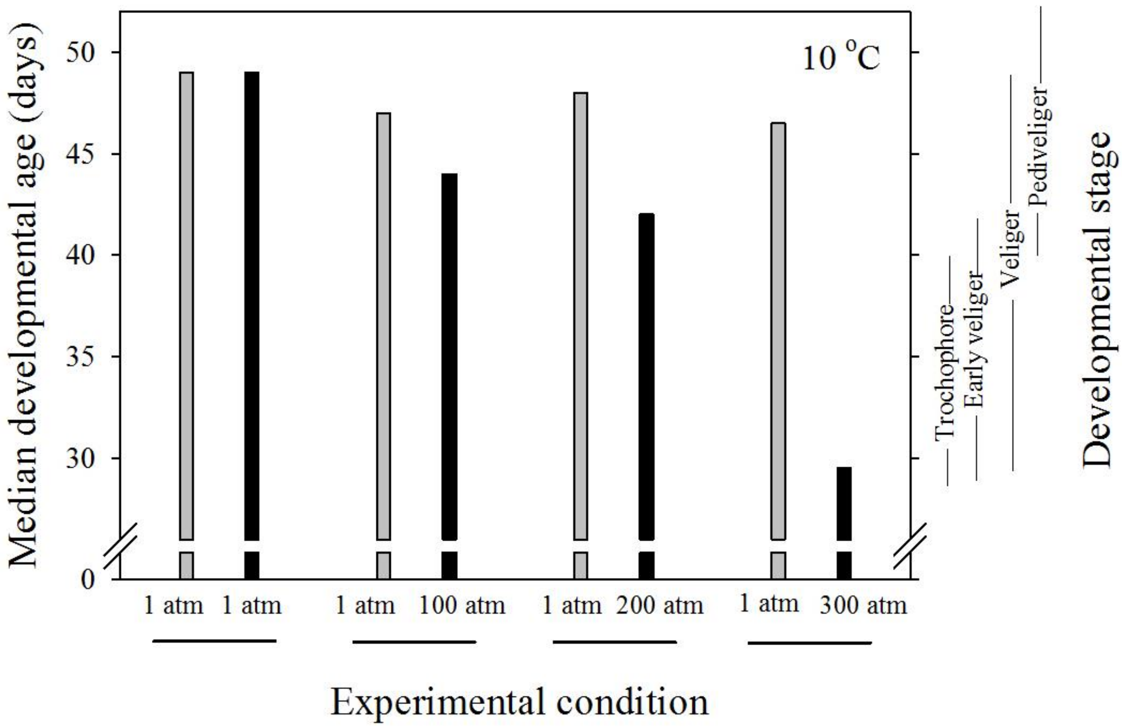

Figure 7.2. Estimated age of *B. undatum* egg masses as determined from masses previously developed at 10 °C, 1atm (Smith et al. 2013). All egg masses are developed at 10 °C but under different pressure treatments. Only results from treatments linked on the x-axis by bars are directly comparable as these estimates are taken from halves of the same egg mass. The age during which each developmental stage was expected to be observed in an egg mass is indicated.

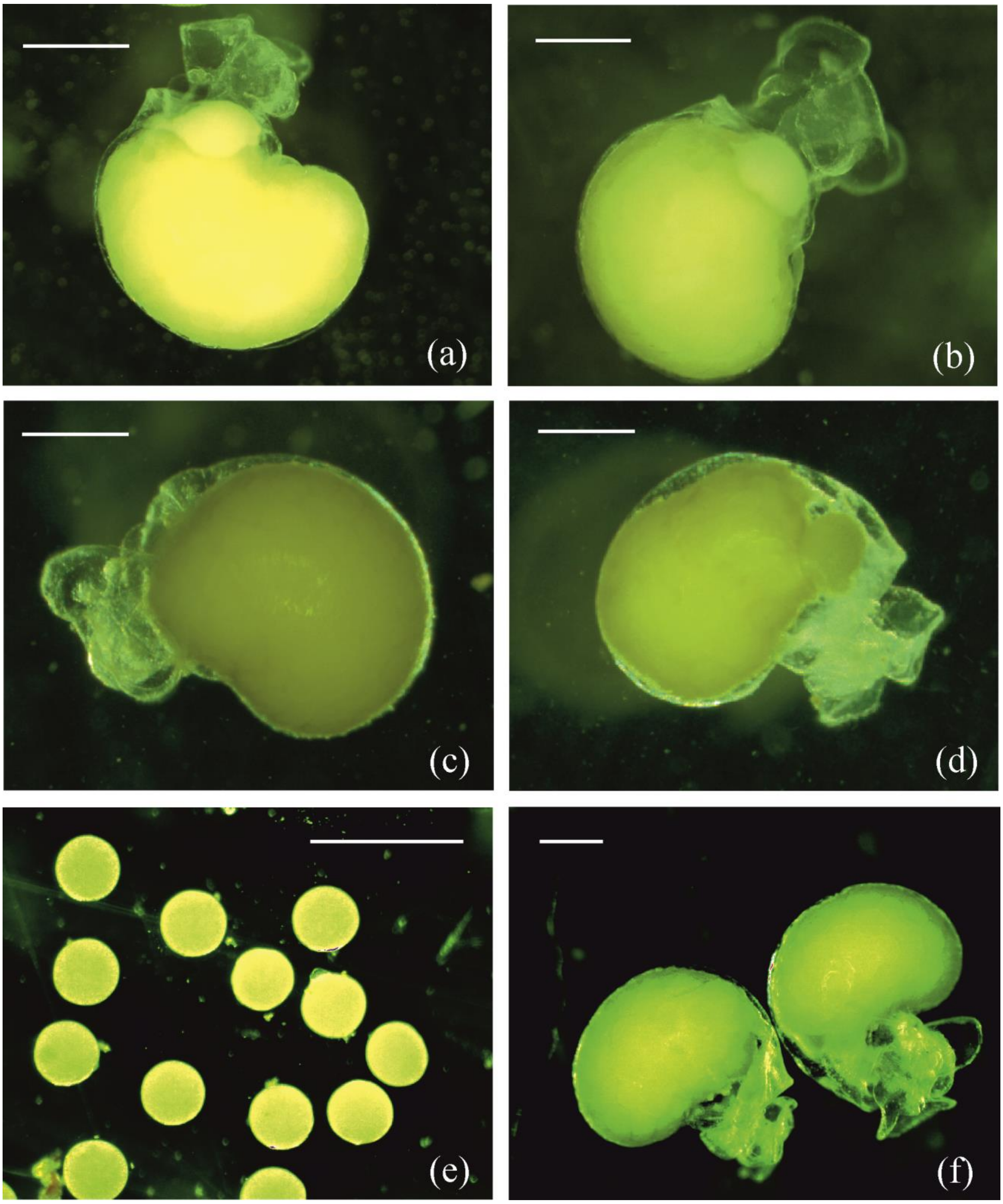

Figure 7.3. Typical *B. undatum* embryos developed under different pressure treatments at 10 °C. (a) 100 atm; veliger. (b) 1 atm; late veliger; control for 100 atm treatment. (c) 200 atm; veliger. (d) 1 atm; early pediveliger; control for 200 atm treatment. (e) 300 atm; egg. (f) 1 atm; pediveliger; control for 300 atm treatment. Each horizontal pair of images are from each half of a single egg mass and are therefore directly comparable because the egg mass age is identical. Images represent typical developmental stages observed at the end of a treatment. Scale bars represent 500 µm.

Table 7.2. Results of analysis by one-way ANOVA for *B. undatum* egg masses developed at 10 °C under different pressure treatments. Each analysis was a direct comparison between control (developed at 1 atm) and experimental (developed under given pressure) egg masses. No analysis was carried out on 300 atm samples due to a lack of development in the experimental condition at this pressure. Analysis of dry weight, C biomass, N biomass and C:N ratio were carried out on individuals sampled at the veliger stage only. For each figure, number of samples (*n*) is indicated. F statistic and p values are shown. Significance levels are indicated by asterisks; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Variable	1 atm			100 atm			200 atm		
	n	F	p	*n*	F	p	*n*	F	p
Number of embryos	(30)	1.20	0.227	(30)	0.00	0.977	(30)	0.32	0.573
DW	(60)	0.01	0.912	(60)	0.86	0.356	(60)	15.34	≤ 0.001***
C µg	(10)	0.14	0.713	(10)	0.59	0.451	(10)	9.55	0.007**
N µg	(10)	0.13	0.721	(10)	0.58	0.455	(10)	9.46	0.006**
C: N	(10)	0.35	0.562	(10)	0.03	0.857	(10)	3.75	0.069

7.5.3. Dry weight and elemental analysis

Due to the low developmental success observed in 300 atm experimental egg masses, no elemental analysis was carried out on these samples. Analysis by ANOVA indicated there was no significant effect of pressure on DW, C biomass, N biomass or C:N ratio at 1 or 100 atm ($p > 0.05$). At 200 atm, ANOVA indicated DW ($p \leq 0.001$), and C and N biomass ($p \leq 0.01$) to be negatively affected by pressure. No effect of pressure was observed on C:N ratio at 200 atm (Fig. 7.5, Table 7.2 and 7.3).

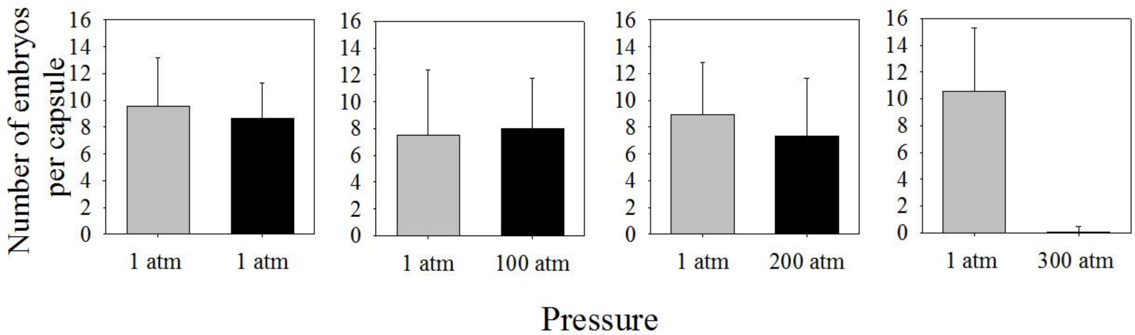

Figure 7.4. Differences in number of embryos developing *per* capsule in *B. undatum* egg masses developing under control (grey) or experimental (black) conditions. Within each plot, control and experimental bars represent halves of the same egg mass. Only the control and experimental treatment bars inside each separate plot are directly comparable. All egg masses were developed at 10 °C. For each bar, *n* = 30. Error bars indicate 1 S.D.

7.6. Discussion

7.6.1. The cost of development under high hydrostatic pressure

Recent technological advances have allowed us the opportunity to investigate the direct effects of pressure on development over considerably longer periods than have previously been achievable (Shillito et al. 2001). This has enabled us, for the first time, to gain insight into the true effects of pressure on the development of a shallow-water species. Short-term studies of 24 to 50 h have previously allowed thresholds in pressure tolerance to be determined for fertilisation (Mestre et al. 2009) or early development (Young et al. 1997; Tyler et al. 2000; Benitez-Villalobos et al. 2006; Aquino-Souza et al. 2008; Mestre et al. 2012). These investigations have indicated a reduction in developmental rate with increased pressure, but they have not revealed the costs associated with sustained exposure to pressure during development, nor whether development *per se* is achievable under pressures outside the species bathymetric distribution. This study confirms a pressure-related decrease in rate of development with retardation observed at 100 atm. Additionally, my results provide evidence of an increase in metabolic expenditure during development with pressure, shown through reduction in dry weight and bioenergetic content. In comparison, 4 h pressurisations of *B. undatum* during development at 10 °C have indicated a pressure tolerance of at least 400 atm in veliger and of 200 atm in juveniles (Smith and Thatje 2012). The short duration of past studies give a much coarser interpretation of pressure thresholds than was achieved during the prolonged exposures used in the present investigation.

The increase in energy demand with depth, indicated by my data, imply a reduced likelihood of successful development under high pressure. When examining bioenergetic content, carbon represents nutritional reserves and nitrogen represents changes in structure and metabolic machinery (Anger 2001). Typically, during lecithotrophic development a shift in C:N ratio is observed as nutritional reserves are depleted and structure and metabolic machinery develop. In the present investigation, the absence of a shift in C:N ratio, despite the reduction in dry weight and both C and N, suggests greater reserves are being used under pressure but without a simultaneous increase in growth structural complexity. A similar rise in energetic use has been

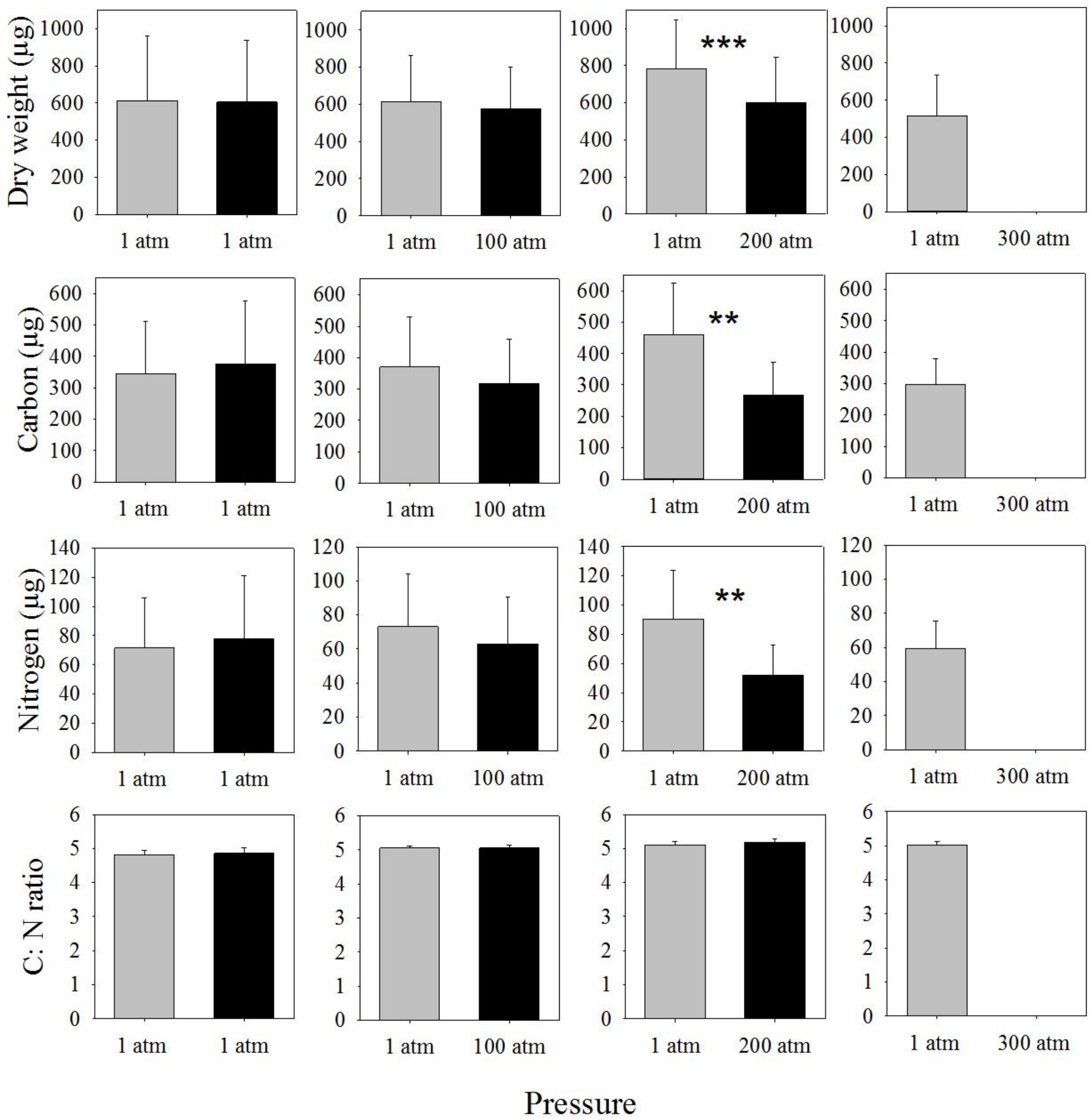

Figure 7.5. Differences in embryo dry weight (µg), carbon (C) and nitrogen (N) biomass, and C:N ratio of *B. undatum* veligers developing under control (grey) or experimental (black) conditions. Within each plot, control and experimental bars represent halves of the same egg mass. Only the control and experimental treatment bars inside each separate plot are directly comparable. All egg masses were developed at 10 °C. For each bar, n = 60 for dry weight and n = 10 for all other variables. Error bars indicate 1 S.D. Significant differences between veliger (V) samples (ANOVA), are indicated in each plot by asterisks; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 7.3. Dry weight (DW), carbon (C), nitrogen (N) and C: N ratio values for *B. undatum* veligers developed at 10 °C under different pressure treatments. Under each experimental condition the two pressures recorded are directly comparable. For each figure, *n* = 60 (dry weight) or *n* = 10 (C biomass, N biomass, C: N ratio). Samples were taken uniformly from three egg masses.

Experimental condition	Pressure (atm)		DW (*n* = 150)		C (*n* = 24)		N (*n* = 24)		C: N mass ratio (*n* = 24)	
			$\bar{x}$	±S.D.	$\bar{x}$	±S.D.	$\bar{x}$	±S.D.	$\bar{x}$	±S.D.
1 atm	1	µg/ind	612.65	346.27	344.71	167.47	71.56	34.45	4.81	0.13
		% of DW	-	-	51.04	1.08	10.61	0.30	-	-
	1	µg/ind	605.81	331.00	375.61	201.20	77.87	42.99	4.85	0.18
		% of DW	-	-	51.82	1.06	10.69	0.33	-	-
100 atm	1	µg/ind	613.97	248.34	369.95	159.52	73.04	31.21	5.05	0.07
		% of DW	-	-	51.80	0.42	10.25	0.19	-	-
	100	µg/ind	574.18	225.29	318.27	139.93	62.96	27.71	5.05	0.10
		% of DW	-	-	51.54	1.42	10.21	0.29	-	-
200 atm	1	µg/ind	781.88	263.23	459.16	165.99	90.29	33.45	5.09	0.11
		% of DW	-	-	51.73	0.96	10.15	0.33	-	-
	200	µg/ind	599.27	247.26	268.20	103.13	51.97	20.83	5.19	0.10
		% of DW	-	-	51.88	1.15	10.00	0.27	-	-
300 atm	1	µg/ind	513.63	221.03	297.83	82.72	59.42	16.02	5.01	0.10
		% of DW	-	-	53.28	1.16	10.64	0.17	-	-
	300	µg/ind	-	-	-	-	-	-	-	-
		% of DW	-	-	-	-	-	-	-	-

observed in gastropods and crustaceans in response to increased temperature during development. This has been shown through an increase in lipid use (García-Guerrero et al. 2003) and metabolic rate (Cancino et al. 2011), and a reduction in embryo size (Fernández et al. 2006). In these examples the effect was evident throughout development and resulted in reduced offspring survival. In *B. undatum* I infer that the observed increase in metabolic expenditure will continue with sustained duration of exposure to high pressure, rapidly reducing the amount of available reserves. In addition to this, I predict the observed lag in development to scale up with duration of exposure to high pressure. A similar delay in development has previously been observed in gastropods in response to hypoxia (Cancino et al. 2003; Fernández et al. 2006; Brante et al. 2009). Under this scenario, the lag in development increased with time (Brante et al. 2009).

A simultaneous increase in developmental time and use of energetic reserves will ultimately result in a mismatch of energetic supply and demand, as the amount of energy necessary for development exceeds the available reserves. Successful development will be increasingly reduced until the effects of pressure become lethal. This may occur at lower pressures than that at which development is prohibited completely. I suggest pressure imposes a physiological limit on bathymetric distribution. Hydrostatic pressure must therefore be considered a significant variable to which shallow-water species must adapt in order to expand their range limits into the deep ocean. Understanding such evolutionary adaptations may give insight into both current and ancestral distribution patterns.

7.6.2. Evolutionary adaptations for high hydrostatic pressure?

Bathymetric patterns in species diversity typically demonstrate a unimodal trend, the peak of which coincides with an area of high species turnover (Etter and Grassle 1992; Carney 2005; Menot et al. 2010). This peak has been reported globally at depths ranging 1000 to 1700 m, with additional smaller peaks occurring below 2000 m (Cartes 1993; Gage et al. 2000; Hayward et al. 2002). The main area of high faunal change indicates a general limit in the distribution of shallow water fauna and upper bathyal biota; only species, which are adapted to tolerate the conditions of the deeper environment, are capable of further depth penetration (Brown and Thatje 2011). Bathymetric trends already recognised within the oceans may therefore represent evolutionary adaptations to depth, which have evolved in order for invading shallow-water species to tolerate the high pressure conditions typical of the deep sea (e.g. Somero 2003). Considering this, the increased egg size or energetic investment *per* egg observed with increasing depth (e.g. Thorson 1950; Van Dover and Williams 1991; Young 2003) may have evolved to compensate for the increased energetic cost associated with development under high pressure. Under this scenario, a large egg may allow successful development at hydrostatic pressures beyond the previous bathymetric limit of a species. The low metabolic rate typical of deep-sea environments may also facilitate this by reducing the energetic requirements necessary for everyday functionality. This provides an alternative explanation for the trend of increased egg size reported in galatheid and lithodid crabs, at depths below 1000 m and in isothermal waters (Van Dover and Williams 1991; Morley et al. 2006). Furthermore, this rationalises the similarity in egg size, observed on and off hydrothermal vents.

In addition to an increased egg size, patterns of abbreviated larval development have also been noted in both high latitude and deep-sea environments, (e.g. Wilson and Hessler 1987; Anger et al. 2003; Lovrich et al. 2003; Thatje et al. 2003, 2004; Buhl-Mortensen and Hoeg 2006). Development *per se* is an energetically expensive process with some aspects, for example moult cycles in Crustacea, being more costly than others. Abbreviated larval development reduces the cost of this process by decreasing the number of ontogenetic stages involved with development. This adaptation, while beneficial for the low temperature and low food availability scenarios it is typically attributed to, may also be valuable in mediating the increased energy demand associated with development under high pressure. Paedomorphosis, where an individual completes its sexual life cycle without metamorphosing into its adult form (e.g. Messing 1984; Rundell and Leander 2010), is occasionally observed in deep-sea invertebrates. Redundancy of metamorphosis will presumably reduce the energetic cost required to reach maturity, and paedomorphosis may therefore be a further energy saving mechanism occurring, in part, in response to high hydrostatic pressure.

Facilitating the migration of a sensitive life stage to a tolerable environment may be an alternative method of adaptation in deep-water species. Patterns of ontogenetic vertical migration (OVM), whereby deep-water species transport eggs or larvae to shallow water to complete development, have been reported in a range of crustaceans (Kelly et al. 1982; Conover 1988; Attrill et al. 1990; Durbin et al. 1995; Kobari and Ikeda 1999, 2001; Padmavati et al. 2004; Yoshiki et al. 2011), echinoderms (Young and Cameron 1987; Cameron et al. 1988) and molluscs (Killingley and Rex 1985; Bouchet and Warén 1979, 1994). These patterns are typically attributed to an increase in temperature and food availability in shallow water. I suggest hydrostatic pressure to be an additional factor explaining these migrations; shallow water development of a deep-water species avoids the energetic cost of development under high pressure, while simultaneously allowing dispersal by water movement. This scenario reduces both the energetic expenditure and the duration of development otherwise expected in a deep-water species. Evolutionary adaptations observed in a species, can give important clues to ancestral origin. Patterns of OVM point toward a shallow-water origin for the species employing this strategy, supporting previous proposals of deep-water invasions by shallow-water species. Such an adaptation may allow a species to migrate to deep water while retaining the greater fecundity typical of shallow water. Although this may enable successful development without additional maternal investment *per* egg, the increased cost of maternal or offspring vertical migration should be considered, together with the potential loss of offspring during time in the plankton. The proposal that OVM is an adaptation to high pressure may, however, explain why migration patterns are not always synchronised to surface conditions (Kobari and Ikeda 2001; Kobari et al. 2010). It may also justify the low pressure tolerance observed in the eggs of some species following this strategy. Typically eggs are released at depth before being exported to the surface for development, but in at least one species of copepod, eggs are unable to tolerate pressures native to adults from the same population and females migrate to the surface to spawn (Yoskiki et al. 2011). Additionally, several species of decapod crustacean have been reported to perform up-slope movement into shallower water at maturity (Kelly et al. 1982; Attrill et al. 1990). The implication that certain developmental stages may have a historical intolerance of high pressure also supports hypotheses of a shallow-water ancestry for many deep-sea species. Although high pressure has never been used before as an explanation for OVM, the idea of an ontogenetic shift in pressure tolerance has previously been proposed to explain patterns of reverse OVM noted in Antarctic krill, where eggs sink and develop at depth (George 1984).

7.6.3. Conclusions

The present study provides evidence that hyperbaric conditions impose an increase in the energetic requirements necessary for successful development in a marine invertebrate. This implies that where a mismatch between energy supply and metabolic cost ensues; the resulting metabolic shortfall will limit the bathymetric distribution of shallow-water species. Previous work comparing shallow-water and deep-sea species has inferred that evolutionary adaptations are dictated by thermal gradients in the water column (e.g. Childress et al. 1990; Thuesen and Childress 1994; Childress 1995). While it is clear that decreasing temperature with depth requires adaptation, I show that hydrostatic pressure is also a significant factor to which species must adapt in order to tolerate deep-sea conditions. These results lead us to conclude that inflated metabolic cost is a consequence of increased hydrostatic pressure during development, and possibly persists throughout other life-history stages. I suggest that this factor is fundamental in determining bathymetric distribution and consequently is a significant evolutionary hurdle for the transition of species from shallow-water to deep-sea.

8. Synopsis

8.1. Summary of findings

This thesis has achieved the most in-depth study to date regarding investigation into the combined temperature and pressure tolerance of a marine invertebrate during early ontogeny (Table 8.1). This includes the first study of its kind to consider a non-planktotrophic reproductive mode, the longest exposure to high pressure ever achieved during development and the first direct evidence of an increased energetic expenditure associated with development under high hydrostatic pressures. Additionally, I have investigated a diverse effect of intracapsular development and described a novel example of sibling cannibalism in the study species *Buccinum undatum* (Linnaeus 1758). In order to integrate the principal findings of the preceding five chapters, I summarise the conclusions of these works with reference to the objectives and hypotheses expressed in the introduction.

<u>**Objective 1.**</u> **Investigate the full intracapsular development of *B. undatum*, describe ontogenetic stages and assess developmental timing.**

<u>Conclusions</u>

- At 6 °C, complete intracapsular development took between 133 and 140 days.
- Seven independent ontogenetic stages were identified during encapsulation; these comprised of egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile and juvenile.
- Nurse egg consumption was very rapid, resulting in large intracapsular variations in the number of eggs consumed by each embryo, and consequently, their size or dry weight.
- ‘Empty’ embryos were regularly observed, which had not successfully consumed any nurse eggs and did not complete development.
- ‘Accidental’ cannibalism was occasionally observed, whereby one embryo ingested another, presumably prior to initial egg development. The result of this ‘trojan horse’ scenario was that the consumed embryo survived but the consumer did not.

<u>Objective 2.</u> **Examine the full thermal tolerance range of *B. undatum* for development, at the south-eastern end of the species distribution.**

<u>Conclusions</u>

- Successful development was observed across a thermal range spanning 6 to 18 °C.
- Across these temperatures, complete intracapsular development took between 49 and 140 days.
- Between 6 and 18 °C, rates of development increased with temperature, allowing hypothesis 1 (developmental rate will increase positively with temperature in *B. undatum* egg masses) to be accepted.
- Developmental success decreased with increasing temperature.
- As temperature increased, number of early veligers developing *per* capsule decreased and mean early veliger weight increased. At the same time, the proportion of 'empty' embryos *per* capsule increased.
- The bioenergetic cost of development increased with temperature.

<u>Objective 3.</u> **Interpret the combined effects of temperature and pressure on development in *B. undatum*, using egg masses collected from across the species range and acclimated to temperatures, which are ecologically relevant throughout the species distribution.**

<u>Conclusion</u>

- Pressure tolerance decreased with increasing ontogenetic stage, allowing hypothesis 2 (*Buccinum undatum* embryos will be less tolerant of high hydrostatic pressure at later development stages, when complexity is greater) to be accepted.
- In veliger, no effect of pressure was observed at 400 atm (equivalent to 4000 m).
- In juveniles, significant detrimental effects of pressure were observed at 200 atm at 10, 14 and 18 °C. This increased to a tolerance exceeding 400 atm at 3 °C.
- Population level differences indicated acclimation to low temperature to increase pressure tolerance, allowing hypothesis 3 (thermal acclimation will result in an increase in pressure tolerance in *B. undatum* embryos with decreasing temperature) to be accepted.

Objective 4. Analyse the effects of increased duration of exposure to high pressure on development.

Conclusions

- Egg masses were exposed to high-pressure treatments for between 16 and 20 days.
- Successful development was observed at pressures of 200 atm, but not 300 atm.
- There was no effect of pressure on the number of embryos developing *per* capsule.
- At 200 atm, a significant decrease in embryo weight was observed relative to 1 atm, indicating a greater metabolic cost for development.
- A lag in development was observed from 100 atm indicating high pressure to have a retarding effect. This subtle effect of pressure allowed hypothesis 4 (an increase in energetic expenditure during development will be observed with increasing pressure in *B. undatum* embryos) to be accepted.

8.2. The importance of experimental duration and acclimation

Physiological tolerances are often determined through periods of exposure lasting only hours. My results highlight subtle differences in tolerance levels, which may only be perceived through long experimental periods. Short experiments identify significant responses to 'acute' exposures, but the subtle effects of 'moderate' exposures (for example retarded growth) are not evident over short periods. These instead become increasingly apparent with time and advance in complexity, highlighting the importance of experimental longevity. In this thesis this is evident with regard to both temperature and pressure. For example, embryos survived at temperatures of 0 °C for approximately 9 months, but did not develop successfully during this period (Chapter 4). If the thermal exposure to 0 °C had been shorter, the detrimental effects of low temperature on development may not have become evident. Additionally, the long-term pressure experiments (Chapter 7) indicated embryo development to be significantly affected by pressure at 200 atm. During a shorter experiment, the reduction in weight and bioenergetic content of embryos may not have been identified. Conversely, although I do not expect development to be lethally impaired at 100 atm (10 °C), it is possible that if the small lag in development seen at this pressure escalated, under longer experimental duration, a significant effect of pressure may have been observed at 100 atm. Essentially, long experiments allow for a period of acclimation. This is an ecologically relevant consideration in experiments, which has previously been shown to both increase (Ravaux et al. 2012) and reduce (Peck et al. 2009) physiological tolerance levels. Increased experimental duration can therefore be assumed to provide a more accurate and realistic response, which may be essential for understanding ecological implications.

Previous studies examining the physiological effects of temperature and pressure have indicated an increase in pressure tolerance with increasing temperature. In contrast, my results show the opposite effect, with low temperature alleviating the effects of high pressure. I propose this trend was observed as a result of the long temperature acclimation that each egg mass was subjected to during development. Considering the comparable adaptations previously reported in response to both low temperature and high pressure (e.g. Somero 1992; Pradillon and Gaill 2007), I suggest a period of acclimation to one factor has the potential to increase an organism's tolerance of the other. During development, new tissue is being rapidly generated and organism plasticity may be at its most flexible within a tolerance range. I propose, therefore, that acclimation to cold temperatures during development, or over multiple generations across the species range, allow minor adaptations to occur which in turn increase pressure tolerance. For example, a common adaptation to both low temperature and high pressure is an increase in membrane fluidity in order to retain cellular function (e.g. Somero 1992; Pradillon and Gaill 2007). This is accomplished through a shift in the ratio of unsaturated to saturated fatty acids. If such a shift occurs to some level during acclimation, to compensate for low temperature, this may in turn increase tolerance of high pressure.

8.3. The capacity for phenotypic plasticity in *Buccinum undatum*: potential for range expansion?

The results of the studies reported in this thesis indicate that during prolonged periods of exposure, *B. undatum* is capable of tolerating both pressures and temperatures outside the range it is exposed to across its current distribution. This suggests that neither of these factors should be limiting in the species current distribution, therefore implying this species to be theoretically capable of range expansion. When these results are examined in detail however, a number of points emerge. A realistic perspective cannot be achieved without considering these aspects collectively.

8.3.1. Limited potential for increased thermal scope?

The thermal tolerance range observed during development indicates *B. undatum* to have the capacity to develop under temperatures, which are ecologically relevant to both lower latitude and deep-sea environments. At increased temperatures typical of lower latitude environments, the rapid nurse egg consumption observed in the common whelk is of significant ecological consequence. If nurse egg consumption rates, and therefore intracapsular competition, are environmentally mediated, as the effects of temperature indicate them to be, then these traits have the capacity to assist, or hinder the species range shift potential. Environmental conditions (such as increased temperature) which promote growth, result in higher levels of competition occurring within an egg capsule. Consequently, earlier developing siblings, which have a competitive advantage, succeed over later developing siblings. When this is combined with the reduction in number of developing embryos observed under the same conditions, the result is a smaller number of larger embryos, each with greater bioenergetic reserves. The consequences of this life history trait are lower relative fecundity, but an increased chance of survival for offspring. This strategy facilitates the increased energetic demands associated with development at high temperature in *B. undatum*, and may be successful, as long as a balance is struck between embryos being large enough to have sufficient reserves for development and plentiful enough to sustain a population. Under this scenario, this trait may be considered to increase fitness in the species.

While the thermal range for development appears relatively wide, the temperatures which are currently understood to be critical for copulation and egg laying in the common whelk, remain below 9 °C for the Solent population, and lower for other populations (Chapter 1, Fig 1.8). In the Solent, this leaves a narrow window over which the complete reproductive cycle can take place and under warmer conditions, it is likely that copulation may not occur. Certainly in this area, I observed an apparent reduction in reproductive success with increasing winter temperatures. It is possible that *B. undatum* is capable of adapting to warmer temperatures over multiple generations, and increased thermal capacity is already evident between northern and southern populations of this species. The lack of presence of this genus in tropical or warm temperate waters however, (Chapter 1, Fig 1.5d) indicates that historically, it has had limited success in achieving such adaptations.

8.3.2. Bathymetric range expansions: do metabolic costs set the limit or is low temperature the key?

Typical deep-sea temperatures are within the critical thermal range for copulation and egg laying, suggesting potential for bathymetric range expansion. A further consideration for deep-sea migration however, is whether development continues to be successful at the low temperatures (average 4 °C) found below 1000 m and at the relevant pressures. The study discussed in Chapter 7 examined long-term pressure treatments carried out at 10 °C. While this temperature is characteristic of 1000 m water depth just off the UK, and therefore ecologically relevant for the Solent population of *B. undatum*, it is not typical of greater ocean depths at any latitude. The results of my short-term pressure treatments show an increase in pressure tolerance with decreasing temperature. Considering this, I infer development under high pressure will be more successful at the lower temperatures typical of higher latitudes and deep-sea environments, suggesting polar waters to be the most likely areas for bathymetric range expansion. This supports theories of high latitude invasions into the deep sea *via* cold isothermal water columns (Wilson 1980; Sepkoski 1988; Young et al. 1997; Tyler and Young 1998; Thatje et al. 2005). Considering my results, I suggest that while isothermal waters *per se* aid bathymetric migrations, polar temperatures increase the rate at which such migrations are possible.

The increased energetic expenditure observed with increased pressure, leads me to argue that metabolic cost is a limiting factor in bathymetric distribution. For any species, a point may occur when the energy required for development exceeds the available reserves. Resource allocation to embryos has often been shown to reflect environmental temperatures, but has rarely been considered to be affected by hydrostatic pressure. In contrast to this, I hypothesise developmental success to be directly impacted by pressure, suggesting this to be an important factor affecting resource partitioning. However, and taking into account a) the increase in pressure tolerance observed with decreasing temperature, identified during short-term experiments (Chapter 6), and b) the reduction in the energetic cost of development observed with decreasing temperature at 1 atm (Chapter 4), I suggest that the energetic requirements for development under high pressure, may reduce at low temperatures. Under this scenario, the increase in energetic requirements for development, between shallow- and deep-water environments, will be lower at high latitudes than at mid or low latitudes. This leads me to hypothesise that although metabolic costs may limit bathymetric distribution, the limits lie at greater depths in cold water. This, once again, indicates range expansions to be most likely to occur at high latitudes, again supporting theories of high-latitude bathymetric invasions.

8.4. Reproductive strategies in the deep sea as a function of hydrostatic pressure

The observed pressure-induced increase in energetic expenditure for development suggests that reproductive strategies reported in the deep sea may occur in part, in response to hydrostatic pressure. Trends of increasing egg size with depth observed in a range of marine invertebrates may be explained by the expected higher energetic requirements of this habitat and energy saving strategies such as abbreviated larval development and paedomorphosis (where an individual completes its sexual life cycle without metamorphosing into its adult form) may be used to reduce this cost. Additionally, patterns of ontogenetic vertical migration (OVM) may help reduce energetic expenditure during development. Considering the metabolic costs associated with development under pressure, it may be more efficient for such species to perform bathymetric migrations during their life history than to develop in deep water. Considering this, I suggest that for some deep sea species, planktotrophy facilitates range shift, not only by increasing the dispersal area of offspring, but also by allowing larvae to undergo ontogenetic migration to a shallower habitat. This also provides a means for development in species, which exhibit a lower pressure tolerance during early ontogeny. Patterns of OVM may therefore facilitate the bathymetric migration of species of shallow water origins, allowing adults to migrate, while offspring continue to exhibit shallow-water traits.

8.5. Future perspectives

Studies into physiological tolerances in invertebrates provide important clues regarding current, historical, and future ecological habitat boundaries, and offer support for theories of ancient and impending evolutionary paths. The results of this thesis have contributed significantly to understanding such eco-physiological thresholds. Several new questions have arisen, which may be addressed in future works. Addressing these questions on the same, or a closely related species, and across multiple populations of the same species, would be complementary to the results presented in this thesis, and may substantially increase our understanding. In addition, it would be beneficial to examine if the concepts put forward in this thesis are a common feature across other taxa, and how strongly they are related to the deep-water origin of Neogastropoda. As a starting point, I suggest the following future lines of research.

1. **Does reduction in developmental temperature lead to a drop in the metabolic cost associated with development under pressure?**

This question can be addressed by repeating the long-term pressure treatments carried out in Chapter 7, at a lower temperature (3 or 6 °C). Ideally this will be completed using egg masses from Iceland, or an area of equivalent water temperature.

2. **Is the complete intracapsular development process (i.e. from egg laying to juveniles hatching) possible under high pressure?**

This question could be approached by repeating the long-term pressure treatments carried out in Chapter 7, for durations of approximately 10 weeks (at 10 °C). This timing is based on the developmental timing established at 1 atm (Chapter 4), and may therefore need to be extended under high-pressure treatments. As previously, an endoscope camera could be used to estimate developmental stage inside the IPOCAMP.

3. **Can the full reproductive cycle be completed under high pressure?**

This is an important step in understanding the physiological limitations of bathymetric migration, or alternatively, the limitations which high hydrostatic pressure imposes on range limits. Due to feeding constraints of adults, it has not previously been possible to test this hypothesis. Recent advances have however been made in the development of feeding chambers, increasing the potential of future investigations of this type.

4. **Is there a physiological compensation, which occurs during exposure to low temperature, and that compensates to some level for the effects of high pressure?**

It would be interesting to examine the effects of low temperature through development on the lipid content of membranes, in order to understand what level of adaptation has occurred. As a suggestion, assessing the effects of long and short exposures to temperatures both above and below that of the normal habitat, would make an exciting comparison. Additionally, examining the effects of thermal acclimation during development, compared to acclimations of equal duration in adults, would make an interesting study.

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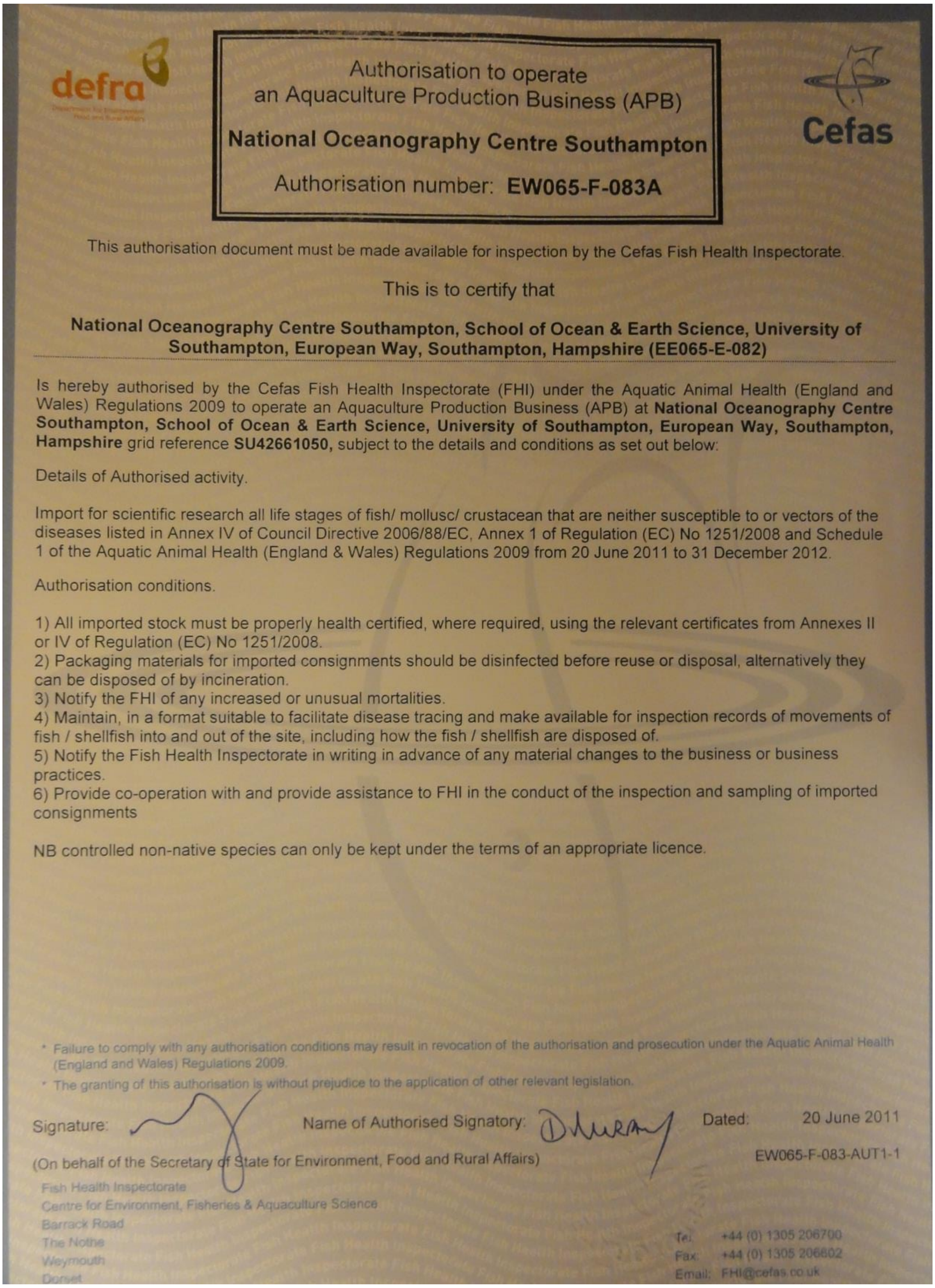
Appendix A: Authorisation to operate an aquaculture production business (APB) certificate



Authorisation to operate
an Aquaculture Production Business (APB)

National Oceanography Centre Southampton

Authorisation number: **EW065-F-083A**

This authorisation document must be made available for inspection by the Cefas Fish Health Inspectorate.

This is to certify that

National Oceanography Centre Southampton, School of Ocean & Earth Science, University of Southampton, European Way, Southampton, Hampshire (EE065-E-082)

Is hereby authorised by the Cefas Fish Health Inspectorate (FHI) under the Aquatic Animal Health (England and Wales) Regulations 2009 to operate an Aquaculture Production Business (APB) at **National Oceanography Centre Southampton, School of Ocean & Earth Science, University of Southampton, European Way, Southampton, Hampshire** grid reference **SU42661050,** subject to the details and conditions as set out below:

Details of Authorised activity.

Import for scientific research all life stages of fish/ mollusc/ crustacean that are neither susceptible to or vectors of the diseases listed in Annex IV of Council Directive 2006/88/EC, Annex 1 of Regulation (EC) No 1251/2008 and Schedule 1 of the Aquatic Animal Health (England & Wales) Regulations 2009 from 20 June 2011 to 31 December 2012.

Authorisation conditions.

1) All imported stock must be properly health certified, where required, using the relevant certificates from Annexes II or IV of Regulation (EC) No 1251/2008.
2) Packaging materials for imported consignments should be disinfected before reuse or disposal, alternatively they can be disposed of by incineration.
3) Notify the FHI of any increased or unusual mortalities.
4) Maintain, in a format suitable to facilitate disease tracing and make available for inspection records of movements of fish / shellfish into and out of the site, including how the fish / shellfish are disposed of.
5) Notify the Fish Health Inspectorate in writing in advance of any material changes to the business or business practices.
6) Provide co-operation with and provide assistance to FHI in the conduct of the inspection and sampling of imported consignments

NB controlled non-native species can only be kept under the terms of an appropriate licence.

- Failure to comply with any authorisation conditions may result in revocation of the authorisation and prosecution under the Aquatic Animal Health (England and Wales) Regulations 2009.
- The granting of this authorisation is without prejudice to the application of other relevant legislation.

Signature: Name of Authorised Signatory: D Murray Dated: 20 June 2011

(On behalf of the Secretary of State for Environment, Food and Rural Affairs) EW065-F-083-AUT1-1

Fish Health Inspectorate
Centre for Environment, Fisheries & Aquaculture Science
Barrack Road
The Nothe
Weymouth
Dorset

Tel: +44 (0) 1305 206700
Fax: +44 (0) 1305 206602
Email: FHI@cefas.co.uk

Appendix B: CEFAS AAH1 form (notification to import live fish and shellfish into England and Wales from another EU territory)

Notification to import live fish and shellfish into England and Wales from another EU territory

This form is used to notify the Fish Health Inspectorate, at Cefas, of your import. You must submit this form at least 24 hours prior to your import. Please note - a form must be completed for each individual import.

Please complete all parts of this form. The form can be returned by fax, post or email. Return address details are listed at the end of this form.

Section A – Importer's details

Authorisation number (if applicable) ☐ Name ☐

Address ☐

Postcode

Tel. No. ☐ Fax No. ☐

Section B – Nature of consignment

You may tick all boxes that are applicable to your import, eg eggs and fish.

Fish ☐ Shellfish ☐ Eggs or gametes ☐

Marine ☐ Coldwater ☐ Freshwater ☐ Tropical (no requirement to list species) ☐

Scientific name (genus and species)	Common name	Total number

Place of origin - business name and address ☐

Postcode

Section C – Place of final destination in England or Wales

Name

Address

Postcode

Tel. No.

Fax No.

Section D – Journey details

Airport of arrival

Flight No

OR

Port of arrival

Vehicle registration (if known)

Time of arrival in GB

Date of arrival in GB

Date of arrival at final destination

Time of arrival at final destination

Signature

Name in block letters

Date

Contact details

Fish Health Inspectorate
Cefas
Barrack Road
Weymouth
Dorset
DT4 8UB

Telephone: 01305 206700
Fax: 01305 206602
Email: fhi@cefas.co.uk

Appendix C: *Reprint* Smith KE, Thatje S (2013) Nurse egg consumption and intracapsular development in the common whelk *Buccinum undatum* (Linnaeus 1758). Helgoland Marine Research 67:109-120

Nurse egg consumption and intracapsular development in the common whelk Buccinum undatum *(Linnaeus 1758)*

Kathryn E. Smith & Sven Thatje

Helgoland Marine Research

ISSN 1438-387X
Volume 67
Number 1

Helgol Mar Res (2013) 67:109-120
DOI 10.1007/s10152-012-0308-1

Springer

Helgol Mar Res (2013) 67:109–120
DOI 10.1007/s10152-012-0308-1

ORIGINAL ARTICLE

Nurse egg consumption and intracapsular development in the common whelk *Buccinum undatum* (Linnaeus 1758)

Kathryn E. Smith · Sven Thatje

Received: 29 November 2011 / Revised: 12 April 2012 / Accepted: 17 April 2012 / Published online: 3 May 2012

Abstract Intracapsular development is common in marine gastropods. In many species, embryos develop alongside nurse eggs, which provide nutrition during ontogeny. The common whelk *Buccinum undatum* is a commercially important North Atlantic shallow-water gastropod. Development is intracapsular in this species, with individuals hatching as crawling juveniles. While its reproductive cycle has been well documented, further work is necessary to provide a complete description of encapsulated development. Here, using *B. undatum* egg masses from the south coast of England intracapsular development at 6 °C is described. Number of eggs, veligers and juveniles per capsule are compared, and nurse egg partitioning, timing of nurse egg consumption and intracapsular size differences through development are discussed. Total development took between 133 and 140 days, over which 7 ontogenetic stages were identified. The number of both eggs and veligers were significantly related to capsule volume, with approximately 1 % of eggs developing per capsule. Each early veliger consumed nurse eggs rapidly over just 3–7 days. Within each capsule, initial development was asynchronous, but it became synchronous during the veliger stage. No evidence for cannibalism was found during development, but large size differences between embryos developing within each capsule were observed, and occasionally 'empty' veligers were seen, which had not successfully consumed any nurse eggs. These results indicate a high level of competition for nurse eggs within each capsule during development in the common whelk. The initial differences observed in nurse egg uptake may affect individual predisposition in later life.

Keywords Intracapsular development · *Buccinum undatum* · Nurse egg partitioning · Competition · Reproduction

Communicated by Martin Thiel.

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Introduction

Many marine gastropods undergo intracapsular development inside egg capsules (Thorson 1950; Natarajan 1957; D'Asaro 1970; Fretter and Graham 1985). Embryos develop within the protective walls of a capsule that safeguards against factors such as physical stress, predation, infection and salinity changes (Thorson 1950; Pechenik 1983, 1999; Strathmann 1985; Rawlings 1995, 1999). Periods of encapsulation vary; some species are released as veligers and undergo a planktonic stage before reaching adult life (mixed development), while others display direct development, hatching from capsules as crawling juveniles (Natarajan 1957; D'Asaro 1970; Pechenik 1979). When direct development occurs, embryos are often accompanied in a capsule by nurse eggs, non-developing food eggs, which provide nutrition during development (Thorson 1950; Spight 1976b; Rivest 1983; Lahbib et al. 2010). These are usually indistinguishable from embryos in the very early stage of ontogeny and are consumed during development, potentially increasing size of juveniles at hatching (Thorson 1950). In some species, nutrition may also be provided by intracapsular fluid or protein from capsule walls (Bayne 1968; Stöckmann-Bosbach 1988; Moran 1999; Ojeda and Chaparro 2004).

Generally speaking, nurse egg consumption occurs over a period of several weeks or months. It commences some weeks into development as embryos form, and nurse eggs are then slowly consumed throughout much of development (Chaparro and Paschke 1990; Ilano et al. 2004; Lahbib et al. 2010). The number of nurse eggs consumed during this period varies across species. Ratios range from 1.7 nurse eggs per embryo in the Pacific shallow-water muricid *Acanthinucella spirata* (Spight 1976a), to between 50,000 and 100,000 nurse eggs per embryo in the North Atlantic deep-sea buccinid *Volutopsius norwegicus* (Thorson 1950). Often, within a species, the nurse egg to embryo ratio varies from capsule to capsule within one clutch (Thorson 1950; Spight 1976a). For example, Rivest (1983) found this ratio in the buccinid *Lirabuccinum dirum* to vary from 11 to 46 across capsules. Similar differences have been reported for other gastropods (Natarajan 1957; Spight 1976a). Within a capsule however, there is usually little variation in the number of nurse eggs ingested by each embryo, with all embryos generally being equal in their ability to consume. Any differences observed are minimal, and juveniles hatching from each capsule are normally of a very similar size (Natarajan 1957; Spight 1976a; Rivest 1983; Chaparro and Paschke 1990; Chaparro et al. 1999; Lloyd and Gosselin 2007). Large size differences amongst capsulemates are unusual, but have been reported in some species of muricid gastropod (Gallardo 1979; González and Gallardo 1999; Cumplido et al. 2011). In gastropods, the number of eggs inside a capsule is usually positively related to capsule size. Within a species, larger capsules hold more eggs and more developing embryos (Gallardo 1979; Pechenik et al. 1984; Miloslavich and Dufresne 1994). The relationship between capsule size and number of eggs (including nurse eggs) has, however, previously been shown to be stronger than the relationship between capsule size and number of developing embryos (Spight 1976b). In some cases, the number of developing embryos within a capsule has been found to be independent of capsule volume. This suggests that embryos are distributed at random, while nurse eggs are regularly placed amongst capsules (Rivest 1983; Chaparro et al. 1999).

Intracapsular development and nurse egg and embryo partitioning have been investigated in several species of marine gastropod (Natarajan 1957; D'Asaro 1970; Spight 1976a; Rivest 1983; Cumplido et al. 2011). While some attempts have been made to examine encapsulated development in the common whelk *Buccinum undatum* (Portmann 1925; Fretter and Graham 1985; Nasution 2003), it has not yet been fully described. Nasution (2003) gives the most in-depth account of development to date, but his descriptions are incomplete and his reports of nurse egg consumption do not match our observations. Descriptions from Portmann (1925) better fit our observations but lack detail. There are also gaps in the current literature, and very limited knowledge exists on nurse egg partitioning and intracapsular embryo size ranges through development. The common whelk is a scavenger found widespread in coastal areas in the North Atlantic. It is generally found from the shallow subtidal down to a few hundred metres of water depth (Valentinsson et al. 1999; Valentinsson 2002; Rosenberg 2009), with a latitudinal range from 38°N to 79°N spanning the North Atlantic and Arctic Oceans (OBIS http://iobis.org/mapper/?taxon=Buccinumundatum). *Buccinum undatum* is an important commercial species, providing locally valuable fisheries in several areas around the North Atlantic including the UK, the USA and Canada (Hancock 1967; Morel and Bossy 2004). It has been suggested as a good candidate for aquaculture (Nasution and Roberts 2004) and globally, demand for it is continuously increasing (Department of Marine Resources www.maine.gov/dmr/rm/whelks.html). Its reproductive cycle has been well documented across its range (Hancock 1967; Martel et al. 1986a, b; Kideys et al. 1993; Valentinsson 2002). Females group to deposit small creamy coloured spherical egg capsules (Martel et al. 1986a). Each lays approximately 80–150, which collectively can create large egg masses of hundreds to thousands of capsules (Fretter and Graham 1985; Valentinsson 2002). The time of year for spawning varies in this species across its distribution. In coastal waters of the UK, egg capsules are laid during the autumn and winter months (predominantly late November–January) as annual water temperatures drop below 9 °C (Hancock 1967; Kideys et al. 1993). In the northwest Atlantic, egg laying instead takes place in spring (late May to mid July) as water temperatures warm (approximately 2–3 °C) (Martel et al. 1986a). Intracapsular development takes between 2.5 and 9 months across the species range (Fretter and Graham 1985; Martel et al. 1986a; Kideys et al. 1993; Nasution 2003). Given the widespread distribution of *B. undatum*, its current commercial importance and its potential as a future candidate for aquaculture, it is important to understand fully the development in this species.

Here, we examine intracapsular development in *B. undatum* using a population from the south coast of England, at the southern end of the species distribution. Number of eggs and number of developing veligers and juveniles are examined through development. Ontogenetic stages are described in detail including nurse egg partitioning, nurse egg consumption and intracapsular ranges in embryo sizes.

Materials and methods

Embryonic development

In order to study the intracapsular development in *B. undatum,* 150 adults were collected from Viviers UK in late November 2009 (www.fishmarketportsmouth.co.uk).

Adults were originally gathered from the Solent, UK (50°39′ N, 001°37′ W) from approximately 15 m water depth by Viviers using whelk traps. They were taken to the aquarium at the National Oceanography Centre, Southampton, and placed in a large outdoor tank with continuous seawater flow through. Whelks were fed scrap fish ad libitum 3 times a week, and the tank was checked daily for laying activity. Egg laying occurred between early December 2009 and early February 2010, predominantly when water temperatures fell below 8 °C. All egg masses were laid on aquarium walls within a few centimetres of the water line.

Three egg masses laid in early January were removed for examination through development. Each was left undisturbed for 24 h after egg laying had ceased before being removed from the aquarium walls and maintained in 1 μm filtered seawater at 6 °C. This was close to local water temperatures, which ranged 4.0–8.3 °C between January and March 2010 (local temperature data obtained bramblemet (www.bramblemet.co.uk/) and CEFAS (www.cefas.defra.gov.uk/our-science/observing-and-modelling/monitoring-programmes/sea-temperature-and-salinity-trends/presentation-of-results/station-22-fawley-ps.aspx) databases). Each week 3 capsules were randomly selected and dissected from each egg mass (Fig. 1a). For each egg mass, the outer layer of egg capsules was removed prior to any examination as these are often empty or hold a very small number of eggs. The contents of each capsule were examined, ontogenetic stage was described and eggs or embryos were measured along their longest axis using an eyepiece graticule. When a capsule contained loose eggs, approximately 20 were measured per capsule. When embryos were present of any age, all were measured (on average 9–11). From the trochophore stage and for the duration of nurse egg feeding, 3 capsules per egg mass were examined daily to determine the duration of short ontogenetic stages and the time taken to consume nurse eggs. Each egg mass was also examined non-invasively each week. Transparency of the capsule wall allowed approximate ontogenetic stage to be determined, and the percentage of the mass at each developmental stage was estimated (Fig. 1b). From this, embryonic development was described, including ontogenetic stages, developmental timing, change in embryo size, nurse egg partitioning and intracapsular size differences during development. Ontogenetic stages were defined as egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile and hatching juvenile (see below for descriptions).

Intracapsular contents through development

In order to investigate the intracapsular contents, *B. undatum* egg masses were collected from Southampton Water (Southampton, UK, 50°50′ N, 001°19′ W) from approximately 10 m water depth between January and March, 2009 and 2010. Seawater temperatures ranged from 4 to 10 °C during these periods. Collection took place using beam trawls deployed by the University of Southampton research vessel *RV Callista*. In total, 35 egg masses were collected, all of which were fixed in 4 % formalin for later investigation.

Capsules were selected at random from all 35 egg masses. As above, the outer layer of each egg mass was removed prior to this. *Buccinum undatum* egg capsules are relatively ellipsoid in shape, with a convex/concave face (Fig. 1a, b). Each capsule was measured in three dimensions (length, width, depth; ±0.01 mm) using digital calipers (Absolute digimatic caliper, Mitutoyo (UK) Ltd, Andover, UK). From these measurements, the volume of each egg capsule was estimated using an adaptation of equations used by Pechenik (1983), Rawlings (1990). The following equation was used.

$$V = (\pi ab) \times c$$

where a = length/2, b = width/2 and c = depth.

Each capsule was then dissected, number of embryos was counted (using a bogorov counting chamber) and ontogenetic stage determined under a compound-microscope. To investigate the relationship between capsule volume and number of eggs or veligers within a capsule, approximately 160 capsules at egg stage (i.e. prior to any development occurring; 15 egg masses; 10–11 capsules from each) and 160 capsules at veliger stage were examined (18 egg masses, 8–9 capsules from each). Capsules ranging from 5.15 to 10.49 mm length (39.0–287.5 mm^3 volume) were compared. Regression analyses were carried out to examine the relationship between capsule volume and number of eggs, and capsule volume and number of veligers.

Change in number of embryos per capsule during development was investigated by examining 100 capsules at veliger stage (12 egg masses, 8–9 capsules from each) and 100 capsules at pre-hatching juvenile stage (9 egg masses, 11–12 capsules from each). Since the number of eggs and embryos per capsule is related to capsule size, for this comparison, capsules of a narrower size range (length 6–8 mm, volume 52.4–146.2 mm^3) were used. This eliminated the possibility of any change in number of embryos per capsule to be influenced by capsule size. Only veligers containing nurse eggs were counted; it was presumed veligers with no nurse eggs would not develop successfully. An unpaired t test was carried out to compare number of veligers per capsule to number of pre-hatching juveniles per capsule.

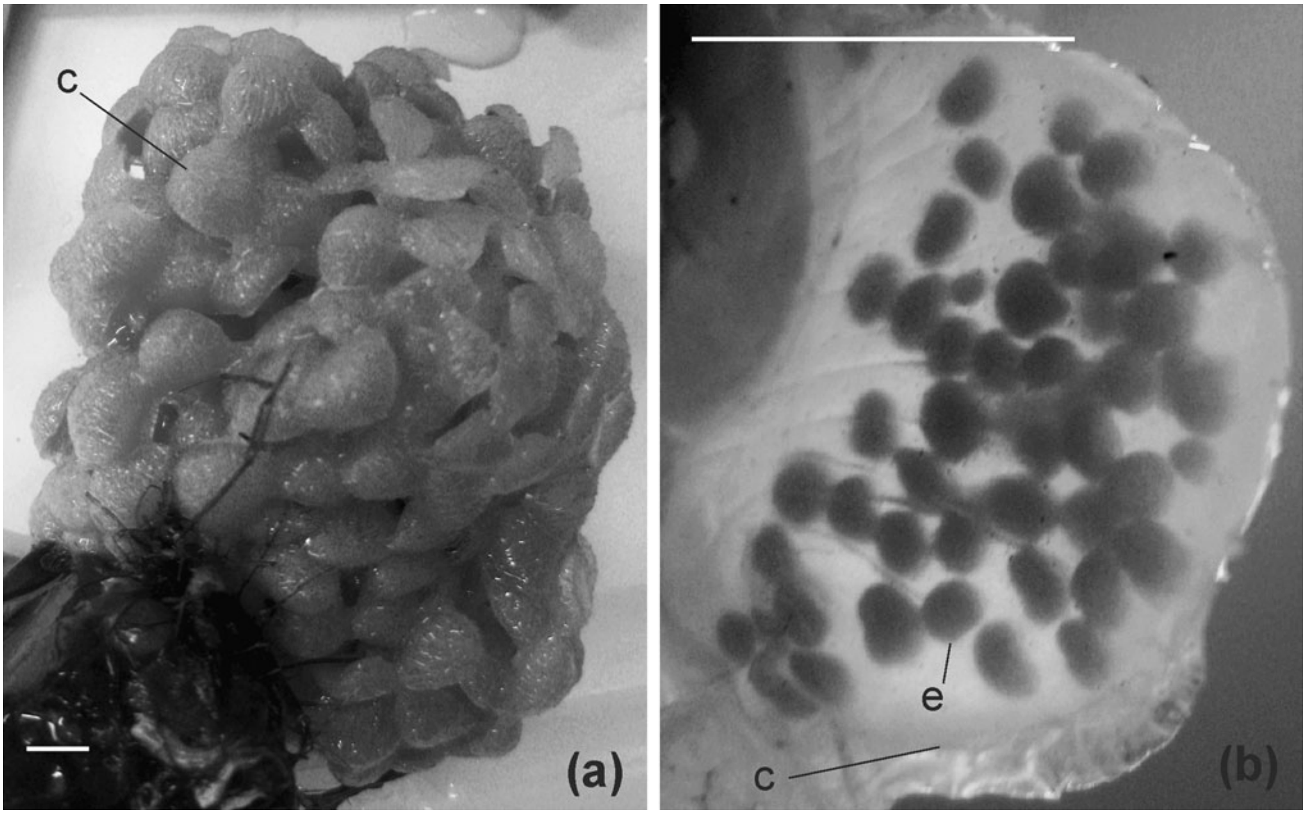

Fig. 1 **a** Egg mass of *B. undatum* showing individual capsules. **b** A large individual egg capsule showing many developed embryos inside, post nurse egg consumption. *Scale bars* represent 5 mm. *c* capsule, *e* embryo

Results

Ontogenetic stages

Seven ontogenetic stages were identified. These are described below.

Egg

Each capsule contains 475–2,639 (mean 1,094) small spherical eggs with no definition. Eggs are cream or yellow in colour and have an average diameter of 234 µm. Within a capsule, egg diameter varies on average by 36 µm. Approximately 1 % of these eggs are developing embryos. The remaining are nurse eggs. At this stage, both developing and nurse eggs are identical (Fig. 2a; Table 1). Egg capsules remain at this stage on average for 49 days.

Trochophore

After 42–56 days developing embryos become globular shaped with a non-circular translucent membrane around the darker embryo. A cilia band (prototroch) is present around approximately one-third to half of the outer circumference of the membrane (Fig. 2b). Each trochophore is a little larger than an egg, with an average length of 321 µm. Each embryo remains at the trochophore stage for just 2–3 days (Table 1).

Early veliger

As the early veliger stage is reached, the prototroch extends laterally to form paired velar lobes with marginal cilia around a central simple mouth. Velar lobes are used for collection of eggs and locomotory movement. Each early veliger is mobile but lacks obvious intentional direction. Behind each lobe and just in front of the main body of the early veliger, paired larval kidneys develop, slightly opaque in colour. Whole (generally nurse) eggs are manipulated into the mouth section using the cilia. These are engulfed and stored in the midgut (Portmann 1925), which forms a circular ball directly behind the mouth section, surrounded by a thin outer membrane. There is some asynchrony in the early development of the embryos from individual capsules. In total, between 2 and 35 veligers develop per capsule (average 11). Each embryo consumes nurse eggs for 3–7 days (at 6 °C). Total consumption by all embryos within a capsule occurs during the early veliger stage, over 4–10 days. Eggs are not damaged during consumption but are stored in the midgut, conserved for later nutritional use. Whole,

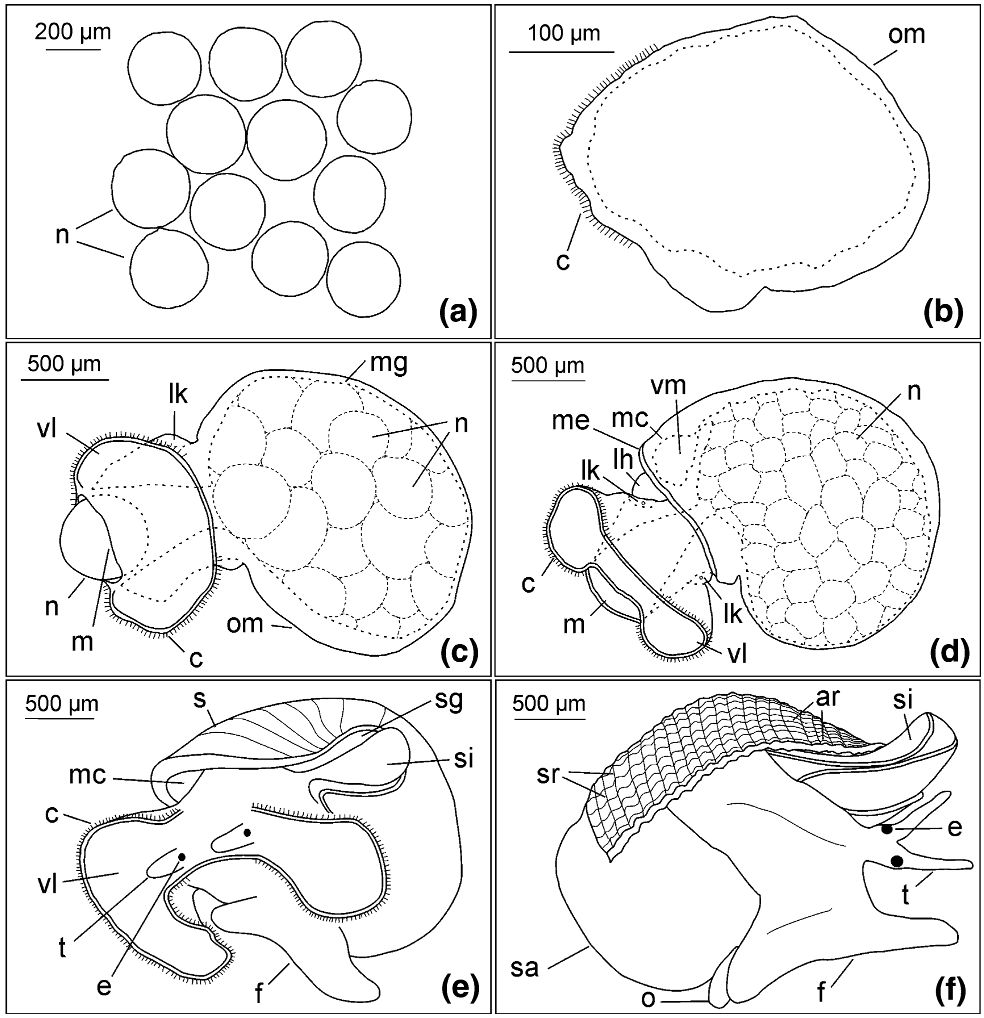

Fig. 2 Intracapsular developmental stages of *B. undatum.* (**a**) Egg, (**b**) trochophore, (**c**) early veliger, (**d**) veliger, (**e**) pediveliger and (**f**) pre-hatching juvenile. *n* nurse egg or undeveloped embryo, *om* outer membrane, *c* cilia, *vl* velar lobe, *m* mouth, *mg* midgut, *me* mantle edge, *mc* mantle cavity, *vm* visceral mass, *lh* larval heart, *lk* larval kidney, *s* shell, *si,* siphon, *sg* siphonal groove, *t* tentacle, *e* eye, *f* foot, *o* operculum, *sa* shell apex, *sr* spiral ribs, *ar* axial ribs

Table 1 Developmental periods for intracapsular development in *B. undatum* from the south coast of England at 6 °C

Ontogenetic stage	Mean time in days spent at each stage (individual)	Time at developmental stage in days (whole egg mass)	Mean size (mm ± SD)	Mean size variation within one capsule (mm)[a]	*n*	*n* (capsules)
Egg	49	0–56	0.23 (±0.01)	0.04	3,235	142
Trochophore	2	42–56	0.32 (±0.02)	0.01	19	12
Early veliger	5	42–56	1.46 (±0.15)	0.33	121	15
Veliger	18	42–77	1.65 (±0.17)	0.27	97	17
Pediveliger	18	70–98	1.91 (±0.32)	0.42	144	20
Pre-hatching juvenile	44	91–140	2.15 (±0.29)	0.38	74	14
Hatching juvenile	n/a	133–140	2.43 (±0.39)	n/a	102	n/a

Mean size at each ontogenetic stage is displayed (mm). Means are determined as an average of *n* measurements; *n* dictates total number of individuals measured. *n* (capsules) dictates number of capsules individuals were measured from and that were examined at each stage. Where n/a is stated, value was inapplicable or not determined

[a] Only capsules with 2 or more individuals included

undamaged nurse eggs can be seen inside the each early veliger. Early veligers average 1.46 mm across their longest axis. Within one capsule, embryo size may vary by as much as 0.85 mm. These size differences continue to be observed throughout development. Once all nurse eggs are consumed, early veligers, veligers and even pediveligers are occasionally found in a capsule, which have consumed no nurse eggs at all (Figs. 1b, 2c, 3a, b; Table 1).

Veliger

In the veliger, the mantle edge thickens and a thin larval shell becomes visible around the midgut, creating a transparent layer. The midgut appears important in dictating the dimensions of this shell. The velar lobes become more separated, and distinct and the larval kidneys continue to be seen, often with a central yellow spot. The central mouth section becomes more opaque, early foot development begins and no further nurse egg consumption is possible. The mantle edge and the visceral mass (white in colour) beneath it become obvious. A transparent pulsating membrane located dorsolaterally in front of the mantle edge becomes evident; this is often named the larval heart (Hughes 1990; Khanna and Yadav 2004). Nurse eggs stored beneath the mantle are still clearly individually discernible at this stage and even going into the pediveliger stage (Figs. 2d, 3b, c; Table 1). It is possible to break the mantle or shell on the back of the veliger or pediveliger and find nurse eggs still inside, which are not degraded and have not yet been digested. Embryos remain at the veliger stage for approximately 14–21 days. During this period, development within a capsule becomes synchronised.

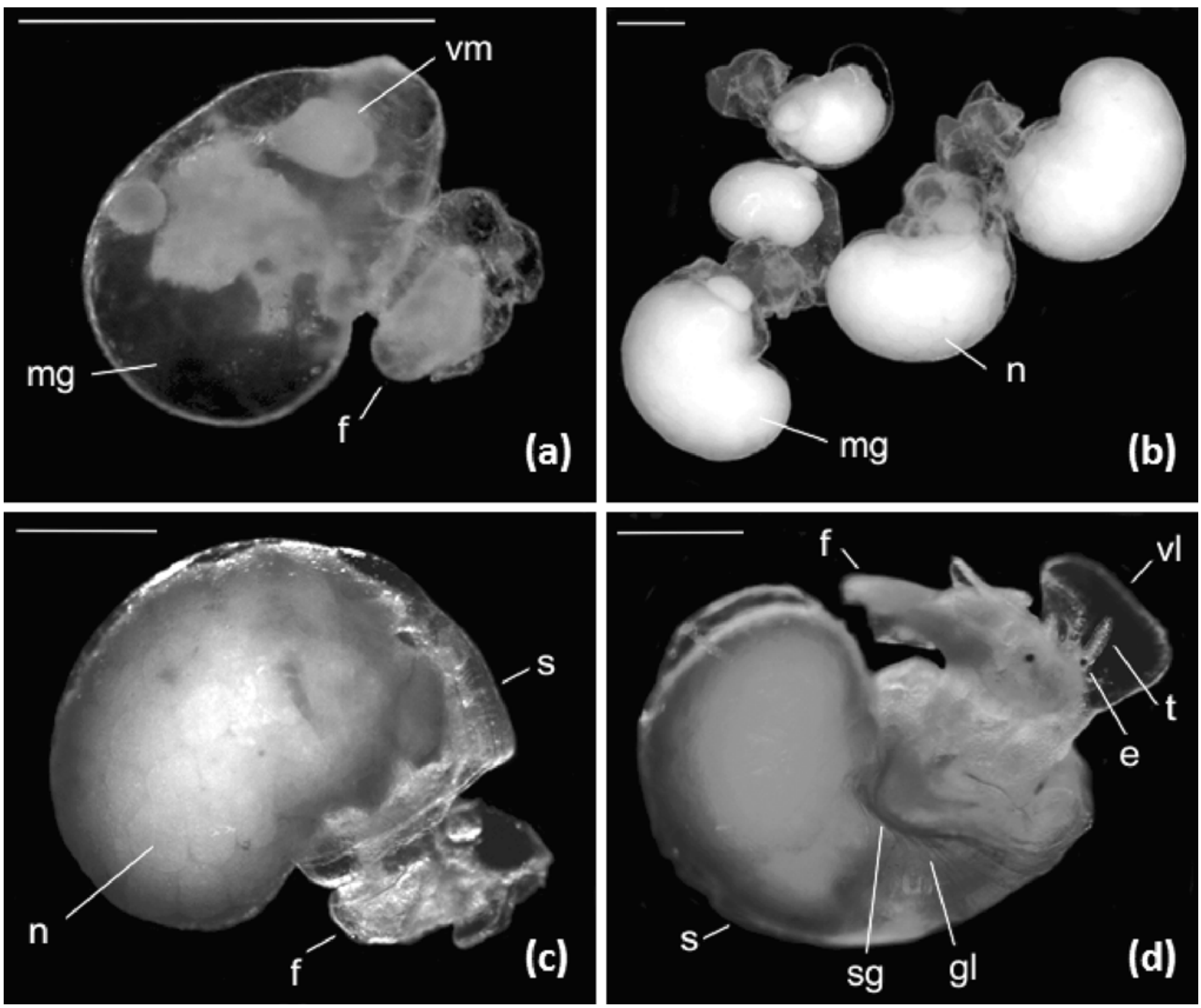

Fig. 3 Early development in *B. undatum.* (**a**) Early pediveliger stage with empty midgut indicating few or no nurse eggs were consumed. (**b**) Veligers of varying sizes developing alongside each other; within one capsule and following nurse egg consumption. (**c**) Early pediveliger stage with individual nurse eggs still clearly discernible under the shell. (**d**) Well-developed mid pediveliger stage with velar lobes still present. Growth lines can be observed on shell. *n* nurse egg, *vl* velar lobe, *mg* midgut, *vm* visceral mass, *s* shell, *sg* siphonal groove, *t* tentacle, *e* eye, *f* foot, *gl* growth lines. *Scale bars* represent 500 µm

Pediveliger

At the pediveliger stage, the shell thickens and becomes increasingly apparent. The mantle cavity is initially visible beneath the mantle edge and the siphonal groove begins to form. The foot, eyes, tentacles and siphon appear. The velum and cilia, which are large at the beginning of this stage, begin to shrink back. They disappear by the end of the pediveliger stage. The larval kidneys and larval heart also disappear. Embryos remain at this stage for approximately 14–21 days (Figs. 2e, 3c, d; Table 1).

Pre-hatching juvenile

Shell growth continues and spiral and axial ribs begin to develop in the shell as the pre-hatching juvenile stage is reached. The shell thickens and colours brown (becomes pigmented). The first whorl becomes obvious and the shell shape elongates. Head, foot, tentacle and siphon features become more prominent and the operculum appears. The feeding proboscis also develops internally during this time. Pre-hatching juveniles complete development over a further 35–49 days before hatching commences. Pre-hatching juvenile size ranges from 1.57 to 3.06 mm. (Fig. 2f; Table 1).

Hatching juvenile

The features described for pre-hatching juveniles become more prominent. The juvenile emerges from the egg capsule through an opening created through radular scraping. They remain on the egg mass for a few days before moving off to feed. Overall hatching size ranged from 1.70 to 3.45 mm (Table 1).

Embryonic development

Each egg mass took between 9 and 11 days to be laid, with complete intracapsular development taking 133–140 days (19–20 weeks) at 6 °C. Within each egg mass, development was asynchronous by up to 14 days throughout the developmental period. Within each capsule, development was initially asynchronous; both trochophore and early veliger stages, and early veliger and veliger stages were observed together in capsules. By late veliger stage development within a capsule was synchronous. Following an initial increase in embryo size as nurse egg consumption occurred, individual size (measured as change in length) increased at a steady rate throughout the remainder of the encapsulated period (Figs. 4, 5; Table 1). Within each capsule, large size differences were observed between embryos at all stages of development. Whole, undamaged nurse eggs were visible inside embryos throughout the veliger and pediveliger stages. Occasional early veligers, veligers and pediveligers were found, which had not consumed any nurse eggs. Apart from the absence of nurse eggs, these embryos were completely normal in their development (Fig. 3a–c; Table 1).

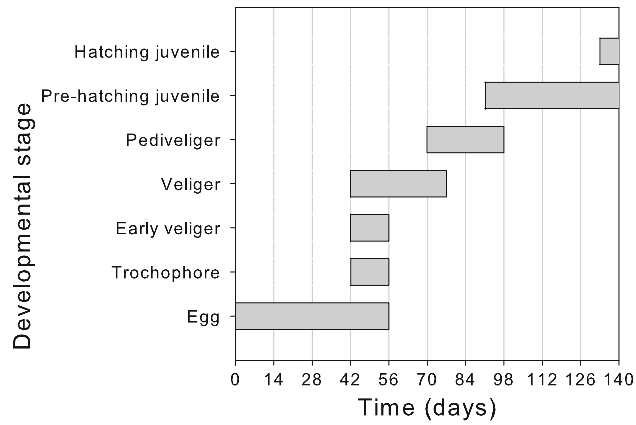

Fig. 4 Developmental time (days) for *B. undatum* from Southampton Water (UK) at 6 °C. *Times* shown represent development across whole egg masses

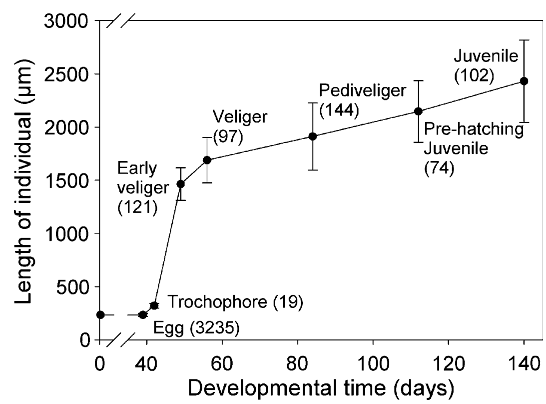

Fig. 5 Change in size of individuals (measured as length along longest axis) during intracapsular development. Size displayed is average length of individual at each stage in µm. Nurse egg consumption occurs between trochophore and early veliger stages. The average size displayed for early veliger is taken post nurse egg consumption. *Error bars* indicate ±1 SD

Intracapsular contents through development

Relationship between capsule volume and number of embryos per capsule

Egg capsule volume ranged from 39.0 to 287.5 mm^3 (capsule length 5.15–10.49 mm). Overall, number of eggs per capsule averaged 1,094 and number of veligers per capsule averaged 11. Regression analysis showed there to be a significant relationship between capsule volume and number of eggs ($r^2 = 0.7646$; $p < 0.001$), and capsule volume and number of veligers ($r^2 = 0.5615$; $p < 0.001$). As a percentage of total eggs, on average 1 %, develop into veligers (Fig. 6a, b).

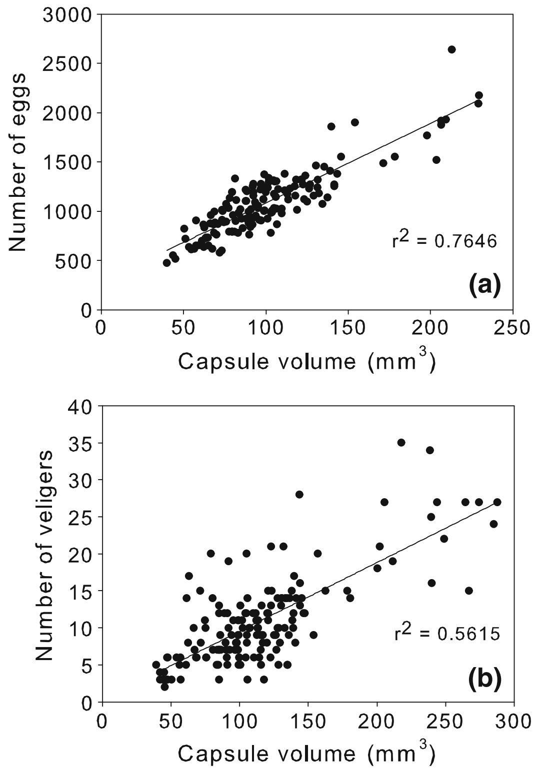

Fig. 6 Relationship between capsule volume and (**a**) number of eggs, (**b**) number of veligers in egg masses of *B. undatum*. Both relationships are significant to $p < 0.001$. The r^2 values are displayed

Change in number of embryos per capsule through development

When examining capsules ranging from 6 to 8 mm in length (volume 52.4–146.2 mm^3), number of developing veligers per capsule ranged from 3 to 21 (average 9) and number of pre-hatching juveniles per capsule ranged from 2 to 20 (average 9). An unpaired *t* test showed there to be no difference between the two groups ($p = 0.772$).

Discussion

Embryonic development and intracapsular contents data

The distribution of *B. undatum* extends from the southern coast of the UK, northwards up into the North Atlantic and Arctic oceans, across a temperature range of −1.5 to 22 °C (Bramblemet; CEFAS; Martel et al. 1986a). For the population used in the present study, annual temperatures vary seasonally from approximately 4–22 °C, and egg laying and development normally occur in water temperatures ranging 4–10 °C. With temperatures maintained at 6 °C, the duration of intracapsular development (4.5–5 months) was similar to previous estimates of *B. undatum* development in British waters (Kideys et al. 1993; Valentinsson 2002). Longer and shorter periods have been reported across the species distribution (e.g. Martel et al. 1986a; Nasution 2003). The observed differences in duration of development can be attributed to the known effects of temperature on metabolic rates in ectotherms.

In the present study, the number of eggs per capsule averaged 1,094 and the number of developing veligers averaged 11. While egg numbers were similar to those indicated in previous studies (Table 2), veliger numbers were similar to figures reported by Hancock (1967), but lower than other estimates (Portmann 1925; Martel et al. 1986a). Since number of veligers is often significantly related to capsule volume (Gallardo 1979; Pechenik et al. 1984; Valentinsson 2002), it is likely that larger capsules were examined in the latter studies. Results indicate approximately 1 % of eggs developed, giving a ratio of 109 nurse eggs per embryo, almost identical to the 110 eggs per embryo reported by Portmann (1925). The percentage of eggs developing was also comparative to other previous estimates for *B. undatum* (Martel et al. 1986a; Valentinsson 2002; Nasution 2003). Similar results have been reported for other buccinids including 1.1–2 % for *Buccinum isaotakki* (Ilano et al. 2004), 0.2–1.8 % for *Buccinum cyaneum* (Miloslavich and Dufresne 1994) and 1 % for *Colus stimpsoni* (West 1979).

Past studies provide conflicting views on the occurrence of intracapsular cannibalism in *B. undatum* (Table 2). Portmann (1925) indicated a reduction in number of individuals per capsule during development (from early veligers to veligers and pre-hatching juveniles), which was suggested to be due to cannibalism (Fretter and Graham 1985). Contrary to this, other studies have shown the number of developing embryos per capsule to remain constant during development, indicating no cannibalism (Hancock 1967; Martel et al. 1986a). Our results were in agreement with these latter studies. Similarly, no cannibalism during development was reported in the buccinids *B. cyaneum* (Miloslavich and Dufresne 1994) and *B. isaotakki* (Ilano et al. 2004), and only very rarely was it observed in the buccinid *L. dirum* (Rivest 1983). It has, however, been reported in some other gastropods including *Crucibulum quiriquinae* (Véliz et al. 2001), *Crepidula coquimbensis* (Véliz et al. 2003; Brante et al. 2009) *Trophon geversianus* (Cumplido et al. 2011) and a vermetid gastropod (Strathmann and Strathmann 2006).

Capsule size or volume has previously been shown to be a good indicator of number of eggs and veligers within a capsule. In the current study, these figures were both significantly related to capsule volume. Number of eggs was more closely related to volume than number of veligers, suggesting eggs are more regularly distributed amongst

Table 2 Reproductive biology of *B. undatum* from present and previous studies

Study	Location	Development temperature (°C)	Time to hatching (months)	Capsule size (length mm)	No. of eggs per capsule	Egg diameter (µm)	% of eggs that develop	No. of veligers per capsule	No. hatching juveniles per capsule	Length of shell at hatching (mm)
Portmann (1925)	Roscoff, France	5–9	n/a	n/a	50–>2,000	n/a	n/a	av. 30	av. 10	n/a
Hancock (1967)	Burnham on Crouch, UK	n/a	3–4	n/a	≤3,000	n/a	n/a	13–14	n/a	n/a
Fretter and Graham (1985)	n/a	n/a	3–9	6–12	500–3,000	200–300	n/a	av. 30	3–10	1–1.4
Martel et al. (1986a)	Gulf of St Lawrence, Canada	2–3	5–8	n/a	2,700	n/a	1.10	av. 30	av. 30	3
Kideys et al. (1993)	Douglas, Isle of Man	n/a	3–5	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Valentinsson (2002)	Skagerrak, Sweden	4–8	3–4	7–9.5	700–2,300	245–285	0.20–1.20	n/a	2–16	1.9–2.8
Nasution (2003)	Irish Sea, Northern Ireland	8–11	2.5–3	n/a	av. 2,360[a]	340	0.47–1.65	n/a	n/a	Nearly 2[b]
Nasution et al. (2010)	Irish Sea, Northern Ireland	10	3	n/a	558–4,196	n/a	0.47–1.65	n/a	n/a	2.1–3.1
Present study	Southampton Water, UK	6	4.5–5	5–10.5	475–2,639	200–260	1.01	av. 9–11	av. 9	1.70–3.45
Author, personal observations	Breidafjordur, Iceland	Approx. 3	n/a	7–10	804–1,441	n/a	1.48	av. 17	n/a	n/a

n/a not available in study, *av.* average, *no.* number

[a] Range stated in journal was not possible

[b] No accurate value was stated in publication

capsules than are developing embryos. This pattern has been reported before for both *B. undatum* (Valentinsson 2002; Nasution et al. 2010) and other gastropods, including *B. cyaneum* (Miloslavich and Dufresne 1994), *B. isaotakki* (Ilano et al. 2004), *Hexaplex (Trunculariopsis) trunculus* (Lahbib et al. 2010), *Acanthina monodon* (Gallardo 1979), *Nucella lapillus* (Pechenik et al. 1984) and *Nucella lamellosa* (Spight 1976b). Contrary to this, number of eggs has been found to be related to, but number of veligers to be independent of capsule size in the buccinid *L. dirum* (Rivest 1983), the calyptraeid *Crepipatella dilatata* (Chaparro et al. 1999) and the muricid *Nucella ostrina* (Lloyd and Gosselin 2007).

An initial rapid increase in embryo size was observed at the early veliger stage in the present investigation. This was followed by a relatively linear increase in size for the remainder of intracapsular development. Similar changes in size during development have been reported for *B. cyaneum* (Miloslavich and Dufresne 1994) and *B. isaotakki* (Ilano et al. 2004). For both, however, the initial increase was slower than was observed in this investigation. In *B. isaotakki*, it is likely that this is reflective of the slower nurse egg consumption rate previously observed in this species (Ilano et al. 2004). Probably, nurse eggs are also taken up at a slower rate in *B. cyaneum.*

Previous hatching sizes for *B. undatum* have been reported ranging from 1.0 to 3.1 mm (e.g. Fretter and Graham 1985; Nasution et al. 2010). These are similar to hatching sizes observed in the present investigation, which averaged just below 2.5 mm in length.

Nurse egg partitioning

Life history theories suggest parental fitness is maximised by investing equally into all offspring (Smith and Fretwell 1974). Traditionally, resource partitioning (in the form of nurse eggs) during intracapsular development follows this trend. Embryos compete for nurse eggs, but within a capsule competitiveness is normally equal. As a result, nurse eggs are consumed quite evenly by all embryos. This does not mean hatchlings are always of a similar size; within one species, or even one clutch, the ratio of nurse eggs to developing embryo may vary greatly between capsules, resulting in large differences in offspring size. This is usually believed to be due to irregular distribution of embryos amongst capsules (Thorson 1950; Rivest 1983; Spight 1976a; Miloslavich and Dufresne 1994). Within a capsule however, generally only small differences in offspring size are reported. For example, Spight (1976a) examined 2 species of muricid gastropod (*Nucella emarginata* and *A. spirata*) and found that although embryo size varied considerably between capsules, within a capsule large differences were rare. Previous studies

Table 3 Periods of development and nurse egg consumption times for different species of gastropods

Species	Temperature (°C)	Duration of intracapsular development (days)[a]	Duration of nurse egg consumption (days)[a]	Percentage of development over which nurse eggs are consumed (%)	Authors
B. isaotakii	2.5–10.2	200	40	20	Ilano et al. (2004)
B. undatum	8–11	70	28	40	Nasution (2003)
B. undatum	6	133–140	3–7	2–5	Present study
C. dilatata	17	18–26	Up to 26	100	Chaparro and Paschke (1990)
Hexaplex (Trunculariopsis) trunculus	22–24	49	35	71	Lahbib et al. (2010)
L. dirum	12	84–98	7–21	8–20	Rivest (1983)
T. geversianus	12–14	112	38	34	Cumplido et al. (2011)

All species included are direct developers

[a] Some timings have been converted from weeks stated in original study

examining development in *B. undatum* have indicated similar results, and comparable observations have also been reported for the gastropods *L. dirum* (Rivest 1983) and *C. dilatata* (Chaparro and Paschke 1990; Chaparro et al. 1999). In contrast, the present study found nurse egg partitioning to be quite different to that previously described for *B. undatum* or other buccinids. Large size differences were continually observed between embryos from any one capsule, and regularly individuals were found alongside a capsulemate four times their size (Fig. 3b). Although to our knowledge, variations in nurse egg consumption have not previously been reported in other buccinids, such intracapsular differences have been described for a small number of gastropods, predominantly from the muricidae family. These include *A. monodon* (Gallardo 1979), *Chorus giganteus* (González and Gallardo 1999) and *T. geversianus* (Cumplido et al. 2011). In *A. monodon* and *C. giganteus*, intracapsular size differences continue to be evident at hatching, presumed to be related to earlier nurse egg consumption (Gallardo 1979; González and Gallardo 1999). In *T. geversianus*, sibling cannibalism (which can also affect offspring size) occurs during later developmental stages, and it is not clear whether hatching sizes vary (Cumplido et al. 2011).

It is widely assumed that offspring quality increases with size (e.g. Thorson 1950; Spight 1976a; Rivest 1983; Gosselin and Rehak 2007; Lloyd and Gosselin 2007; Przeslawski 2011). Larger hatchlings are less likely to be affected by factors such as physical stress, predation and starvation. While intracapsular size differences are generally believed to be due to competition (Gallardo 1979; González and Gallardo 1999), in the present investigation, they are probably enhanced by a combination of asynchrony in development and short nurse egg consumption periods. We found nurse egg feeding to be very rapid, with each early veliger consuming eggs for just 3–7 days. This relates to 2–5 % of the developmental period. In comparison, in most gastropods, nurse egg consumption occurs over a large proportion of intracapsular development (Table 3). Even the shortest uptake periods previously reported (8–20 % of the developmental period) (Rivest 1983) are still more than double the length of the consumption period observed by us. Within a capsule, the potential to take up nurse eggs is limited by the amount already consumed by earlier developers. Thus, while intracapsular asynchrony in early development is not uncommon (e.g. Vasconcelos et al. 2004; Fernández et al. 2006; Lahbib et al. 2010), when it is combined with the short nurse egg consumption period seen in *B. undatum,* it follows that even a 24-h lag in initial embryonic development will put individuals at a distinct disadvantage. Rapid nurse egg consumption in *B. undatum* is consistent with findings by Portmann (1925), but contradictory to those of Nasution (2003). Additionally, 6 °C is towards the lower end of the temperature range that southern populations of *B. undatum* naturally develop in. Nurse egg consumption is even faster at warmer temperatures (Authors, unpublished data). This may lead to larger intracapsular size differences during development, and with predicted sea temperature elevations, intracapsular size ranges may increase.

Normal veligers and pediveligers that had not successfully consumed any nurse eggs were occasionally found within a capsule in the present investigation (Fig. 3a). It is likely that these individuals reached the feeding stage after all resources had been consumed. Since no further feeding occurs between nurse egg consumption and hatching, these embryos had no nutrition available to them for development and we assumed they did not survive. This in itself is very unusual and even in the few reported cases of large intracapsular size differences between embryos (Gallardo 1979;

González and Gallardo 1999; Cumplido et al. 2011), to our knowledge completely 'empty' embryos have not been observed.

In the current study, it was noted that for several weeks following consumption, individual nurse eggs could still be observed through the thin veliger mantle and early shell (Fig. 3c). Throughout this period, if the mantle or shell was broken, whole eggs would spill out. This indicated that although eggs were rapidly consumed, they were not immediately utilised but instead were stored for later nutritional use. This phenomenon was also noted by Portmann (1925), who recognised that nurse eggs stayed intact inside *B. undatum* veligers for long periods of time. In comparison, he found they disintegrated directly after consumption in *N. lapillus*. Nurse eggs have also been shown to be visible internally throughout the feeding period in *A. monodon* (Gallardo 1979), *L. dirum* (Rivest 1983) and *C. dilatata* (Chaparro and Paschke 1990). In each case however, the literature suggests nurse eggs begin to be assimilated shortly following consumption. In other species such as *T. geversianus,* nurse eggs break down prior to consumption by embryos (Cumplido et al. 2011).

The range in size of embryos within a capsule and the occurrence of 'empty' embryos observed in this investigation indicates that a higher level of competition is occurring in *B. undatum* than is normally observed during intracapsular development in gastropods. While large intracapsular size differences have been observed in some muricid gastropods, to our knowledge, competition for nurse eggs to the degree that some embryos are left with no nutrition for development has never previously been reported.

Acknowledgments Thanks are given to the skipper and crew of *RV Callista* for their help with sample collection. This work was supported by grants from the Total Foundation (Abyss2100) to ST and the Malacological Society to KS.

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Appendix D: *Reprint* Smith KE, Thatje S, Hauton C (2013) Thermal tolerance during early ontogeny in the common whelk *Buccinum undatum* (Linnaeus 1785): bioenergetics, nurse egg partitioning and developmental success. Journal of Sea Research 79:32-39

Journal of Sea Research 79 (2013) 32–39

Contents lists available at SciVerse ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Thermal tolerance during early ontogeny in the common whelk *Buccinum undatum* (Linnaeus 1785): Bioenergetics, nurse egg partitioning and developmental success

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ARTICLE INFO

Article history:
Received 13 September 2012
Received in revised form 8 January 2013
Accepted 29 January 2013
Available online 14 February 2013

Keywords:
Intracapsular development
Thermal tolerance
Bioenergetics
Plasticity
Buccinidae
Buccinum

ABSTRACT

Temperature is arguably the primary factor affecting development in ectotherms and, as a result, may be the driving force behind setting species' geographic limits. The shallow-water gastropod *Buccinum undatum* is distributed widely throughout the North Atlantic, with an overall annual thermal range of below zero to above 22 °C. In UK waters this species is a winter spawner. Egg masses are laid and develop when sea temperatures are at their coolest (4 to 10 °C) indicating future climate warming may have the potential to cause range shifts in this species. In order to examine the potential impacts of ocean warming, we investigate the effects of temperature on the early ontogeny of *B. undatum* across a thermal range of 0 to 22 °C. Each egg mass consists of approximately 100 capsules, in which embryos undergo direct development. Successful development was observed at temperatures ranging from 6 to 18 °C. Rates of development increased with temperature, but the proportion of each egg mass developing successfully decreased at the same time. With increasing temperature, the mean early veliger weight increased, but the number of early veligers developing per capsule decreased, suggesting a negative impact on the number of crawl-away juveniles produced per capsule. Elemental analysis showed both carbon (C) and nitrogen (N) to increase with temperature in early veligers but not in hatching juveniles, indicating greater energy reserves are accumulated during early ontogeny to compensate for the higher energetic demands of development at higher temperature. The developmental plasticity observed in *B. undatum* suggests this species to be capable of adapting to temperatures above those it currently experiences in nature. *B. undatum* may possess a thermal resilience to ocean warming at its current upper temperature distribution limit. This thermal resilience, however, may come at the cost of a reduced offspring number.

1. Introduction

Thermal tolerance plays a significant role throughout an organism's life history. In marine invertebrates, temperatures outside a species tolerance range cause negative physiological effects (e.g. Pörtner et al., 2005; Somero, 2010), impacting growth, survival and development throughout an individual's life. This thermal tolerance range may vary across a species' distribution, with population specific thermal ranges regularly being observed, generally varying with latitude. The thermal tolerance range of a species during development is often narrower than that which adults from the same population can tolerate. Within this, rates of growth and development scale with temperature (Sewell and Young, 1999; Weiss et al., 2009). This pattern has observed globally in marine invertebrates and latitudinal trends can be observed indicating rates of growth and development in many shallow water organisms to largely increase from the poles to the tropics as seawater temperatures rise (Clarke, 1983; Hoegh-Guldberg and Pearse, 1995; Stanwell-Smith and Peck, 1998). Outside a population's developmental thermal range, developmental success is usually impaired (Anger et al., 2003; Lillie and Knowlton, 1897).

As is now well documented, the results of global warming have led to increasing temperatures throughout the oceans (Barnett et al., 2005; Harley et al., 2006; IPCC, 2007). Median increases in sea surface temperature are currently 0.07 °C per decade (Burrows et al., 2011), with some areas being more affected than others. In the UK and north-east Atlantic for example, temperatures have risen by 0.2–0.8 °C per decade (Hughes et al., 2010; MCCIP, 2010). Recent predictions suggest the oceans will continue to warm until at least 2080 (Hughes et al., 2010; MCCIP, 2010). Changing seawater temperatures are likely to negatively impact marine species, affecting developmental success and limiting distribution. In response to this, species range-shifts are predicted to occur globally, tracking isotherms characteristic of their current distribution (Ackerley et al., 2010; Burrows et al., 2011; Loarie et al., 2009). Such migrations have already been observed in a range of marine species, including crustaceans (Southward et al., 1995), gastropods (Zacherl et al., 2003) and fish (Dulvy et al., 2008; Nye et al., 2009; Perry et al., 2005). These are however, and among other ecological factors, dependent on suitable habitat being available (Burrows et al., 2011).

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http://dx.doi.org/10.1016/j.seares.2013.01.008

The effects of temperature on larval and juvenile development is of particular concern and as a result a growing number of studies have recently contributed to this topic (e.g. Anger et al., 2004; Johns, 1981; Sewell and Young, 1999; Stanwell-Smith and Peck, 1998). To date, the majority of studies have examined species that exhibit fully or partially planktonic development (Anger et al., 2004; Cancino et al., 2003; Johns, 1981; Lima and Pechenik, 1985; Pechenik et al., 2003; Roller and Stickle, 1989; Sewell and Young, 1999; Stanwell-Smith and Peck, 1998) and only rarely have the effects of temperature on development been described in a species with direct development (Fernandez et al., 2006). Such species have limited dispersal abilities, and typically migrate or radiate at a slower rate (Jablonski, 1986; Thatje, 2012), suggesting that species following a direct mode in development may be more "at risk" from temperature change than those with planktonic development.

Species undergoing non-planktonic development often develop within egg capsules which protect against factors such as physical stress, predation, infection and salinity changes (Pechenik, 1983, 1999; Rawlings, 1995, 1999; Strathmann, 1985; Thorson, 1950). Within each capsule, embryos are usually provided with a source of nutrition for development. This is most commonly found in the form of nurse eggs (Chaparro and Paschke, 1990; Ilano et al., 2004; Lahbib et al., 2010; Thorson, 1950), but additional nutrition may also occasionally be gained from intracapsular fluid (Bayne, 1968; Moran, 1999; Pechenik et al., 1984; Stöckmann-Bosbach, 1988) or capsule walls (Ojeda and Chaparro, 2004).

The common whelk *Buccinum undatum* is a shallow-water gastropod, which exhibits direct encapsulated development using nurse eggs for nutrition. It is common in the North Atlantic and Arctic oceans, provides locally valuable fisheries across these areas (Hancock, 1967; Morel and Bossy, 2004) and has been suggested as a candidate species for aquaculture (Nasution and Roberts, 2004). Its reproductive cycle (Hancock, 1967; Kideys et al., 1993; Martel et al., 1986a, 1986b; Valentinsson, 2002) and intracapsular development (Portmann, 1925; Nasution, 2003; for discussion see Smith and Thatje, 2013) are well documented with egg laying and development taking between 2.5 and 9 months across its distribution range (Kideys et al., 1993; Martel et al., 1986a). *B. undatum* is a cold-water spawner and at the southern end of its distribution development predominantly occurs during winter months when water temperatures are at their coolest; approximately 4 to 10 °C around the UK (Kideys et al., 1993; Smith and Thatje, 2013). This indicates that unless this species is capable of developing under warmer temperatures, its distribution is likely to be impacted by increasing seawater temperatures. The widespread distribution, commercial importance and knowledge of intracapsular development in the common whelk make it a good model species for investigating the effects of temperature on development.

Here, we examine the full thermal scope for intracapsular development in *B. undatum* from its southernmost distribution range from the south coast of England. Reproductive trade-offs per capsule and bioenergetic changes in offspring development in response to the temperature are assessed, and discussed within a macroecological context of thermal adaptation.

2. Materials and method

2.1. Egg mass collection

In order to examine the effects of temperature during development in *B. undatum*, egg masses were collected between December 2009 and February 2010, and December 2010 and February 2011. Two methods of collection were used, as described below.

2.1.1. Trawling

Egg masses were collected from the Solent (50°47′ N, 001°15′ W). During the collection periods stated above, seawater temperatures ranged from 4 to 10 °C. Local temperature data were obtained from long-term monitoring data from bramblemet (www.bramblemet.co.uk/) and CEFAS (www.cefas.defra.gov.uk/our-science/observing-and-modelling/monitoring-programmes/sea-temperature-and-salinity-trends/presentation-of-results/station-22-fawley-ps.aspx). Collection took place using beam trawls deployed from on board *RV Callista* at depths of 5 to 10 m.

2.1.2. Farmed in seawater aquarium

Approximately 150 adult *B. undatum* were collected by Viviers UK in late November 2009 and 2010 (www.fishmarketportsmouth.co.uk). Adults were originally gathered from the Solent (50°47′ N, 001°15′ W) by Viviers using whelk traps. They were maintained in a large outdoor tank with continuous seawater flow through at the National Oceanography Centre, Southampton, and fed scrap fish *ad libitum* three times a week. The tank was checked daily for laying activity. Egg laying took place between December 2009 and February 2010, and December 2010 and February 2011. It predominantly occurred when water temperatures fell below 8 °C. All egg masses were laid on aquarium walls within a few cm of the water line. Once egg laying was complete, each egg mass was left undisturbed for 24 hours before being removed from the aquarium walls.

2.2. Egg mass maintenance and investigation

Since adult whelks were obtained from the same population, water temperatures at time of collection were similar, and no difference was observed in egg capsule size or number of eggs per capsule between egg masses collected via trawl and those farmed in the aquarium, all egg masses collected were combined. A one-way ANOVA ($p \geq 0.05$) was used to confirm there was no difference in egg capsule size or number of eggs per capsule between the collection methods used. All egg masses used during the investigation had capsules of a similar volume (100 to 150 mm^3), and each mass was made up of approximately 80 to 140 individual egg capsules. Capsule volume was determined through measurements of capsule length, width and depth (±0.01 mm), using the following equation taken from Smith and Thatje (2013);

$$V = (\pi ab) * c$$

where a = length/2, b = width/2 and c = depth.

Upon collection, three capsules from each egg mass were dissected and their contents examined to assess developmental stage (according to Smith and Thatje, 2013). Only egg masses not yet showing embryonic development were used in the investigation. The number of eggs was counted for each capsule used. Each mass was also examined non-invasively to confirm no development had occurred. The capsule walls of *B. undatum* are relatively transparent (Smith and Thatje, 2013), allowing approximate ontogenetic stage to be determined. A total of 7 trawled and 14 farmed egg masses were used in the investigation. A one-way ANOVA was carried out to confirm there was no difference in number of eggs per capsule between the egg masses used in the investigation ($p \geq 0.05$; mean number of eggs per capsule 1175). Each egg mass was maintained in a 1.8 L incubation tank containing aerated, 1 µm filtered seawater. Egg masses were acclimated to one of seven temperatures (0, 2, 6, 10, 14, 18, 22 °C; 1 trawled and 2 farmed egg masses maintained at each temperature). Acclimation took place by adjusting the temperature of each incubation tank by 1 °C every 24 hours from the initial water temperature at egg mass collection. A 100% water change was carried out on each tank 3 times a week.

Every week for the initial 14 weeks and every fortnight for the remaining developmental period, 3 capsules were randomly selected and dissected from each egg mass, the contents were examined and the developmental stage determined. Ontogenetic stage was determined according to Smith and Thatje (2013) and defined as egg, trochophore, early veliger, veliger, pediveliger, pre-hatching juvenile or hatching juvenile. Nurse eggs were consumed through the early veliger stage. For each mass, the outer layer of egg capsules was removed prior to any examination as these were often empty or held a very small number of eggs. From the trochophore stage and throughout nurse egg consumption (Smith and Thatje, 2013), egg masses were examined daily to determine the duration of short ontogenetic stages. Each egg mass was also examined non-invasively every week. From this, the percentage of the mass at each developmental stage was estimated. When an egg mass had completed early veliger development, a minimum of 10 capsules from each egg mass were opened and the number of developing embryos counted. Each early veliger was stored individually in a pre-weighed (6 mm × 4 mm) tin capsule and frozen at −80 °C. Samples were freeze-dried over 24 hours and then dry weight was determined (±1 µg). Hatching juveniles at each temperature were sampled and dried and weighed in the same fashion. Each juvenile was then de-calcified using RDC rapid decalcifier (Cellpath, Powys, UK), rinsed in distilled water and then dried and weighed a second time. This allowed total, shell and flesh weights and shell:flesh weight ratios to be determined. Any abnormal individuals were not sampled. Abnormal embryos included those with malformed heads, misshapen bodies or those lacking any mantle or shell development. All samples weighing more than 200 µg were used for later elemental (C and N) analysis. Elemental analysis was carried out using a Fison (Carlo Erba) 1108 Elemental Analyser. The elemental analyser was calibrated using chitin as a standard (%C = 44.71; %N = 6.79). Carbon (C) and Nitrogen (N) percentages were determined during analysis and the C:N ratio was calculated.

Data did not have equal variance and therefore a non-parametric Kruskal–Wallis was used to analyse the effect of temperature on number of early veligers per capsule, early veliger and juvenile weights, and early veliger and juvenile elemental composition.

3. Results

3.1. Embryonic development

3.1.1. Duration of development and developmental success

Egg masses were observed for a total of 36 weeks (252 days). Within each capsule, development was initially asynchronous but was synchronised by the end of the veliger stage. Within an egg mass, some asynchrony in ontogenetic timing was observed between capsules throughout development. Between all egg masses maintained at the same temperature, the level of asynchrony and the overall developmental timing observed was equal (Fig. 1, Table 1). At the highest temperature (22 °C) no development occurred; after 42 days all eggs had begun to degrade and no further samples were collected. At temperatures ranging from 6 to 18 °C, intracapsular development was successful and took between 49 and 140 days. At the lowest two temperatures

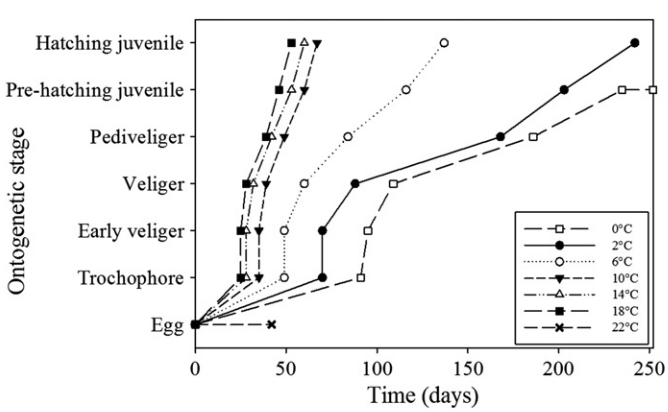

Fig. 1. Mean developmental timing (days) for intracapsular development in *Buccinum undatum*. Egg masses are from the Solent (50°47′ N, 001°15′ W), off the south coast of the UK, and developed at temperatures ranging 0 to 22 °C.

(0 and 2 °C) development was very slow and a high number of abnormal embryos were observed (61.6% at 2 °C; 51.8% at 0 °C). At these two temperatures some individuals had reached pre-hatching juvenile stage in every capsule examined, but even juveniles deemed "normal" generally possessed very thin, transparent shells, which were often broken with limited or no colouring. Asynchrony in development was observed throughout the investigation within each capsule examined at 0 and 2 °C; pre-hatching juveniles, pediveligers and occasionally veligers were found together in individual capsules. After 36 weeks no hatching was observed at 0 °C and only 11 juveniles had successfully hatched at 2 °C, from an estimated 400 capsules developed at this temperature. Observations ceased at this point and individuals were deemed unviable. Of the temperatures at which successful development occurred (6 to 18 °C), rates of development were similar at 10 °C (63 to 70 days), 14 °C (56 to 63 days) and 18 °C (49 to 56 days) but took approximately twice as long at 6 °C (133 to 140 days). Across these temperatures, the percentage of egg mass, which successfully completed development, varied from 20% at 18 °C to 100% at 6 and 10 °C.

3.1.2. Nurse egg consumption

Nurse eggs were consumed during the early veliger stage at all temperatures at which development occurred. Consumption time (classified as the duration of the early veliger stage) within a capsule decreased with increasing temperature and ranged from 16 days on average at 0 °C to 2 days on average at 18 °C (Fig. 1, Table 1). In capsules developing at temperatures ranging 6 to 14 °C, all nurse eggs were consumed by developing embryos in every capsule examined. All capsules examined developing at 18 °C, and occasional capsules examined developing at 0 or 2 °C, contained a number of unconsumed nurse eggs throughout the duration of development.

3.1.3. Embryo size

At all temperatures large size differences were observed between the embryos developing within any one capsule. At the early veliger stage, these differences were confirmed through examination of individual weight (see below). Early veligers that had not successfully consumed any nurse eggs were observed quite regularly after all nurse eggs had been consumed in a capsule. These "empty" individuals were observed at the early veliger, veliger and occasionally the pediveliger stage, but no later in development. Although not quantified, frequency of "empty" embryos appeared to increase with temperature.

3.2. Intracapsular content through early ontogeny

The number and weight of early veligers were examined across developmental temperatures ranging from 0 to 18 °C (Fig. 2a,b). Weight of hatching juveniles was examined across developmental temperatures ranging 2 to 18 °C (Fig. 2c). Number of early veligers per capsule was significantly affected by developmental temperature ($p \leq 0.001$). Numbers first increased from 0 to 6 °C and then decreased again from 6 to 18 °C. Early veliger weights were also significantly affected by temperature ($p \leq 0.001$), but an opposite pattern was observed (Fig. 2b). Average weight decreased as temperature increased from 0 to 6 °C and then increased again as temperature increased from 6 to 14 °C, before decreasing at 18 °C. Within a capsule, early veliger weights varied between 75 and 603 µg at 0 °C (mean 334 µg), 74 and 759 µg at 2 °C (mean 326 µg), 473 and 1025 µg at 6 °C (mean 739 µg), 416 and 1240 µg at 10 °C (mean 913 µg), 332 and 1325 µg at 14 °C (mean 761 µg), and 74 and 809 µg at 18 °C (mean 354 µg). Across all individuals developing at each temperature (i.e. across all capsules), early veliger weights varied by between 900 and 1331 µg. In juveniles, temperature significantly affected total weight, shell weight, flesh weight and shell:flesh ratios ($p \leq 0.001$), but no correlation was observed between juvenile weight and temperature. At each temperature

Table 1
Developmental periods in days for intracapsular development in *Buccinum undatum* from the Solent (50°47′ N, 001°15′ W), off the south coast of the UK, at temperatures ranging 0 to 22 °C. n/a indicates lack of development prevented average time from being determined.

	Temperature	Time at developmental stage in days — whole egg mass (mean number of days spent at stage)						
		0 °C	2 °C	6 °C	10 °C	14 °C	18 °C	22 °C
Developmental stage	Egg	0 to 105 (91)	0 to 77 (70)	0 to 56 (49)	0 to 42 (33)	0 to 35 (28)	0 to 28 (24)	0 to 42[a] (n/a)
	Trochophore	63 to 112 (4)	56 to 84 (3)	42 to 56 (2)	28 to 42 (2)	21 to 35 (1–2)	21 to 28 (1–2)	n/a
	Early veliger	63 to 119(16)	56 to 84 (12)	42 to 56 (5)	28 to 42 (4)	21 to 35 (3)	21 to 28 (2)	n/a
	Veliger	70 to 252 (n/a)	63 to 252 (n/a)	42 to 77 (18)	28 to 49 (7)	21 to 42 (6)	21 to 35 (5)	n/a
	Pediveliger	105 to 252 (n/a)	98 to 252 (n/a)	70 to 98 (18)	42 to 56 (7)	35 to 49 (7)	28 to 49 (6)	n/a
	Pre-hatching juvenile	217 to 252 (n/a)	154 to 252 (n/a)	91 to 140 (44)	49 to 70 (16)	42 to 63 (14)	35 to 56 (14)	n/a
	Hatching juvenile	n/a[b]	231 to 252[c]	133 to 140	63 to 70	56 to 63	49 to 56	n/a
Percentage of egg mass to successfully develop		0	0.2[c]	100	100	95	20	0

[a] All egg masses degraded after 42 days.
[b] No hatching had occurred after 252 days.
[c] A total of 11 juveniles hatched from approximately 500 capsules across 3 egg masses.

and across all individuals, hatching juvenile weight varied by between 1381 and 4661 μg.

3.3. Bioenergetic changes through early development

Elemental analysis was carried out on early veligers developed at temperatures ranging 0 to 18 °C and juveniles developed at temperatures ranging 2 to 18 °C (Fig. 3). At 2 °C, due to the low number of hatchlings, only 3 juveniles were analysed in total. Throughout development the carbon mass fraction was higher than the nitrogen mass fraction. In early veligers a trend of increasing C and N with temperature was observed. Percentages of C and N and C:N ratios were all significantly affected by developmental temperature at the early veliger stage ($p \leq 0.001$). Upon reaching the juvenile stage, no differences were observed between developmental temperatures in percentages of C ($p = 0.997$) or N ($p = 0.998$), or C:N ratios ($p = 0.619$). Significant decreases in C, N and C:N ratio values were observed during development (from early veliger to hatching juvenile) at every temperature investigated. All changes were significant to $p \leq 0.001$ except for changes in C and C:N ratio at 2 °C (both significant to $p \leq 0.01$) and changes in N at 2 °C and C:N at 10 °C (both significant to $p \leq 0.05$). For C and N, percentage depletion increased as developmental temperature increased from 17.9% (C) and 12.3% (N) at 2 °C to 32.5% (C) and 29.6% (N) at 14 °C. Reported depletion of C and N, while still significant, was lower at 18 °C than at 14 °C. Rate of depletion in C:N ratio decreased between developmental temperatures of 2 and 10 °C, before increasing again as temperatures increased further.

4. Discussion

4.1. Embryonic development

4.1.1. Thermal tolerance during development

Within a species' distribution, thermal tolerance ranges are often reported to vary between populations. Such ranges are ultimately dependent on temperature, and thus, shifts may occur with latitude, or in association with ocean currents or other factors affecting local water temperatures. This illustrates a high level of thermal plasticity in response to local temperatures, indicating that population level differences in reproductive adaptations exist. (e.g. Storch et al., 2009; Thatje et al., 2005; Zippay and Hofmann, 2010). Our results indicate this trend to be evident in *B. undatum*. In the present study, complete development was observed between 6 and 18 °C for a population at the southern end of the distribution, from the south coast of the UK. In comparison, populations of the common whelk from the Gulf of St. Lawrence, Canada, where the Labrador current causes low annual temperatures, develop in water temperatures of 2 to 3 °C (Martel et al., 1986a), and in Breidafjordur, Iceland, at the northern end of the species distribution, between 3 and 6 °C (Smith and Thatje, 2012; Smith, unpublished results). Similar fluctuations in thermal tolerance have been noted previously in gastropods (Zippay and Hofmann, 2010) and crustaceans (Storch et al., 2009; Thatje et al., 2005), with different populations having a narrow thermal tolerance range, which scaled with temperature and was specific to the latitude at which it was found. Within a single species population, previous studies have indicated thermal tolerance to often be lower during development than throughout adult life (Dawirs, 1985; deRivera et al., 2007; Gosselin and Chia, 1995; Weiss et al., 2009). For example, hatched juveniles of the gastropod *Nucella emarginata* were negatively impacted by temperatures of 30 °C, while adults of the same species could easily withstand such temperatures (Gosselin and Chia, 1995). In the present study, we found similar results with the thermal tolerance range identified during development (6 to 18 °C) being narrower than annual local water temperatures for the sampled population (4 to 22 °C). Such findings suggest that thermal tolerance during early ontogeny may be fundamental in setting a species' geographic limits.

Developmental success (to hatching) was greatest within the natural developmental temperature range of the local populations (6 and 10 °C) and decreased at temperatures outside this. Similar scenarios have been observed on many previous occasions in marine ectotherms including temperate and sub-polar crustaceans (Anger et al., 2003; Johns, 1981) and tropical and polar echinoderms (Sewell and Young, 1999; Stanwell-Smith and Peck, 1998). Interestingly, in each of the above studies, optimum success was observed at temperatures towards the middle or top of the thermal range investigated. In comparison, in the present investigation peak survivorship was at temperatures at the bottom of the thermal tolerance window for development and at the lower end of the habitat temperature limits for adults of this population. This may be related to the cold-water spawning observed in *B. undatum*. Several authors have linked spawning to falling temperatures, indicating low temperature to induce spawning in this species (Hancock, 1967; Smith and Thatje, 2013; Valentinsson, 2002). The preference for colder temperatures, evident in *B. undatum* is likely linked to the deep sea and cold-water origin of neogastropods (Hickman, 1984; Jablonski and Bottjer, 1991).

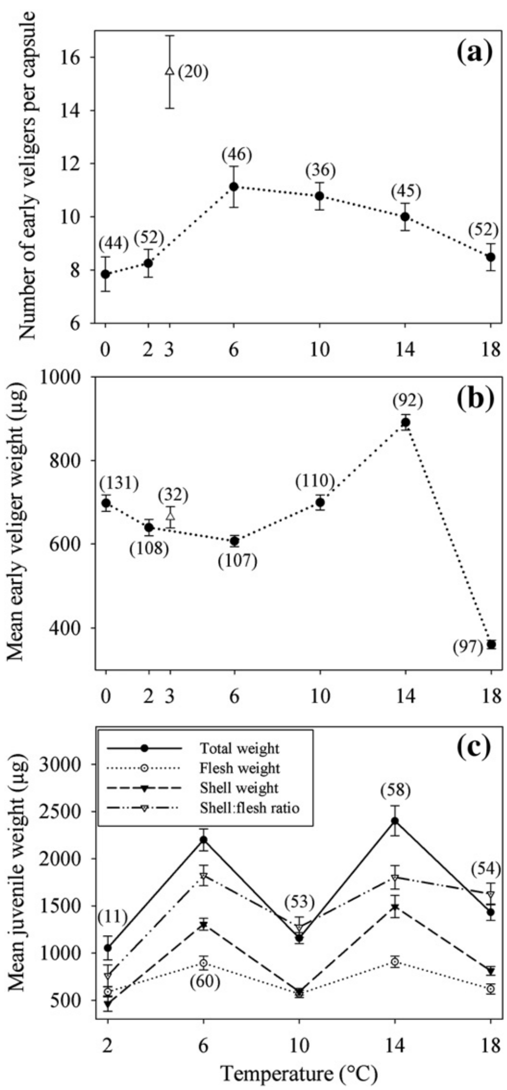

Fig. 2. (a) Number of early veligers per capsule, (b) early veliger weight (post nurse egg consumption) and (c) juvenile weights for *Buccinum undatum*. For (a) and (b), filled circled represent samples collected from the Solent (50°47′ N, 001°15′ W), off the south coast of the UK, and developed at temperatures ranging 0 to 18 °C. Open triangles represent samples collected from Breidafjordur (65°00′ N, 023°30′ W), Iceland, and developed at approximately 3 °C. For (c), symbols are displayed in the legend. Analysis by Kruskal–Wallis indicated number of early veligers per capsule, early veliger weight and juvenile total, flesh and shell weights and shell:flesh ratio to all be significantly affected by temperature ($p \leq 0.001$). Error bars display standard error. Bracketed numbers indicate sample size n.

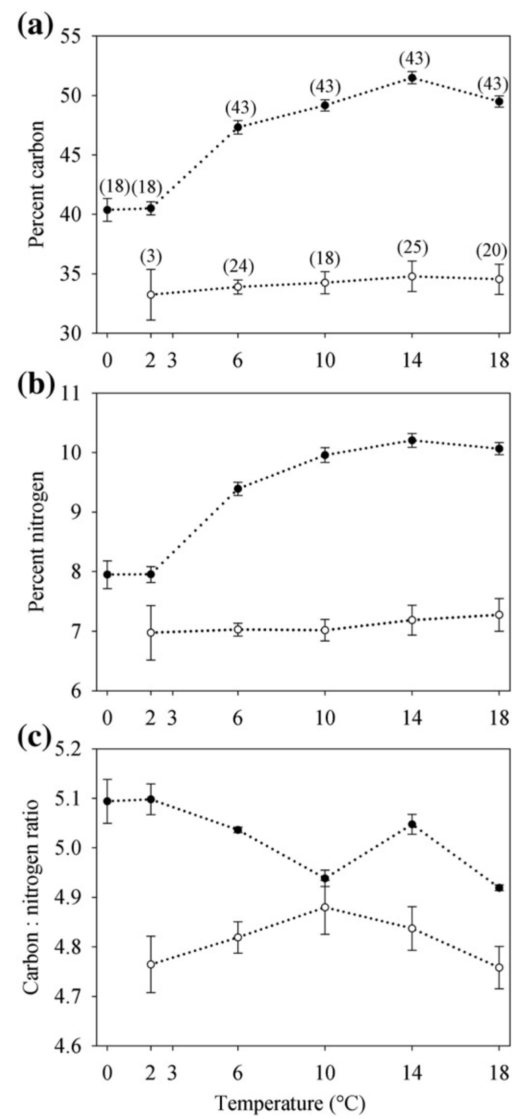

Fig. 3. Changes in (a) carbon, (b) nitrogen and (c) C:N ratio between early veliger (closed circles) and hatching juvenile (open circles) *Buccinum undatum*. Samples are from the Solent (50°47′ N, 001°15′ W), off the south coast of the UK, and developed at temperatures ranging 0 to 18 °C. Analysis by Kruskal–Wallis indicated carbon, nitrogen and C:N ratio to be significantly affected by temperature in early veligers ($p \leq 0.001$) but not in hatching juveniles. At each temperature, significant differences in carbon, nitrogen and C:N ratios were found between early veligers and juveniles ($p \leq 0.05$). Error bars display standard error. Bracketed numbers on plot (a) display sample size *n* values, and are identical for all 3 plots.

4.1.2. Developmental timing

The difference in developmental timing between 6 and 10 °C was large compared to other temperatures, with egg masses taking an additional 70 days to develop at 6 °C. In comparison, total time to hatching only varied by 7 days in duration between 10 and 14 °C, and 14 and 18 °C. Other studies have reported similar results, with small increases in temperature at the lower end of the thermal range causing much larger reductions in development time than similar changes at the upper end of the thermal range (Anger et al., 2003; Johns, 1981). For example, Anger et al. (2003) reported that time from hatching to metamorphosis in the lithodid crab *Paralomis granulosa* decreased from 116 days at 3 °C to 53, 40, 31 and 24 days at 6, 9, 12 and 15 °C, respectively. In *B. undatum*, egg masses laid in late December begin development in January and February as temperatures are reaching their lowest, whereas those laid in late February develop as temperatures are warming again. The difference in developmental timing between 6 and 10 °C suggests egg masses may hatch at approximately the same time (late spring, early summer), despite a two-month lag in laying time. This suggests there are ecological benefits to hatching at this time of year, probably related to obtaining optimum growth and survival. Amongst other things this may include factors such as temperature, food availability, and predatory pressures (Giese, 1959; Pechenik, 1999; Thorson, 1950).

4.2. Intracapsular content through early ontogeny

4.2.1. Number and size of early veligers

In the present investigation, the number of early veligers developing per capsule was used as a proxy for number of hatching juveniles, and therefore reproductive output. In *B. undatum*, number of embryos per capsule does not vary between early veliger development and juveniles hatching (Hancock, 1967; Martel et al., 1986a; Smith and Thatje, 2013). On rare occasions where an embryo does not complete development, soft and hard (shell) body parts remain obvious inside the capsule; although a scavenger when in adult form, developing *B. undatum* has been suggested to be unable to consume for the majority of intracapsular development (Smith and Thatje, 2013; Smith, unpublished results). The unconsumed nurse eggs observed in capsules throughout, at 0, 2 and 18 °C give support for this assumption. While change in number of embryos per capsule has never been investigated in *B. undatum* across a wide thermal range such as that examined in the present study, we would expect to observe any deceased embryos during capsule dissection. In the present study, none were seen, indicating there to be no change in number of developing embryos through ontogeny at any temperature. We therefore considered number of early veligers per capsule to be a good proxy for reproductive output regardless of developmental temperature.

A general trend was observed for the total number of early veligers per capsule to decrease with increasing temperatures. In contrast, the occurrence of "empty" embryos appeared to increase with temperature. Since these embryos had not taken up any nutrition for development, and were not observed past the pediveliger stage, it was presumed they did not develop successfully. The increase in empty embryos and the decrease in number of developing embryos will potentially reduce the number of offspring completing development at higher temperatures and indicates that within each capsule, individuals developing later due to asynchrony in timings are at a greater disadvantage as temperatures increase. This suggests that despite the potential ecological benefits of rapid development to hatching during later, warmer months of the year, hatchling number and quality may be reduced under such conditions.

Both high and low temperatures have previously been shown to retard early development (Anger et al., 2004; Byrne et al., 2009; Fernandez et al., 2006; Gallardo and Cancino, 2009; Sewell and Young, 1999). As postulated above, it is likely that the present study population of *B. undatum* has adapted to develop optimally at local temperatures and any deviation above or below this is unfavourable. The observed trend of increasing early veliger weight with increasing temperature can be explained by examining the number of embryos per capsule. Since in this study there was no difference in the number of nurse eggs per capsule, in capsules where a smaller number of embryos developed a higher number of nurse eggs was available for each developing embryo, thus leading to a greater mean embryo weight. As well as the number of nurse eggs consumed, embryo weight may also be affected by nurse egg size, and this factor should therefore be briefly considered. In *B. undatum*, nurse egg size is significantly related to capsule volume (Smith, unpublished results). However, since capsules of a narrow range of volumes were used in the present investigation, nurse egg size was expected to be homogenous across the samples and was therefore not considered a significant factor affecting embryo weight. In contrast to early veliger weight, no clear pattern was found between juvenile weight and temperature. The double-peak observed in juvenile weight was unexpected and remains subject to further investigation.

Interestingly, developmental data from egg masses collected from Breidafjordur, Iceland, at the northern end of the population distribution, fitted in with trends observed in the present investigation (Smith, unpublished results). Capsules of an equal size to those used in the present study, developed at approximately 3 °C, were found to have a greater number of embryos developing per capsule than those observed in the present investigation in egg masses developing between 6 and 18 °C. Mean early veliger weight for the northern population was similar to that reported in the present investigation for embryos from the southern population developed at 6 and 10 °C. This indicates such weights to be optimal for development and again highlights local adaptations, which may have occurred (Fig. 2a–b).

4.2.2. Intracapsular nurse egg partitioning

During early development, embryos of the common whelk consume nurse eggs at a rapid rate, storing them in the mid-gut for later use (Portmann, 1925; Smith and Thatje, 2013). As an example, at 6 °C, nurse egg consumption within a capsule was completed over 5 days out of a 140-day intracapsular development period and escalated as temperature increased. The intracapsular asynchrony observed during early development, however, means not all embryos begin nurse egg consumption at the same time. This results in large differences in the number of nurse eggs taken up by each embryo within the same capsule, leading to considerable variations in both early veliger and hatching juvenile weights. While large differences in nurse egg consumption and offspring size have previously been reported for *B. undatum* (Smith and Thatje, 2013) and also for a small number of muricid gastropods (Cumplido et al., 2011; Gallardo, 1979; González and Gallardo, 1999), the incidence of this appears rare. Rather, nurse egg partitioning is usually quite regular within a capsule (Chaparro and Paschke, 1990; Chaparro et al., 1999; Rivest, 1983; Spight, 1976). The findings reported here give support for previous suggestions that a high level of competition occurs during development in *B. undatum* and indicate that a juvenile's predisposition for later life is highly dependent on how well it competes during these early days. Individuals that consume a higher number of nurse eggs inevitably hatch at a larger size. Larger offspring are widely assumed to be of greater quality than smaller siblings, being less prone to factors like predation, starvation and physical stress (e.g. Gosselin and Rehak, 2007; Lloyd and Gosselin, 2007; Przelawski, 2004, 2011; Rivest, 1983; Spight, 1976; Thorson, 1950).

4.3. Bioenergetics through early development

Following the initial positive relationship observed in early veligers between temperature and proportions of C and N, by hatching, proportions were comparable across all temperatures. Thus, although at higher temperatures more energy reserves are accumulated during early development, a greater metabolic loss is incurred, probably related to larger metabolic demands at higher temperatures. These results indicate that in *B. undatum* shifts in the energy budget occur across the species range during early development to allow for external temperature differences. This allows all juveniles to be at the same relative bioenergetic predisposition at hatching, regardless of temperature. Similar findings have previously been reported for the crustaceans *Artemia salina* over a four-day period (Evjemo et al., 2001) and *Cherax quadricarinatus* over a 20 to 37-day period (García-Guerrero et al., 2003). In both these studies bioenergetics continued to differ with temperature, regardless of developmental stage, demonstrating high plasticity in these species with embryos rapidly adapting to ambient temperatures.

The decreases in C:N ratio observed in the present investigation, imply lipids to be used preferentially over proteins. The observed decrease was greatest at high and low temperatures, indicating lipid metabolism to be higher at the extremes of the thermal range. This suggests that while some adaptations have taken place, the cost of development continues to be higher at temperatures outside the species natural ambient range.

5. Conclusions

Given current rates of seawater warming (Hughes et al., 2010; MCCIP, 2010) it is possible that temperatures of 14 °C will be observed during the current developmental period for southern populations within the next four decades. The developmental success observed for *B. undatum* at this temperature indicates the possibility that range shifts may not be observed in southern populations of this species. Increasing temperatures may, however, impair initial spawning and are detrimental to total reproductive output. While development was successful at 14 °C, costs were incurred. Offspring numbers were reduced and each embryo required a greater amount of energetic reserves to reach the same relative condition at hatching. Ultimately, at the current upper thermal limit for development in *B. undatum* reproductive output may be impacted, negatively affecting population size at the southern extreme of the species distribution.

Acknowledgments

Thanks are given to the skipper and crew of RV Callista (University of Southampton) for their help with sample collection. Thanks are also given to Shir Akbari (University of Southampton) for his help with elemental analysis, and to Adam Reed, Alastair Brown, and Andrew Oliphant for help with animal maintenance. This work was supported by grants from the Total Foundation (Abyss2100) to S.T. and the Malacological Society to K.S.

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Appendix E: Smith KE, Thatje S (*in press*) The subtle intracapsular survival of the fittest: maternal investment, sibling conflict or environmental effects? Ecology http://dx.doi.org/10.1890/12-1701.1

Running head: Intracapsular survival of the fittest

The subtle intracapsular survival of the fittest: maternal investment, sibling conflict or environmental effects?

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Abstract

Developmental resource partitioning and the consequent offspring size variations are of fundamental importance for marine invertebrates, in both an ecological and evolutionary context. Typically, differences are attributed to maternal investment and the environmental factors determining this; additional variables, such as environmental factors affecting development, are rarely discussed. During intracapsular development for example, sibling conflict has the potential to affect resource partitioning. Here, we investigate encapsulated development in the marine gastropod *Buccinum undatum.* We examine the effects of maternal investment and temperature on intracapsular resource partitioning in this species. Reproductive output was positively influenced by maternal investment but additionally, temperature and sibling conflict significantly affected offspring size, number and quality *during* development. Increased temperature led to reduced offspring number, and a combination of high sibling competition and asynchronous early development resulted in a common occurrence of 'empty' embryos, which received no nutrition at all. The proportion of 'empty' embryos increased with both temperature and capsule size. Additionally, a novel example of a risk in sibling conflict was observed; embryos cannibalised by others during early development ingested nurse eggs from inside the consumer, killing it in a 'Trojan horse' scenario. Our results highlight the complexity surrounding offspring fitness. Encapsulation should be considered as significant in determining maternal output. Considering predicted increases in ocean temperatures, this may impact offspring quality and consequently species distribution and abundance.

Key words: cannibalism; embryology; life-history theory; maternal investment; nurse egg consumption; resource partitioning; sibling rivalry; temperature**.**

Introduction

Offspring fitness is of fundamental importance in selection and consequently, a species' success depends on its ability to reproduce effectively. Selection favours those that produce the largest possible number of surviving young. Traditionally, quality is believed to increase with size, with larger offspring regularly (although not always) showing higher growth rates and survival in marine invertebrates (Rivest 1983; Moran and Emlet 2001; Marshall et al. 2006) and other taxa including birds (Rhymer 1988), insects, reptiles and plants (Kingsolver and Huey 2008). The increased maternal investment associated with producing larger, fitter young (Vance 1973) does however result in energetic trade-offs occurring (Vance 1973; Smith and Fretwell 1974). Most contemporary life history theory suggests 'optimal' offspring size may vary with environmental conditions, and to maximise fitness, mothers will adjust the size and number of offspring accordingly (McGinley et al. 1987; Bernardo 1996; Mousseau and Fox 1998). Amongst other variables, stress, habitat quality, temperature, maternal size and maternal nutritional status have all been identified as factors affecting the trade-off between size and number of offspring (Hadfield 1989; Marshall and Keough 2007; Crean and Marshall 2009; Kamel et al. 2010b).

Traditionally, maternal output is perceived to be greatest if maternal investment (i.e. the resources invested into offspring by the mother) is even amongst all offspring; consequently, the assumption is regularly made that the offspring produced by one female are of an equal size and fitness (Thorson 1950; Smith and Fretwell 1974; Bernardo 1996; Parker et al. 2002). In reality, however, intra-clutch variations in offspring size are often observed, and over the past decade have become a popular topic for debate. Within-brood variations in offspring size have now been recognised in diverse species, occurring both between co-habiting embryos exhibiting one developmental mode (e.g. Fox and Czesak 2000; Kudo 2001; Dziminski and Alford 2005; Marshall et al. 2008b), and between co-habiting embryos exhibiting different developmental modes (Poecilogony) (e.g. Gibson et al. 1999; Oyarzun and Strathmann 2011; Kesäniemi et al. 2012; Gibson and Carver 2013).

Bet-hedging, in which females inhabiting variable environments produce offspring of a range of sizes or developmental modes, is one of several explanations that have been suggested to explain within-brood variations in offspring size (Slatkin 1974; Philippi and Seger 1989; Koops et al. 2003; Marshall et al. 2008a; Crean and Marshall 2009). The reported differences are typically understood to be a maternal effect, occurring as a direct result of variations in the provisioning allocated to each embryo (Krug et al. 2012). Explanations for this often focus on the environmental factors affecting maternal provisioning (Bernardo 1996; Moran and Emlet 2001), and only rarely have other variables been considered (e.g. Lardies and Fernandez 2002; Fernandez et al. 2006; Parker et al. 2002; Kamel et al. 2010a, 2010b; Oyarzyn and Strathmann 2011). Assuming no poecilogony, species exhibiting intracapsular development often receive maternal provisioning during development in the form of nurse eggs, which are consumed for nutrition. Nutrients may alternatively be allocated at production to each embryo, or be obtained from intracapsular fluids or capsule walls (Ojeda and Chaparro 2004). Within each capsule, embryos are usually equal in their ability to consume, and therefore, intracapsular size variations are rarely observed (Spight 1976; Rivest 1983; Chaparro and Paschke 1990; Strathmann 1995). Within-brood variation in offspring size is instead achieved through irregular allocation of nurse eggs and embryos to capsules (Thorson 1950; Spight 1976; Rivest 1983). However, in a handful of species, size differences have been observed between the adelphophagic or direct developing embryos in one capsule (Portmann 1925; Gallardo 1979; González and Gallardo 1999; Strathmann and Strathmann 2006; Cumplido et al. 2011; Smith and Thatje 2013). Within a capsule, individuals, which consume more nutritional reserves, inevitably hatch at a larger size, and are typically understood to have a greater chance of survival. In these examples, the differences typically occur as a result of competition for intracapsular resources, or occasionally through cannibalism of partly developed siblings. Some maternal control may exist, for example some spionid polychaetes manually open egg capsules to limit cannibalism (e.g. Kamel et al. 2010b; Oyarzun and Strathmann 2011), but maternal effect may not always be the primary determinate of individual offspring fitness. An alternative source of variation is sibling conflict, although in the oceans this is rarely considered a significant factor, and as a result has received little attention to date (e.g. Parker et al. 2002; Kamel et al. 2010a, 2010b).

Of particular interest is the intracapsular resource partitioning in *Buccinum undatum,* a common and commercially important North Atlantic gastropod. This species exhibits direct development, which is initially asynchronous, with no observed poecilogony. Nurse eggs are the primary nutritional reserve available throughout this period (Valentinsson 2002; Smith and Thatje 2013). To our knowledge, in other species exhibiting intracapsular development, all embryos obtain nutrition of some form, either incorporated into the egg during production, during intracapsular development, or during a later planktotrophic developmental stage (Gallardo 1979; González and Gallardo 1999; Kamel et al. 2010b; Cumplido et al. 2011). In contrast to most species however, large intracapsular size differences have been observed between offspring, resulting in large size differences at hatching (Fig. 1a) (Portmann 1925; Smith and Thatje 2013). Additionally, common occurrences of ‘empty’ embryos have been reported (but not quantified) inside capsules (Figure 1b), whereby some embryos do not consume any nurse eggs during development (Smith and Thatje 2013). These embryos often successfully complete early development, but do not survive to hatching, indicating an unusual example of sibling conflict. Lack of survival has been observed through the presence of small, thin empty shells, and the absence of live ‘empty’ embryos after the pediveliger stage (Smith and Thatje, 2013; authors, unpublished data). Sibling conflict may be reflected in the variety in number of nurse eggs consumed by different offspring and the occurrence of 'empty' embryos; embryos that did not succeed in acquiring any nutrition at all. Furthermore, evidence suggests the proportion of 'empty' embryos to be accentuated by increased temperature (Smith et al. 2013), suggesting environmental factors may play a significant role in intracapsular resource partitioning, contributing to the variations resulting from sibling conflict. Considering this, predicted increases in seawater temperature (Hughes et al. 2010) could lead to a reduction in the number of individuals’ successfully developing, potentially affecting species abundance. This rare example demonstrates a different effect of encapsulation, the unusual nature of which remains un-described.

Understanding the dynamics contributing to developmental resource partitioning in offspring is of great significance, in both the evolutionary and ecological context. Here, we contribute to discussion of the evolution of egg size and offspring size in marine invertebrates, with emphasis on the rarely considered effects of sibling conflict. We investigate the unusual intracapsular resource partitioning reported in *B. undatum.* We use this species to compare the consequences of maternal investment and environmental effects on sibling conflict during intracapsular development.

Materials and methods

Study species

Buccinum undatum is a common North Atlantic shallow-water gastropod. Over the past few decades it has become an increasingly important commercial species in North America and the UK, with global demand continuously rising (Lawlor and Vause 2009; Department of Marine Resources www.maine.gov/dmr/rm/whelks.html). The southern end of its distribution falls around the south coast of the UK, where many local fisheries depend on income from this species (Lawlor and Vause 2009). Here, the common whelk is a winter spawner, with egg laying and complete development occurring when water temperatures are at their lowest, ranging approximately 4 to 10°C (Smith and Thatje 2013). Females group to lay; each one produces between 80 and 150 egg capsules, which collectively create large egg masses containing up to thousands of capsules. Maternal provisioning to each capsule increases with female size; larger females produce larger capsules, which contain a greater number of nurse eggs and embryos. Each capsule contains between approximately 500 and 2600 eggs, of which between approximately 2 and 21 develop into embryos. Initial development is asynchronous and within a capsule there may be several days between the first and last embryos beginning nurse egg consumption. Following this, total nurse egg consumption within a capsule occurs over approximately 2 to 5 days, depending on temperature. Development becomes synchronous during the veliger stage, following nurse egg consumption. Across the entire species range, total intracapsular development takes between 2.5 and 8 months (Martel et al. 1986; Smith and Thatje 2013; Smith et al. 2013).

Egg mass farming and maintenance

Buccinum undatum egg masses were farmed in the Seawater aquarium at the National Oceanography Centre, Southampton, as described by Smith and Thatje (2013). Adult whelks were collected by Viviers from the Solent, UK (50°39’ N, 001°37’ W) in late November 2010 using whelk traps (~15m depth) (www.fishmarketportsmouth.co.uk). Egg laying occurred predominantly at water temperatures below 8˚C, with each egg mass being contributed to by several females. Egg masses were removed from aquarium walls 24 hours after laying had ceased.

Since the number of nurse eggs and embryos per capsule is positively correlated with capsule size in *B. undatum,* (Valentinsson 2002; Smith and Thatje 2013), egg masses were divided into size classes depending on capsule volume (calculated according to Smith and Thatje 2013). Capsules with volumes of less than 100 mm^3 were classified as small, between 100 and 200 mm^3 were classified as medium and above 200 mm^3, as large. Egg masses were maintained in separate 1.8L incubation tanks containing aerated, 1µm filtered seawater. Each tank was acclimated stepwise to one of four temperatures (6, 10, 14, 18˚C; 1˚C temperature change every 24 hours), with capsules from every size class being developed at 10˚C and only medium capsules being developed at all other temperatures.

Intracapsular resource partitioning

Development was assessed according to Smith and Thatje (2013). Egg masses were inspected twice a week until early veligers became visible through the semi-transparent capsule walls. They were then examined daily until all nurse eggs had been consumed. At temperatures ranging 6 to 18˚C, total nurse egg consumption in *B. undatum* has been shown to take between approximately 2 and 5 days (Smith et al. 2013). If nurse egg consumption appeared to not have ended 24 hours after the expected completion time for the experimental temperature, then when dissected only capsules containing veligers which were too developed to continue nurse egg consumption were examined (see Smith and Thatje (2013) for developmental stages). Fifteen capsules were randomly dissected from every mass, the number of embryos inside each was counted and the proportion of 'empty' embryos determined (classified as individuals who had consumed ≤ 1 nurse egg). Any nurse eggs, which had been consumed, were visible inside an embryo as yellow balls inside the translucent embryo body. Empty embryos could therefore be determined without dissection (Figure 1b).

Nurse egg partitioning

In order to assess nurse egg partitioning, the embryos from 10 of these capsules were individually dissected. Following initial consumption, in *B. undatum* nurse eggs are stored undamaged in the mid-gut prior to use, allowing number of eggs consumed to be easily distinguished. The outer membrane of the embryo was pierced, letting the consumed nurse eggs spill out (Figure 1c), and allowing the number of nurse eggs to be counted. For each capsule, the range in number of eggs consumed within a capsule was also established, by subtracting the lowest number of eggs consumed by an embryo from the highest number of eggs consumed by an embryo.

Embryo dry weight

The embryos from the remaining 5 capsules were removed, stored individually in pre-weighed (6mm x 4mm) tin capsules and frozen at -80˚C. Only embryos containing more than 1 nurse egg were sampled, since 'empty' embryos were very fragile and were regularly damaged during sampling. A minimum of 30 embryos was weighed from each mass. If this number was not achieved from the 5 capsules already open, additional capsules were opened to obtain sample numbers. Samples were later freeze-dried over 24 hours and then dry weight (DW) was determined on a microbalance (accurate to 1μg) to assess changes in mean embryo weight with temperature.

Elemental composition

Elemental analysis was carried out on embryos in order to assess bioenergetics. Elemental carbon (C) and nitrogen (N) correlate to lipid and protein levels, and therefore indicate the physiological condition of an organism. Changes in structure and metabolic machinery are reflected by N, and information on nutritional reserves provided by C (Anger 2001). Twenty-five of the freeze-dried samples from each condition were analysed using a Fison (Carlo Erba) 1108 Elemental Analyser. The analyser was calibrated using chitin as a standard (% C = 44.71; % N = 6.79). Proportions of C and N were determined during analysis, and biomass of each was then calculated.

Maximum consumption

The maximum number of nurse eggs a veliger was capable of consuming was assessed from the content of 15 medium capsules randomly selected from an egg mass developed at 10˚C. As soon as early veligers became visible inside a capsule, the translucent marginally concave face of the capsule was removed, leaving the deeper, ribbed face. All but one of the early veligers was removed from the capsule. This embryo remained with the unconsumed nurse eggs in a covered petri dish filled with pre-incubated (10˚C; 1 µm, 12h UV filtered) seawater until it had reached the veliger stage and nurse egg consumption was no longer possible. At this point the embryo was dissected as described previously, and the number of consumed eggs determined.

Statistics

‘Empty’ embryos were included in counts for all data except dry weights and elemental (C, N), due to the small size, and therefore weight of these individuals.

A non-parametric Kruskal-Wallis one-way analysis of variance was used to compare number of embryos developing per capsule, number of nurse eggs consumed per embryo, embryo DW and proportion of ‘empty’ embryos to developmental temperature and capsule size. The number of nurse eggs consumed by embryos developing in small, medium and large capsules at 10˚C was also compared to the number of nurse eggs consumed by the maximum consumption embryos. A non-parametric test was used due to unbalanced and un-matched data sets for number of nurse eggs consumed per embryo and embryo DW. *Post hoc* analysis was carried out using Dunn's test. Since both C and N weights were determined using DW, an analysis of covariance was carried out (using DW as a covariate), to examine relationships between C and N, and developmental temperature or capsule size. Data transformation (1/x) was carried out prior to analysis of covariance. Regression analysis was used on all 10˚C samples (all capsule sizes) to examine relationships between proportion of empty embryos, and number of embryos per capsule and capsule volume. Range in number of eggs consumed by embryos was also compared to number of embryos per capsule, capsule volume and total number of nurse eggs per capsule (determined by summing the number of nurse eggs consumed by all embryos in a capsule). Multiple and independent regression analysis was carried out to compare the dependent variables (proportion of empty embryos and range in number of eggs consumed) to the above factors.

***Buccinum undatum* from Breiðafjörður, Iceland**

Four egg masses (medium capsule size) were hand collected from beaches around Breiðafjörður, Iceland, near the northern end of the species distribution. These samples had developed naturally at temperatures ranging 3 to 4°C, and were used for an inter-population comparison. Number of early veligers per capsule (26 capsules) and early veliger dry weights (7 capsules, 8 samples per capsule) were sampled from capsules with a volume of 100 to 200 mm^3. These data were used only as a comparison and no statistical analysis was carried out.

Results

Embryo midgut content

During development, *B. undatum* embryos engulf nurse eggs and store them in the midgut, conserved whole for later nutritional use. When embryos were pierced to enable nurse eggs to be counted, on three separate occasions an early veliger was found inside, indicating it to have been consumed by the more developed embryo.

Intracapsular resource partitioning

In total, 170, 116, 124 and 78 embryos were dissected from medium capsules at 6, 10, 14 and 18°C respectively (10 capsules per temperature), and 47 and 257 embryos were dissected from small and large capsules, respectively (10°C; 10 capsules per size class). All embryos from every capsule examined were alive. Weights were collected from 52, 56, 38 and 30 embryos from medium capsules at 6, 10, 14 and 18°C respectively (5 capsules per temperature), and 31 and 78 embryos from small and large capsules, respectively (10°C; 9 small capsules, 5 large capsules) and 56 embryos from the Iceland population. Patterns showed mean number of embryos to decrease with increasing temperature (across both populations) and increased with capsule size (Figure 2a, b). In contrast, proportion of 'empty' embryos increased with both increasing temperature and capsule size. Mean number of nurse eggs per embryo, embryo DW, and C and N increased between 6 and 14°C, before decreasing at 18°C. When comparing capsule size, each of these variables was greatest in medium capsules (Table 1, Figure 2c, d). Mean embryo dry weight for the Iceland population was between that observed at 6 and 10°C for the Solent population. In every capsule examined, of each size and at each developmental temperature, a large range was observed in number of eggs consumed by any one embryo (Table 2; Figure 3, 4a, b). Across all capsules, (excluding the maximum consumption experiment), individual embryos consumed between 0 and 260 nurse eggs. 'Empty' embryos were commonly observed at every temperature and in all capsule sizes. At 18°C, every capsule opened contained additional nurse eggs, which had not been consumed by developing embryos. These embryos had developed to the point at which no further nurse egg consumption was possible, before all nurse eggs had been consumed. Embryos developing at 18°C consumed fewer nurse eggs than embryos developing at any other temperature.

Analysis indicated number of embryos per capsule ($H = 23.48$; $p \leq 0.001$), number of nurse eggs per embryo ($H = 144.78$; $p \leq 0.001$), DW ($H = 37.61$; $p \leq 0.001$) and proportion of 'empty' embryos ($H = 25.59$; $p \leq 0.001$) to be significantly affected by temperature. Post hoc analysis (Figure 2a, b) showed all 18°C data to be significantly different to all other temperatures ($p \leq 0.05$). For all variables except number of nurse eggs per embryo, 6°C data also differed significantly from 14°C data ($p \leq 0.05$). Analysis indicated number of embryos per capsule ($H = 38.91$; $p \leq 0.001$), number of nurse eggs per embryo ($H = 35.54$; $p \leq 0.001$), DW ($H = 10.87$; $p \leq 0.005$) and proportion of 'empty' embryos ($H = 15.40$; $p \leq 0.001$) to be significantly affected by capsule size. Post hoc analysis (Figure 2a, b) showed all data to differ significantly between all capsule sizes ($p \leq 0.05$).

Multiple regression analysis, which considered all independent variables in one test, indicated at least one independent variable (number of embryos per capsule, capsule volume or total number of nurse eggs per capsule) to significantly affect range in number of eggs consumed by embryos within a capsule ($p \leq 0.01$). Multiple regression analysis also indicated at least one independent variable (number of embryos per capsule or capsule volume) to significantly affect proportion of 'empty' embryos ($p \leq 0.001$). Independent regression analysis, comparing dependent variables to each independent variable separately, confirmed a significant relationship between each of the above independent variables and either range in number of eggs consumed by embryos within a capsule or proportion of 'empty' embryos ($p \leq 0.001$).

Analysis of covariance indicated both C and N weights to be significantly affected to embryo DW under all conditions ($p \leq 0.001$). Both values were also significantly affected by developmental temperature ($p \leq 0.001$). A significant effect for capsule size was not demonstrated for C ($p = 0.064$) and no effect of capsule size was observed for N ($p = 0.729$).

Maximum consumption

'Maximum consumption' embryos consumed between 245 and 325 nurse eggs (Figure 4a, b). This was significantly different from the number of eggs consumed by embryos developing naturally at 10°C, in either small, medium or large capsules ($p \leq 0.001$). All maximum consumption embryos consumed more nurse eggs than any individual that developed naturally at any temperature, apart from one individual that developed at 14°C who consumed 260 eggs.

Discussion

Developmental resource partitioning in marine invertebrates is of fundamental importance and the resulting variations in offspring size may be of both ecological and evolutionary significance. One hypothesis for the fluctuation of maternal provisioning given to offspring from one clutch in a variable environment is bet-hedging (Slatkin 1974; Philippi and Seger 1989; Koops et al. 2003; Marshall et al. 2008a; Crean and Marshall 2009). Other hypotheses include selection for differing capabilities, as is observed with poecilogony, and unequal allocation of resources to offspring (e.g. Rivest 1983; Gibson et al. 1999; Oyarzun and Strathmann 2011). Explanations for these patterns often focus on maternal investment, and the influence that environmental factors have on maternal investment (Bernardo 1996; Moran and Emlet 2001), with less attention being paid to the effect of competition among siblings on size variation (e.g. Parker et al. 2002; Kamel et al. 2010a, 2010b). Our results highlight the adverse effects that sibling rivalry can have on offspring size, number and quality, and additionally, emphasise that not only maternal investment, but also environmental factors to significantly affect resource partitioning during intracapsular development. *Buccinum undatum* provides an example of the varied potential outcomes of encapsulation and also an unusual and novel kind of sibling cannibalism.

The process of encapsulation allows for an elevated occurrence of sibling-to-sibling interaction, which presents a high potential for competition (Marshall and Keough 2007). Examples of species with variable resource partitioning within a capsule are rare, but have been reported in cases of poecilogony (Kamel et al. 2010b; Oyarzun and Strathmann 2011), sibling cannibalism or arrested development in siblings (Strathmann and Strathmann 2006; Kamel et al. 2010b; Oyarzun and Strathmann 2011), or through varied nurse egg consumption by embryos (Portmann 1925; Gallardo 1979; González and Gallardo 1999; Cumplido et al. 2011; Smith and Thatje 2013). In the Muricidae, in which this latter example is typically observed (e.g. Gallardo 1979; González and Gallardo 1999; Cumplido et al. 2011), intracapsular sizes are variable, but to our knowledge all offspring receive some nutrition. The differences seen have been attributed to competition, which occurs over the weeks or months of nurse egg consumption during development (e.g. Cumplido et al. 2011). In the buccinid *B. undatum,* intracapsular size differences are accentuated by the particularly rapid nurse egg consumption (typically 2 to 5 days) and the asynchrony in early development, observed in this species. Asynchronous development may result from the process of fertilisation and egg distribution during encapsulation. The resulting inequality in developmental stage and nurse egg consumption, can be hypothesised to be favoured by selection, or simply to be a by-product of reproduction in *B. undatum*. The earliest developers have a significant advantage over later developers, and the most delayed embryos regularly develop after all nurse eggs have been consumed (Smith and Thatje 2013). Although asynchronous development is not uncommon (e.g. Fernandez et al. 2006), nurse egg consumption is typically slower. Usually, asynchrony within a capsule is compensated for by the slower nurse egg consumption, leading to smaller intracapsular size variations than is observed in *B. undatum*. This selection for the fittest inhibits the number of embryo's successfully developing, as some out-compete others for the limited resources available.

Maternal provisioning to a capsule typically increases with both female and capsule size (Valentinsson et al 2002; Smith and Thatje 2013). In the present study, a higher total number of embryos developing per capsule was observed, but this was offset to a large degree by the increased proportion of 'empty' embryos observed in conjunction. The increased occurrence of 'empty' embryos and the lower mean weight observed in larger capsules suggests a lower nurse egg to embryo ratio. Consequently, embryos developing in medium capsules appeared to be better off; these individuals were on average larger and fitter than individuals in either large or small capsules. This may indicate that medium capsule sizes, and therefore intermediate numbers of embryos, are most beneficial for developing offspring. However, future work is necessary to test if this is an adaptive trend. Interestingly, the high number of eggs consumed by each embryo in the maximum consumption experiment indicated embryos to be capable of consuming many more nurse eggs than they naturally succeeded to do inside a capsule of any size. No 'large' embryos appear to develop naturally, in *B. undatum*; only 'medium' and 'small'.

Environmental factors play an important role in shaping life history. They affect not only maternal investment but also impact offspring growth and survival (Koops et al. 2003; Anger et al. 2004; Marshall and Keough 2007; Crean and Marshall 2009). Environmentally induced changes in offspring size or number occurring *during* embryonic development have, however, rarely been discussed in the context of life history theory (Lardies and Fernandez 2002; Kamel et al. 2010b; Oyarzun and Strathmann 2011). Our results indicate a temperature-mediated shift in number of embryos developing, embryo weight and bioenergetic content, and proportion of empty embryos within a capsule. Comparisons can be drawn to the poecilogonic spionid polychaete *Boccardia proboscidea.* Cannibalism of small planktotrophic offspring by large adelphophagic (nurse egg consuming) offspring increases with duration of encapsulation, and consequently, the number of individuals hatching is reduced, but their average size increases. Time of hatching is dependent on environmental temperature and is decided by the mother, who tears open each capsule (Kamel et al. 2010b; Oyarzun and Strathmann 2011). In this example, and as is typically seen in marine invertebrates, a larger number of smaller offspring were produced under warmer condition. This observation is regularly attributed to maternal investment (Thorson 1950; Clarke 1992; Marshall and Keough 2007; Marshall et al. 2008b) and for *B. proboscidea,* is also ultimately controlled by the mother. In contrast, in *B. undatum* a larger number of smaller offspring are produced under *lower* temperature conditions, suggesting that in colder years a higher number of less energetically fit offspring will hatch. This resource allocation, although atypical, generally results in offspring receiving a higher level of reserves under warmer conditions, which could compensate for the greater bioenergetic demand necessary for development at higher temperatures, as has previously been reported in this species (Smith et al. 2013) and other marine invertebrates (Evjemo et al. 2001; García-Guerrero et al. 2003). This result is further supported by the greater number of offspring observed developing in the *B. undatum* egg masses from Iceland, where temperatures are naturally lower than in the UK. Similar patterns have also been observed in other gastropods (e.g. Fernandez et al. 2006; Collin 2012). Rapid nurse egg consumption and asynchrony in early development may be adaptations, which have evolved within *B. undatum* to allow for optimal survival under different thermal scenarios. Although more 'empty' embryos were observed under warmer conditions, considering the increased bioenergetic demand at high temperature, this may promote an overall increase in survivors. Temperature clearly plays an important role in intracapsular resource partitioning in *B. undatum.* It could be argued that asynchronous development is a maternal strategy to allow for differing numbers of successful offspring under varying developmental temperatures; this may explain patterns of inequality in other species (e.g. Fernandez et al. 2006). This would not however, explain the increase in proportion of 'empty' embryos observed with increasing maternal investment, which appears to be of no maternal benefit.

Interestingly, increased developmental rates known to occur at higher temperatures eventually appear to limit nurse egg consumption. Head development, which begins at the veliger stage, prevents further nurse egg consumption (Smith and Thatje 2013). The data suggest that at 18°C, the rate at which individuals develop is greater than the rate at which nurse eggs can be consumed. As a result, only a small proportion of eggs are consumed before embryos are no longer able to feed, meaning a limited amount of energy is available to them for development to the juvenile stage. The existence of 'empty' embryos at this temperature, despite the unconsumed nurse eggs, highlights just how rapid development is.

The common occurrence of 'empty' embryos reported here provides a unique example of the risks involved with encapsulation. In the rare examples of species exhibiting intracapsular poecilogony, a similar occurrence is observed, with seemingly 'empty' embryos developing alongside adelphophagic embryos (e.g. Kamel et al. 2010b; Gibson and Carver 2013). In these examples however, the 'empty' embryos are planktotrophic, and feed post hatching. In contrast, in *B. undatum* the 'empty' embryos observed are direct developers and do not survive to hatching. During encapsulation, lack of survival by embryos has been noted in several species, including some vermetid gastropods (e.g. Strathmann and Strathmann 2006). In these examples however, the deceased embryos are understood to be consumed by surviving embryos. In this study in contrast, embryos were not consumed by siblings; between the veliger and hatching juvenile stages, embryos appear unable to feed and even the occasional dead, nurse-egg containing embryo observed within a capsule was not ingested (Authors, unpublished data). Instead, 'empty' embryos appear to be an unfortunate consequence of development and are effectively wasted. They therefore represent an unusual loss of maternal investment, uncommon in encapsulated developments studied to date.

Although rare, other examples of unexploited reproductive resources exist in marine invertebrates. For example, the gastropod *Lirabuccinum dirum* occasionally lays capsules, which contain nurse eggs but no embryos (Rivest 1983). Additionally, capsules of *B. proboscidea* are sometimes opened before all nurse eggs have been ingested (Kamel et al. 2010b). In the latter example however, mothers have been proposed to consume uneaten nurse eggs following the opening of capsules. Considering the low temperature origin of neogastropods (Jablonski and Bottjer 1991), and the reduction in number of 'empty' embryos with decreasing temperature seen here, we propose 'empty' embryos to be an evolutionary consequence of adaptation to warmer temperatures.

A curious feature to mention is the 'accidental' cannibalism observed in *B. undatum.* Early veligers appear to be relatively non-selective in what they consume, and occasionally, one was observed to have ingested another embryo, which had not yet begun development. Cannibalism is not uncommon during intracapsular development, and has been previously described in many species of gastropods and other marine invertebrates (e.g. Strathmann and Strathmann 2006; Kamel et al. 2010b). Generally, when cannibalism occurs, one individual will consume another in order to increase energy reserves for development. In *B. undatum*, however, the observed cannibalism does not have this result (Authors, unpublished data). Once ingested, embryos continued development inside the mid-gut of the consumer. They then took up nurse eggs from inside the mid-gut, and eventually burst out through the thin mantle surrounding the consumer (Figure 1d). In this 'Trojan horse' scenario the victim became the 'winner' and the cannibal did not survive. This appears to be a defence mechanism against sibling cannibalism, and also presents a scenario where later development can be beneficial to offspring. Here, the cannibalised embryo gains a surplus of nurse eggs without having to compete for them. A disadvantage of this is the possibility that that particular egg is digested before commencing development.

This paper contributes a unique example of the diverse outcomes of encapsulated development, contributing to discussions of the trade-off between offspring size and number, in life history theory. It illustrates the adverse effects that both sibling rivalry and environmental factors can play in defining embryonic development, highlighting the importance of understanding the roles that different variables play during development as well as how they affect maternal investment. The combined effects of temperature and competition on intracapsular resource partitioning, and ultimately offspring quality, are of particular importance given the growing attention, which has been given to climate warming over recent decades. If current sea temperatures continue to rise at similar rates as those observed today (the English Channel and the southern North Sea, 0.8°C increase per decade; Hughes et al. 2010), temperature increases of 4 to 8 degrees, as employed in this study, could become relevant in the next 50 to 100 years. Such increases in seawater temperature have the potential to cause population level changes in distribution and abundance. Initially however, the developmental plasticity observed in this species may facilitate survival, providing a balance is struck between embryos being large enough to have sufficient reserves for development and plentiful enough to sustain a population.

Acknowledgements

Thanks are given to Viviers, UK for their help with adult sample collection. Thanks also go to Adam Reed, Alastair Brown, and Andrew Oliphant for help with animal maintenance, and to Andrew Oliphant for helpful discussions on the topic. This work was supported by grants from the Total Foundation (Abyss2100) to ST and the Malacological Society to KS. We thank two anonymous reviewers for constructive criticism on the draft manuscript.

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Table 1. Number of embryos per capsule, dry weight (DW), contents of carbon (C), and nitrogen (N) (all in µg/individual) for *Buccinum undatum* embryos. Mean embryo weights are taken only from embryos, which have consumed □1 nurse egg.

Capsule size	Developmental temperature (˚C)	Number of embryos per capsule*		DW (µg)*		C (µg/ind)		N (µg/ind)		C:N ratio	
			S.D. (*n*)		S.D. (*n*)		S.D. (*n*)		S.D. (*n*)		S.D. (*n*)
Small	10	4.2	1.52 (15)	595.2	148.23 (31)	317.2	57.98 (25)	65.3	12.09 (25)	4.9	0.04 (25)
Medium	6	14.9	5.28 (15)	673.1	203.72 (52)	354.5	86.93 (25)	69.1	17.04 (25)	5.1	0.06 (25)
	10	12.2	2.60 (15)	764.7	294.47 (56)	411.0	132.07 (25)	82.6	27.20 (25)	5.0	0.07 (25)

	14	11.1	2.85 (15)	857.2	274.87 (38)	474.4	147.44 (25)	93.8	30.18 (25)	5.1	0.11 (25)
	18	7.8	2.51 (15)	367.0	202.15 (30)	231.0	88.21 (25)	46.8	18.07 (25)	4.9	0.11 (25)
Large	10	24.3	5.89 (15)	629.7	259.84 (78)	359.9	139.05 (25)	73.4	29.45 (25)	4.9	0.08 (25)
	3 (Iceland data)	15.2	6.01 (26)	742.8	276.56 (56)	-	-	-	-	-	-

*Kruskal-Wallis one-way analysis of variance indicates results to be significantly affected by both developmental temperature and by capsule size ($p \leq 0.005$).

Table 2. Range and number of nurse eggs consumed by *Buccinum undatum* embryos developed at temperatures ranging from 6°C to 18°C and in small, medium and large capsules. Number of embryos dissected (*n*) refers to all columns except the furthest right hand column. For the percentage of capsules containing embryos with ≤ 1 nurse egg, *n* = 15 for all conditions.

			Number of eggs consumed per embryo						
				Minimum range in one capsule		Maximum range in one capsule			
Capsule size	Developmental temperature (˚C)	Number of embryos dissected (*n*)	Range across all capsules	Number of embryos	Range (number of eggs consumed)	Number of embryos	Range (number of eggs consumed)	Mean number of eggs consumed by one embryo in one capsule*	Percent of capsules containing embryos with ≤ 1 nurse egg*
Small	10	47	0-174	6	30 (48-78)	4	173 (1-174)	77.0	20%
Medium	6	170	1-239	15	38 (20-58)	14	138 (20-158)	102.3	13%

	10	116	0-182	10	67 (76-143)	9	182 (0-182)	112.6	40%
	14	124	0-260	10	60 (49-109)	16	243 (17-260)	148.9	67%
	18	78	0-92	4	22 (5-27)	12	92 (0-92)	42.4	87%
Large	10	257	0-187	35	86 (0-86)	27	186 (0-186)	62.22	87%

*Kruskal-Wallis one-way analysis of variance indicates results to be significantly affected by both developmental temperature and by capsule size ($p \leq 0.005$).

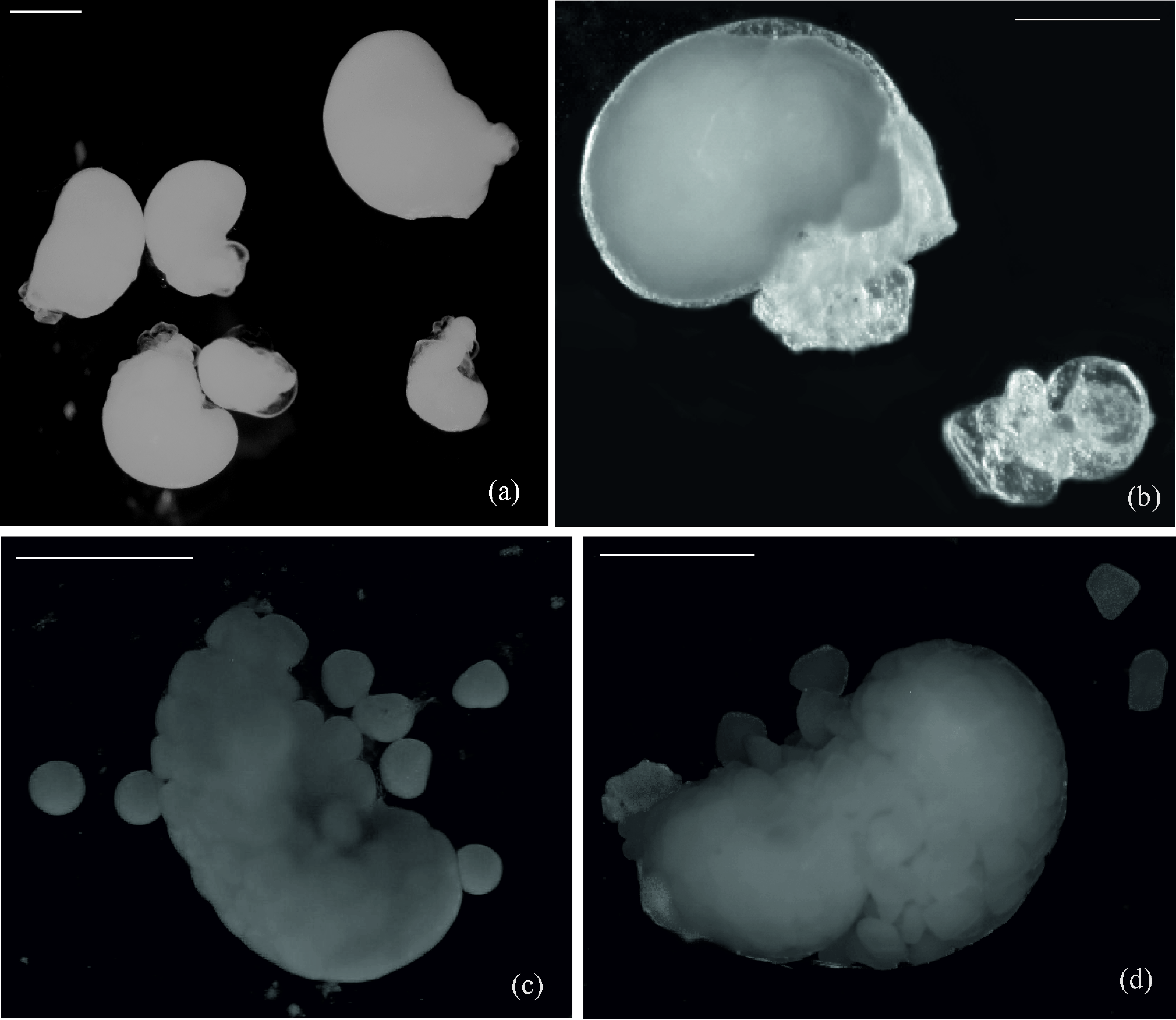
Figure 1: Images of *Buccinum undatum* during development. (a) embryos of varying sizes developing alongside each other; within one capsule and following nurse egg consumption. (b) a normal veliger next to an 'empty' veliger from the same capsule. The 'empty' veliger is visibly lacking nurse eggs inside its midgut. (c) a pierced embryo displaying intact nurse eggs spilling out. (d) a 'victim' of cannibalism bursting out of the midgut of its consumer. Scale bars represent 500 μm.

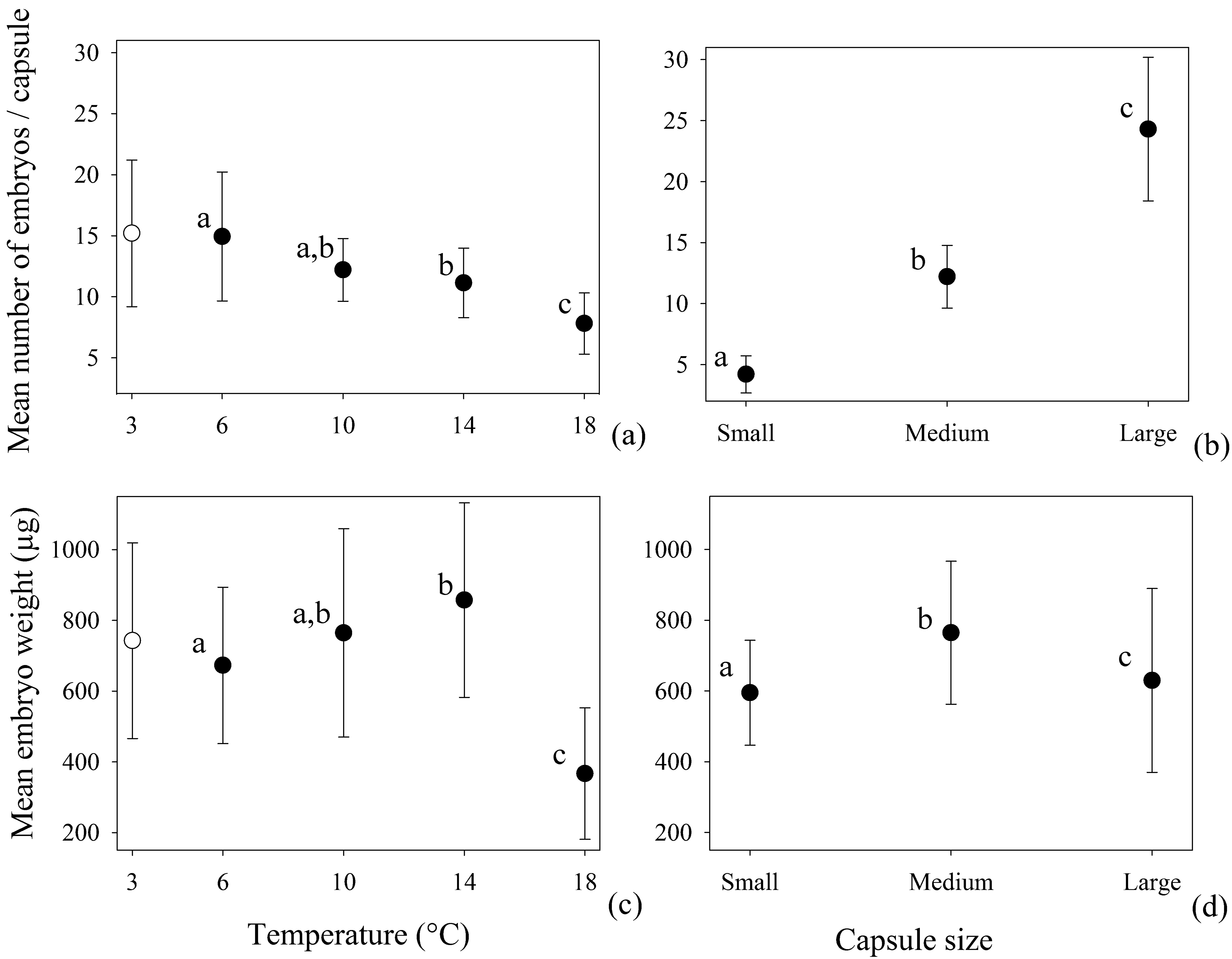
Figure 2: Number of *Buccinum undatum* embryos per capsule developing a) under different temperatures and b) in different sized capsules, and embryo weights from c) different temperatures and d) different sized capsules. Closed circles represent data collected from egg masses from the Solent, UK. Open circles represent data collected from Breiðafjörður, Iceland. For each plot (a-d), different letters indicate values that are significantly different as indicated using a non-parametric Kruskal-Wallis one-way ANOVA with *post hoc* analysis (Dunn's test). Analysis was carried out on Solent samples only. Error bars indicate standard deviation. 'Empty' embryos are included in embryo per capsule counts but not embryo weights.

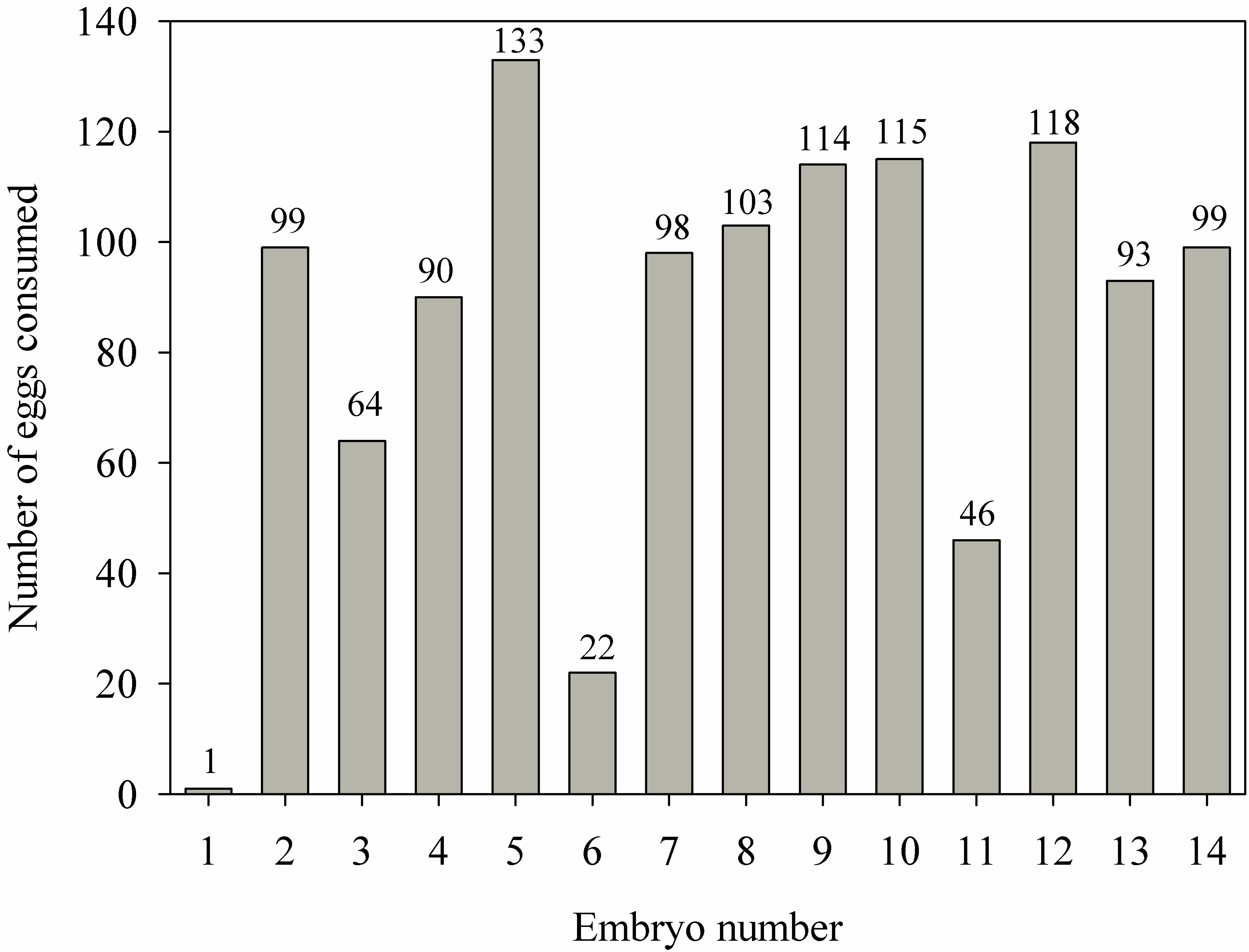
Figure 3: Typical nurse egg partitioning between embryos in one *Buccinum undatum* capsule. Numbers along the x-axis represent individual embryos from within one capsule. Each bar indicates the number of nurse eggs consumed by an individual embryo. Embryos developed at 10°C, medium sized capsule.

Figure 4: Proportion of *Buccinum undatum* embryos consuming different quantities of nurse eggs. (a) Bars with solid colouring depict embryos developed at temperatures ranging from 6°C to 18°C. (b) Bars with solid colours depict embryos developed in small, medium or large capsules. (a) and (b) Bars with diagonal lines depict embryos from the maximum consumption experiment. Data include 'empty' embryo counts. Statistical analysis indicated number of nurse eggs consumed per embryo to be significantly affected by temperature and

capsule size, and number of eggs consumed by maximum consumption embryos to be significantly greater than those consumed by embryos developing naturally at 10°C from any sized capsule ($p \leq 0.001$).


(a)
(b)
(c)
(d)


Mean number of embryos / capsule
30
25
20
15
10
5
a
a,b
b
c
3
6
10
14
18
(a)
30
25
20
15
10
5
a
b
c
Small
Medium
Large
(b)
Mean embryo weight (µg)
1000
800
600
400
200
a
a,b
b
c
3
6
10
14
18
(c)
Temperature (°C)
1000
800
600
400
200
a
b
c
Small
Medium
Large
(d)
Capsule size



Number of eggs consumed
Embryo number
0
20
40
60
80
100
120
140
1
2
3
4
5
6
7
8
9
10
11
12
13
14
1
99
64
90
133
22
98
103
114
115
46
118
93
99

6°C
10°C
14°C
18°C
Maximum consumption embryos
Percentage of embryos
0
10
20
30
40
50
60
0-20
21-40
41-60
61-80
81-100
101-120
121-140
141-160
161-180
181-200
201-220
221-240
241-260
261-280
281-300
301-320
321-340
(a)
Small
Medium
Large
Maximum consumption embryos
Percentage of embryos
0
5
10
15
20
25
30
35
(b)
Number of eggs consumed by one embryo

Appendix F: *Reprint* Smith KE, Thatje S(2012) The secret to successful deep-sea invasion: does low temperature hold the key? PLoS ONE. 7(12): e51219. doi:10.1371/journal.pone.0051219

The Secret to Successful Deep-Sea Invasion: Does Low Temperature Hold the Key?

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Abstract

There is a general consensus that today's deep-sea biodiversity has largely resulted from recurrent invasions and speciations occurring through homogenous waters during periods of the Phanerozoic eon. Migrations likely continue today, primarily via isothermal water columns, such as those typical of Polar Regions, but the necessary ecological and physiological adaptations behind them are poorly understood. In an evolutionary context, understanding the adaptations, which allow for colonisation to high-pressure environments, may enable us to predict future events. In this investigation, we examine pressure tolerance during development, in the shallow-water neogastropod *Buccinum undatum* using thermally acclimated egg masses from temperate and sub-polar regions across the species range. Fossil records indicate neogastropods to have a deep-water origin, suggesting shallow-water species may be likely candidates for re-emergence into the deep sea. Our results show population level differences in physiological thresholds, which indicate low temperature acclimation to increase pressure tolerance. These findings imply this species is capable of deep-sea penetration through isothermal water columns prevailing at high latitudes. This study gives new insight into the fundamentals behind past and future colonisation events. Such knowledge is instrumental to understand better how changes in climate envelopes affect the distribution and radiation of species along latitudinal as well as bathymetric temperature gradients.

Citation: Smith KE, Thatje S (2012) The Secret to Successful Deep-Sea Invasion: Does Low Temperature Hold the Key? PLoS ONE 7(12): e51219. doi:10.1371/journal.pone.0051219

Editor: Paul Eckhard Witten, Ghent University, Belgium

Received September 7, 2012; **Accepted** October 31, 2012; **Published** December 5, 2012

Funding: This work was made possible through a grant (Abyss2100) from the Total Foundation to Sven Thatje and from the Malacological Society of London to Kathryn Smith. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Throughout their evolutionary history marine invertebrates have colonised the oceans by extension of physiological boundaries. Phylogenetic links can be found between species in every ocean and throughout latitudinal and bathymetric ranges [1]. It is generally accepted that past colonisations to new latitudes have taken place during geological periods of cooling or warming (e.g. temperature declines during the Pliocene and Pleistocene eras) or through gradual adaptation to warmer or colder temperatures [2–4]. Patterns indicate such shifts to predominantly occur from the tropics, towards higher latitudes, with poleward declines in species diversity being observed throughout the water column [5–9]. Generally, invasions into, and emergences from, bathyal and abyssal depths are believed to primarily occur via isothermal water columns. Peak changes in diversity between shallow-water and deep-sea, appear in-line with certain geological periods when such isothermal waters were widespread (such as the Mesozoic and early Cenozoic eras), and more recently in high latitude areas through regions of deep-water formation [10–16]. Both fossil records and molecular phylogeny offer insight into such evolutionary paths. For example, Weddell Sea molluscs from the southern hemisphere can be tracked slowly north through fossil records from the Pliocene and Pleistocene periods [2] and close phylogenetic relationships have been proposed between shallow-water and deep-sea caridean shrimp [17], mytilid mussels [18] and anomuran decapods [19].

Many factors (biological, physical and chemical) affect the dispersal and colonisation of species [20], but for marine invertebrates, temperature and hydrostatic pressure create two substantial challenges. In shallow water temperature changes with latitude, and throughout the oceans both temperature and pressure change with depth. While both are known to affect biological systems throughout the whole animal, and are capable of causing major physiological disruptions, the impacts of these two variables have been found to be antagonistic, with pressure increases and temperature decreases often showing similar results [21–23]. A species combined tolerance to these two factors may therefore be vital in determining the upper and lower limits in its vertical distribution [24]. While peak changes in faunal assemblage are believed to have primarily occurred during past geological periods, new colonisations likely continue to take place today [3,4,13,25,26]. Current bathymetric migrations are being reported in areas with low water temperatures [13,26], with recent studies indicating such events to be occurring in response to increasing surface water temperatures [27–29]. However, the growing amount of literature describing the combined effects of temperature and pressure on marine invertebrates typically indicates an increased sensitivity to pressure with decreasing temperature, with temperature being the dominant of the two factors. Work to date examining the combined effects of temperature and pressure on marine invertebrates typically focuses on echinoderms and crustaceans [12,15,24,30–33]. Both adult and developmental stages have been investigated, but this includes only species with planktonic larvae. These studies generally exercise a small number of acute temperature and pressure treatments, with few studies examining the full physiological scope of an invertebrate with regard to both factors [32,33]. Therefore, conclusions should not yet be drawn from the limited dataset available.

Understanding how the physiological scope of an organism is affected by thermal and hyperbaric changes may help us to realise the parameters that set its ecological boundaries. In turn this will aid us in predicting forthcoming bathymetric radiations, migrations and new evolutionary paths, which may occur within the oceans in response to the future effects of climate change [13]. In marine invertebrates, physiological tolerances often vary through ontogeny [e.g. 13,16]. A species combined tolerance throughout development is therefore key in dictating how successful future vertical migrations may be. In particular, it is important to understand where thresholds lie for species with non-planktonic development. Such species have limited dispersal capabilities, and migrations and radiations typically occur at a slower rate [34].

Neogastropods are a large group of molluscs, which are indicated by fossil records to have a cold, deep-water origin [35,36]. Shallow-water species from this order may therefore be likely candidates for re-emergence into the deep sea. Species from certain families, including the Buccinidae, exist today throughout every ocean, from the intertidal to the abyssal zone [37]. Buccinidae regularly exhibit non-planktonic intracapsular development, the food-independent nature of which makes them ideal candidates for studying early ontogeny. Here, using *Buccinum undatum*, a widely distributed North Atlantic shallow-water buccinid, we examine the combined effects of temperature and pressure on early ontogeny. We use the novel approach of allowing thermal acclimation through development, and examining populations from different climates, across the species distribution range. Using respiration as an indicator of physical fitness, we investigate ecophysiological adaptations, which may indicate the potential for future deep-water colonisation.

Materials and Methods

Ethics Statement

All experiments were conducted in accordance with the legal requirements of the United Kingdom. The use of Molluscs is unregulated in the United Kingdom and subsequently does not require ethics approval by a specific committee. No specific permits were required for the described field studies.

Study Species

The common whelk *B. undatum* is a shallow-water gastropod, which is found widespread across a latitudinal range running through the North Atlantic and Arctic Oceans. Across its range, it is annually exposed to temperatures ranging from below zero to above 22°C. Egg laying and intracapsular development occur across a narrower thermal range of 2 to 11°C [38,39], although development is possible at temperatures up to 18°C [40]. Egg masses naturally take between 2.5 and 9 months to develop. At the southern end of its distribution this species is a winter spawner, with egg masses being laid as seawater temperatures cool and reach their lowest levels. In comparison, at the northern end of the distribution, or in colder waters, egg masses are laid in spring as water temperatures are warming [41–43]. *Buccinum undatum* is an important commercial species, providing locally valuable fisheries in several areas around the North Atlantic including the UK, the USA and Canada [44,45]. Demand for this species is continuously increasing globally (Department of Marine Resources www.maine.gov/dmr/rm/whelks.html) and it has been suggested as an aquaculture candidate [46]. It has a close taxonomic relationship with several deep-sea species, for example *Buccinum abyssorum, Beringius turtoni, Belomitra quadruplex* [47] and *Buccinum thermophilium* [37].

Egg Mass Collection

Buccinum undatum egg masses were collected from the Solent, UK (50°47′ N, 001°15′ W) between December 2009 and February 2010, and December 2010 and February 2011, and from Breiðafjörður, Iceland (65°00′ N, 023°30′ W) between April and May 2011. The methods of collection used are described below.

The Solent, UK. Egg masses were collected as described by Smith *et al.* [40]. In brief, collection took place using beam trawls deployed from on board *RV Callista* (5–10 m depth; water temperatures 4 to 10°C; www.bramblemet.co.uk/) or through farming in the seawater aquarium at the National Oceanography Centre, Southampton (water temperatures <8°C). For the latter, adult whelks were collected by Viviers from the Solent using whelk traps (~15 m depth) (www.fishmarketportsmouth.co.uk). Egg masses were removed from aquarium walls 24 hours after laying had ceased.

Breiðafjörður, Iceland. Egg masses, which had been detached from substrate by heavy weather, were collected from the intertidal area by hand, from beaches around Breiðafjörður. During the collection period, seawater temperatures ranged from 3 to 4°C.

Egg Mass Maintenance

Egg masses were maintained individually in 1.8 L incubation tanks containing aerated, 1 μm filtered seawater (three 100% water changes per week). Egg masses from the southern location (the Solent) were acclimated to 6, 10, 14 or 18°C (classified *n*°C (S)), and from the northern location (Breiðafjörður) to 3 or 6°C (classified *n*°C (N)). Temperatures were chosen because previous investigations on thermal tolerance during development have shown populations from the southern end of the distribution to be able to develop successfully under temperatures of 6 to 18°C [40]. Since these egg masses successfully developed at temperatures above, but not below natural developmental temperatures, it was assumed the same pattern would be observed from egg masses throughout the distribution. Therefore, a minimum temperature of 3°C was chosen for egg masses from the northern end of the distribution because this is the lowest developmental temperature observed in this area (Smith, personal observations). Three egg masses were maintained at each temperature. Acclimation was stepwise (1°C every 24 hours) from the initial water temperature at collection. Egg masses were maintained at each temperature throughout intracapsular development, and for a minimum of 21 days prior further pressure experimentation. Developmental timing was estimated using the results of Smith et al. [40] and ontogenetic stage, using the results of Smith & Thatje [39]. All experimental work was carried out at the National Oceanography Centre, Southampton, UK. Pressure experiments were carried out on two ontogenetic stages; veliger and hatching juvenile [39]. These stages were chosen as representative of the oldest and youngest ontogenetic stages, which could be manipulated without damage.

Effects of Pressure on Respiration Rates

The effect of pressure on respiration rates was measured by transferring three individuals into a 2.8 ml plastic vial containing pre-incubated 1 μm filtered seawater. Veligers were sampled by gently flushing the content of an open capsule into a petri dish and transferring individuals by pipette. Individual veligers were randomly selected from three separate capsules for each vial. Juveniles were collected directly from the incubation tank walls. Vials were sealed under water to prevent air from being trapped inside. This allowed seawater to be pressurised without risking air being forced into the liquid, thus preventing the partial pressure of dissolved gases from being affected. A further two vials were filled containing animals, followed by four control vials, containing no animals. The seven vials were placed inside a pressure vessel (Stauff, UK), which was then filled with freshwater. Both the vessel and the freshwater were pre-incubated to experimental temperature. The vessel was pressurised to the selected experimental treatment (1, 100, 200, 300, 400 atm) using a Maximator model manual hydraulic pump [after 16,24]. Pressurisation was continuous and took a maximum of 30 seconds. Pressures ranging 1 to 400 atm (equivalent to 0 to 4000 m water depth) were chosen to represent shallow water through to average ocean depths. The pressure vessel was then incubated at the experimental temperature for 4 hours, before being rapidly depressurised [after 16,24]. Pressure remained continuous for the entire experimental period. Prior to experimentation, preliminary investigations were carried out to ascertain the duration of the experiment (4 hours), with regards to vial oxygen levels. Hypoxic conditions were considered to occur if oxygen levels fell below 60% air saturation and this figure therefore dictated experimental duration.

Upon depressurisation the oxygen concentration of the water (% air saturation) was recorded for each vial. Measurements were obtained using a temperature and atmospheric pressure adjusted oxygen meter and microoptode (Microx TX3, Presens, Germany). This equipment was calibrated daily using fully aerated seawater (100% O_2 saturation), and seawater deoxygenated through oversaturation with sodium sulfite anhydrous (0% O_2 saturation), both pre-incubated to temperature [24]. Each vial was gently agitated prior to the measurement being taken to ensure the oxygen level was constant throughout. The % air saturation was then continuously logged for a minimum of one minute or until a constant level of air saturation was observed. Readings from the control vials were averaged for further calculations. Animals from each vial were stored together in a pre-weighed (6 mm×4 mm) tin capsule and frozen at −80°C. Samples were freeze-dried over 24 hours and then whole-animal dry weight was determined (±1 μg).

The final respiration rate was calculated following the method used by Brown and Thatje [32], an adaptation of the protocol described by Thatje *et al.* [24]. Here, the difference in readings between control and experimental vials was determined and oxygen consumption assessed per unit of dry weight. This method accounts for any oxygen consumption occurring through microbial action. In this method, the concentration of oxygen in 100% saturated seawater was calculated according to Benson and Krause [48]. This protocol was repeated for every temperature/pressure combination, for 3 egg masses at each temperature, and for veliger and hatching juveniles from each egg mass.

Statistics

Data were analysed using General Linear Model ANOVA (two factors, crossed; temperature and pressure). *Post hoc,* analysis was carried out using the Sidak simultaneous test. Prior to analysis all data were subject to square root transformation to attain homoscedasticity (Levenes test, $p>0.05$).

Results

Oxygen consumptions recorded below, are stated as μmol O_2 mg^{-1} h^{-1} (±1 S.D.).

Effects of Temperature on Respiration Rates

Analysis indicated respiration rates in both veligers and hatching juveniles to be significantly affected by temperature ($p\leq0.001$) (table 1, figs. 1a&2a). In veligers, a trend was observed at 1 atm of oxygen consumption increasing as temperature increased in samples from Breiðafjörður, but decreasing with increasing temperature in samples from the Solent. In juveniles, the trend was for oxygen consumption to decrease as temperature dropped, across both populations. When all pressures were averaged for each temperature, *post hoc* analysis ($p\leq0.05$) indicated several groupings of similar temperatures for both veligers and juveniles (figs. 1a&2a).

Effects of Pressure on Respiration Rates

Analysis indicated pressure to significantly affect respiration rates in all hatching juveniles ($p\leq0.001$), but not in veligers ($p=0.440$) (table 1, figs. 1b&2b). A trend was observed of oxygen consumption to decrease with increasing pressure in juveniles from both Breiðafjörður and the Solent. When all temperatures were averaged for each pressure, *post hoc* analysis ($p\leq0.05$) indicated hatching juveniles to be significantly affected by pressures of 200 atm or more (when compared to 1 atm). *Post hoc* analysis indicated there to be no difference in oxygen consumption in veligers between any pressures ($p\leq0.05$).

Temperature and Pressure Interaction Effects on Respiration Rates

Interaction effects of temperature and pressure were found to significantly impact oxygen consumption in hatching juveniles ($p\leq0.001$), but not in veligers ($p=0.934$) (table 1, figs. 1c&2c). *Post hoc* analysis ($p\leq0.05$) indicated there to be no difference in oxygen consumption in veligers between 1 atm, and any other pressure at any temperature (table 2a). In hatching juveniles, *post hoc* analysis ($p\leq0.05$) indicated that the effects of increasing pressure on oxygen consumption were reduced with decreasing temperature (table 2b). In veligers, the largest fluctuation in respiration rates across all pressures was observed at 6°C (S), where oxygen consumption ranged from 0.012 (±0.004) μmol O_2 mg^{-1} h^{-1} to 0.017 (±0.009) μmol O_2 mg^{-1} h^{-1}. The smallest difference was at 3°C (N) [range 0.004 (±0.002) to 0.005 (±0.002) μmol O_2 mg^{-1} h^{-1}]. In hatching juveniles, the largest change in oxygen consumption across all pressures occurred at 14°C (S) [range 0.002 (±0.001) to 0.012 (±0.005) μmol O_2 mg^{-1} h^{-1}] and the smallest was again at 3°C (N) [range 0.002 (±0.001) to 0.004 (±0.001) μmol O_2 mg^{-1} h^{-1}].

Discussion

Cold Water Preference in Buccinidae?

Historically, neogastropods are believed to have a cold, deep-water origin, first appearing in fossil record on the 'deep' outer shelf during the mid cretaceous period [11,35,36]. Since then, they have evolved over millions of years to inhabit every corner of the oceans, across the full bathymetric and latitudinal range [37]. In particular within the Buccinidae, a distinct cold-water preference remains evident today. Species abundance is greatest in sub-polar and temperate areas (http://iobis.org/mapper/), and many species (including *B. undatum*) with ranges covering temperate or sub-tropical climates, show preference for cold water in their breeding cycle, with spawning and development occurring when annual water temperatures are at their lowest [39,41,49]. Recent work on thermal acclimation indicates that although a species can shift the limits of its thermal tolerance range to colonise new environments, optimal performance rarely acclimates to changes

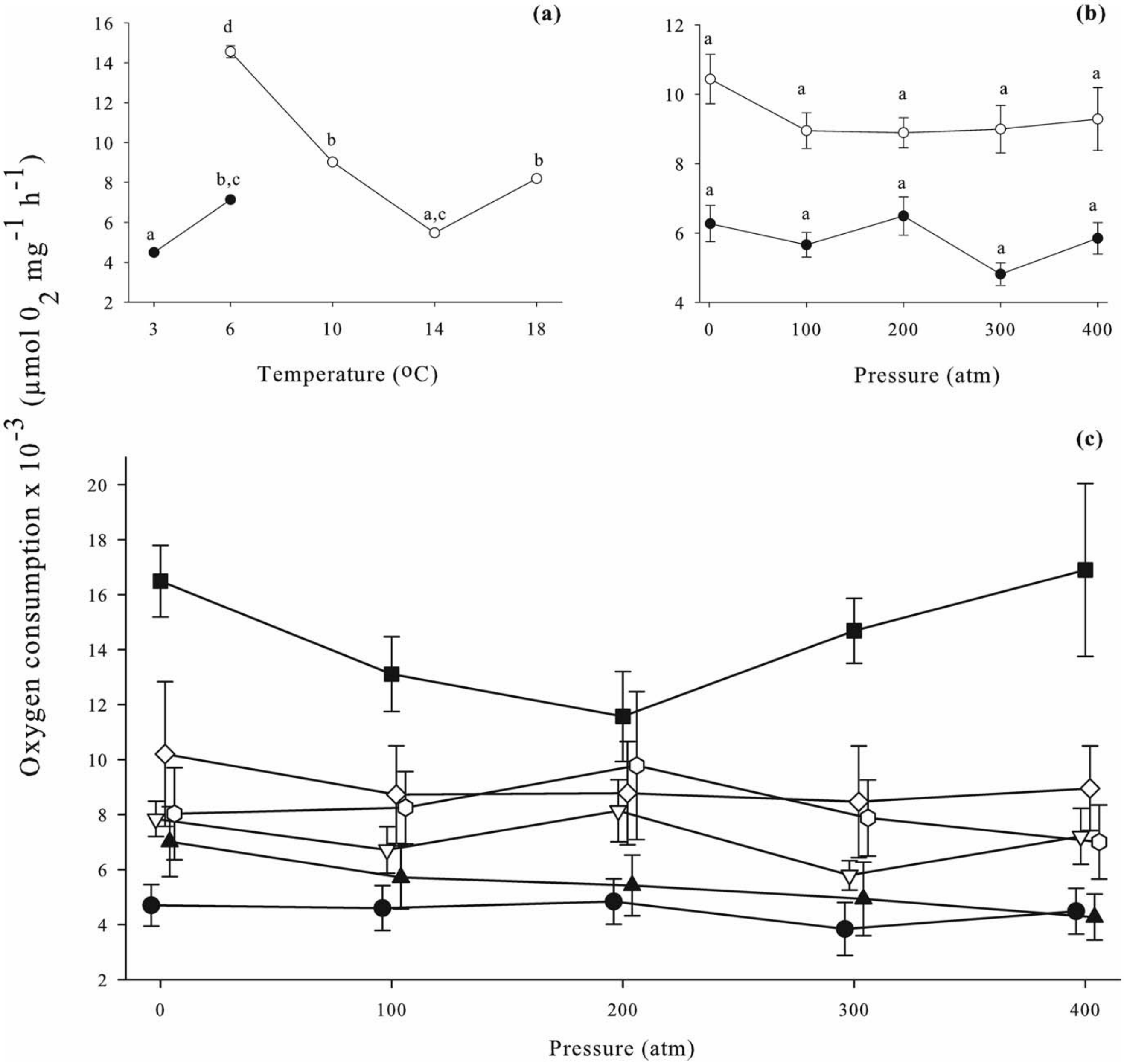

Figure 1. Oxygen consumption in *Buccinum undatum* veliger from the Solent (UK) and Breiðafjörður (Iceland). (a) Effects of temperature on oxygen consumption. Data for each temperature is averaged from 5 pressures (1, 100, 200, 300, 400 atm). Closed circles, Breiðafjörður data; open circles, Solent data. General Linear Model ANOVA indicated oxygen consumption to be significantly affected by temperature ($p \leq 0.001$); different letters indicate values that are significantly different. For each data point n = 45. (b) Effects of hydrostatic pressure on oxygen consumption. Data for each pressure is averaged across temperatures (6, 10, 14, 18°C for Solent samples; 3, 6°C for Breiðafjörður samples). Closed circles, Breiðafjörður data; open circles, Solent data. Oxygen consumption was not affected by pressure; different letters indicate values that are significantly different. For each data point n = 54. (c) Change in oxygen consumption with pressure, at 6 temperatures. Closed circles, 3°C Breiðafjörður; open circles, 6°C Breiðafjörður; closed triangles, 6°C Solent; open triangles, 10°C Solent; closed squares, 14°C Solent; open squares, 18°C Solent. Oxygen consumption was not affected by temperature – pressure interactions; see table 2 for *post hoc* analysis (Sidak simultaneous test). Error bars display standard error. For each point, n = 9.
doi:10.1371/journal.pone.0051219.g001

in environmental temperature [50,51]. It is therefore likely that the historical low-temperature bias observed during breeding in this family may remain beneficial for optimal performance. Amongst others, cold-water spawning and development in neogastropods have been observed in species from the Buccinidae, Conidae, Fasciolariidae and Muricidae [41,49]. Our results indicate that in *B. undatum* this historical cold-water preference is particularly prevalent during early development. In juveniles, oxygen consumption scaled with temperature, as has been observed on many previous occasions for invertebrates [24,32,33,52–55]. In veligers, in comparison, oxygen consumption increased with temperature until it exceeded 6°C, at which point it reduced, indicating thermal optima, and potentially also thermal maxima, to be lower at this developmental stage. These results are also supported by

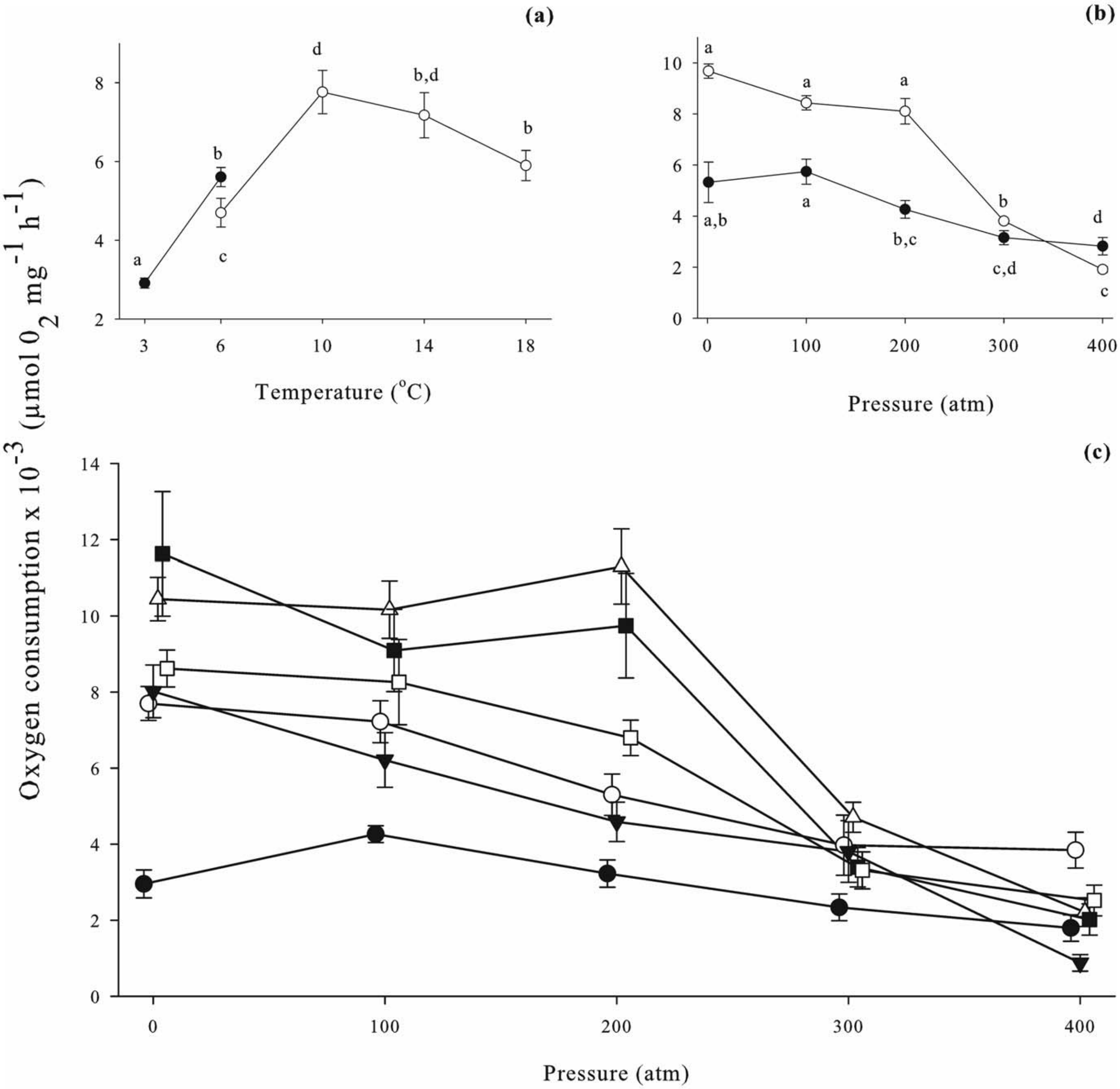

Figure 2. Oxygen consumption in hatching juvenile *Buccinum undatum* from the Solent (UK) and Breiðafjörður (Iceland). (a) Effects of temperature on oxygen consumption. Data for each temperature is averaged from 5 pressures (1, 100, 200, 300, 400 atm). Closed circles, Breiðafjörður data; open circles, Solent data. General Linear Model ANOVA indicated oxygen consumption to be significantly affected by temperature ($p\leq0.001$); different letters indicate values that are significantly different. For each data point n = 45. (b) Effects of hydrostatic pressure on oxygen consumption. Data for each pressure is averaged across temperatures (6, 10, 14, 18°C for Solent samples; 3, 6°C for Breiðafjörður samples). Closed circles, Breiðafjörður data; open circles, Solent data. Oxygen consumption was significantly affected by pressure ($p\leq0.001$); different letters indicate values that are significantly different. For each data point n = 54. (c) Change in oxygen consumption with pressure, at 6 temperatures. Closed circles, 3°C Breiðafjörður; open circles, 6°C Breiðafjörður; closed triangles, 6°C Solent; open triangles, 10°C Solent; closed squares, 14°C Solent; open squares, 18°C Solent. Oxygen consumption was significantly affected by temperature - pressure interactions ($p\leq0.001$); see table 2 for *post hoc* analysis (Sidak simultaneous test). For each data point, n = 9. Data were analysed using General Linear Model ANOVA. Error bars display standard error.
doi:10.1371/journal.pone.0051219.g002

observations which indicate *B. undatum* to have a narrower thermal tolerance range during development than during adult life [40]. For each population, the highest oxygen consumption readings were taken at 6°C, indicating thermal optima may be relatively constant across the distribution range regardless of habitat temperature. The thermal optima we observed, also coincide with the minimum, and most successful, temperature at which complete intracapsular development occurs in *B. undatum* at the southern end of their distribution [40]. Northern populations, in comparison, and those which are historically adapted to sub-polar or polar climates, are also able to develop at temperatures below this [43,56].

Table 1. General Linear Model ANOVA results.

		Veliger		Hatching juvenile	
	n, df	F statistic	*p*-value	F statistic	*p*-value
Temperature	45, 5	30.14	≤0.001†††	30.63	≤0.001†††
Pressure	54, 4	0.94	0.440	105.01	≤0.001†††
Temperature* pressure	9, 20	0.56	0.934	4.42	≤0.001†††

Analysis testing the effects of temperature, pressure and temperature – pressure interactions on oxygen consumption in veliger and hatching juvenile *Buccinum undatum* from the Solent (UK) and Breiðafjörður (Iceland).
Significance level is indicated by asterisks, † $p \leq 0.05$; †† $p \leq 0.01$; ††† $p \leq 0.001$.
n = number of replicates, df = degrees of freedom.
doi:10.1371/journal.pone.0051219.t001

Thermal and Hyperbaric Effects on Physiological Thresholds and the Importance of Complexity

Temperature and pressure have both frequently been argued to be major factors affecting physiology in invertebrates, thus playing a pivotal role in their distribution patterns [21,23,24,50,57]. Growth, survival and developmental success may all be affected by these factors, and internally, all membrane based processes have the potential to be disrupted by them [21,23,58,59]. Organism complexity plays a key role in determining the intensity of such impacts, in particular in response to pressure. Quite simply, as an organism develops its complexity increases, making it more susceptible to the negative effects of pressure and often narrowing its tolerance window. Studies carried out on echinoderms indicate that when individuals are in their most complex form (i.e. adults) pressure tolerance is much lower than during development [13]. The effects of both temperature and pressure have also been shown to vary during development in invertebrates [16,30,60,61].

While both temperature and pressure create substantial physiological obstacles for marine invertebrates, when comparing the effects of the two variables, temperature is commonly observed to be the dominant factor. Changes in temperature continuously affect invertebrates [e.g. 50,57,62], but past authors have demonstrated the effects of pressure on shallow-water invertebrates (crustaceans and echinoderms) to only become significant when equivalent to 2000 m or more [24,31,33]. The results of our study support these past investigations; temperature significantly affected veligers and juveniles, whereas pressure only affected juveniles, and only when greater than 200 atm. These findings also show similar ontogenetic shift in pressure tolerance as have been previously reported [16,30], with juveniles being more susceptible than veligers.

Polar Climates; the Optimum Environment for Deep-sea Invasion?

Several theories exist regarding the evolutionary expansion of ocean biodiversity. It is generally accepted that recurrent bathymetric colonisations and speciations have occurred, primarily via isothermal water columns. Ocean temperature profiles have, however, varied across geological periods, and with this, shifts in biodiversity. For example, well established patterns indicate latitudinal expansions in both shallow-water and deep-sea species

Table 2. *Post hoc* analysis (Sidak simultaneous test).

(a) *Veliger*		Pressure (atm)							
		100		200		300		400	
Temperature (°C)		T	*p*-value	T	*p*-value	T	*p*-value	T	*p*-value
The Solent, UK	**18**	0.167	1.000	0.588	1.000	−0.036	1.000	−0.571	1.000
	14	−0.768	1.000	−0.924	1.000	−1.344	1.000	−1.631	1.000
	10	−0.512	1.000	−0.517	1.000	−0.731	1.000	−0.177	1.000
	6	−1.341	1.000	−2.004	1.000	−0.652	1.000	0.246	1.000
Breiðafjörður, Iceland	**6**	−0.674	1.000	0.053	1.000	−1.117	1.000	−0.459	1.000
	3	−0.052	1.000	0.140	1.000	−0.765	1.000	−0.210	1.000
(b) *Juveniles*		Pressure (atm)							
		100		200		300		400	
Temperature (°C)		T	*p*-value	T	*p*-value	T	*p*-value	T	*p*-value
The Solent, UK	**18**	−0.590	1.000	−1.620	1.000	−5.570	<0.001†††	−6.860	<0.001†††
	14	−1.800	1.000	−1.310	1.000	−7.490	<0.001†††	−9.700	<0.001†††
	10	−0.250	1.000	0.540	1.000	−5.220	<0.001†††	−8.840	<0.001†††
	6	−1.746	1.000	−3.426	1.000	−4.706	0.002††	−9.530	<0.001†††
Breiðafjörður, Iceland	**6**	−0.459	1.000	−2.375	1.000	−4.080	0.026†	−4.075	0.027†
	3	1.786	1.000	0.379	1.000	−0.995	1.000	−1.970	1.000

The effects of pressure on oxygen consumption (when compared to 1 atm) across 6 temperatures (3 to 18°C) in (a) veliger and (N) hatching juvenile *Buccinum undatum* from the Solent (UK) and Breiðafjörður (Iceland).
Significance level is indicated by asterisks, † $p \leq 0.05$; †† $p \leq 0.01$; ††† $p \leq 0.001$.
n = 9.
T = test statistic.
doi:10.1371/journal.pone.0051219.t002

diversity, with overall decreases from the tropics to the poles [5,8,9]. Such gradients are believed to have commenced in line with global cooling events such as that in the early Cenozoic, when ocean waters ceased to be homogenous, potentially limiting bathymetric migrations, and have been linked to the onset of seasonally fluctuating food supplies [6,7]. Fossil records also show increases in speciations and biodiversity following glacial retreat. Theories suggest opportunistic taxa may go through rapid adaptive radiation in order to fill empty niches [63]. Such opportunistic taxa include species, which have been hypothesised to survive glacial advances through migration to deeper waters [64]. Conflicting views exist regarding the expansion of extant fauna of deep-sea chemosynthetic environments. While previous studies have suggested deep-sea vents and seeps to have been colonised by fauna from shallow-water seeps, pre-adapted to such environments [65], recent works instead suggest invasions primarily occur latitudinally from adjacent deep-sea environments, indicating isothermal water columns to contribute minimally in facilitating such invasions [66,67].

Here, we focus on the widely accepted hypothesis of bathymetric migrations occurring via isothermal water columns [10–16]. While during past geological periods (e.g. the late Mesozoic and early Cenozoic), ocean waters were warm and relatively homogenous throughout, today ocean temperatures average ~4°C below 1000 m [10–13,68]. Regions where surface waters are of a similar temperature to this, make up the largest areas of isothermal waters. If we consider bathymetric colonisations and speciations to primarily occur via such waters, these areas are therefore the most likely locations for modern bathymetric migrations.

Since the effects of temperature and pressure go hand in hand, these two variables should be evaluated collectively when considering the potential for deep-water colonisation. Our results imply *B. undatum* to theoretically be capable of surviving the combined thermal and hyperbaric conditions characteristic of the deep sea. While ontogenetic shifts were evident in both pressure and temperature thresholds, contrary to past studies [e.g. 15,24,30,32,33], our results indicate pressure sensitivity to decrease at low temperature, with individuals being capable throughout development of withstanding pressures equivalent to at least 4000 m at the lowest experimental temperature of 3°C.

Previous studies indicate theoretical depth penetration by shallow-water crustaceans and echinoderms to be possible at temperatures close to the upper limits of, or above, those experienced in their natural environment [e.g. 12,15,24,30,32,33]. The antagonistic effects of temperature and pressure imply that increases in one, may compensate to some degree for increases in the other. For example, membrane fluidity is affected by both variables but while high pressure decreases fluidity, high temperature increases it. If both variables increase simultaneously therefore, membrane fluidity may be minimally affected [for reviews see 21,23]. Environmental changes, however, rarely follow this pattern, and adaptations often occur as a result of changes in just one variable. Since similar adaptations develop as a result of increased pressure or decreased temperature, organisms, which have already adapted to low temperatures, for example, may be pre-adapted to some degree for high pressure. Growth at low temperatures has been shown to increase pressure resistance in the bacterium *Escherichia coli* [69,70]. Similarly, studies examining the effects of pressure on shallow-water echinoderm embryos, indicate pressure tolerance at native temperatures to be greater in polar species [30] than in temperate species [31]. We suggest that in other marine invertebrates a similar phenomenon occurs, with cold-adapted populations being less affected by increases in pressure than warm-adapted populations. Under this scenario, as ocean surface waters continue to warm, *B. undatum* and similar cold-adapted species may migrate along isotherms, taking refuge in deeper waters which appear optimal for their physiology and using this as a mechanism to survive. Such migrations have already been reported in a range of cold-water marine fish, in response to warming surface waters [26–28]. Similar migrations have also been suggested in response to decreasing temperatures; evidence of glacial refugia indicates some species to have previously seeked refuge in deeper waters in order to avoid advancing ice sheets [14,64]. The relationship between depth range and latitude often observed in marine invertebrates gives support for this, with several species showing patterns of increasing depth limits at high latitudes [71]. While *B. undatum* is known to exist in waters shallower than 250 m [47], to our knowledge, records detailing variations in depth distribution across the species range are incomplete and further study is needed to determine whether this species follows the same pattern.

While theoretically, this study shows *B. undatum* to be capable of cold, deep-water penetration, the shallow-water distribution of this species suggests factors other than temperature and pressure may currently limit its distribution. Species range limits are set by a combination of many biological, physical and chemical factors [15,29]. Such factors include food availability, predation, competition, habitat and water chemistry [15,29]. Alternatively, the thermal and hyperbaric tolerance observed throughout early ontogeny in this species may not be representative of that which it can tolerate throughout the duration of its entire life history; an important aspect that remains subject to future study, including the challenge of long-term maintenance of invertebrates under high hydrostatic pressures.

Our study provides evidence that if thermally acclimated throughout development, high-pressure tolerance is possible at the low temperatures typical of deep-sea environments. Although questions remain regarding the potential for complete egg mass development and successful growth to adult life under high pressure, or the impact of additional factors such as species interactions, our findings suggest populations of *B. undatum* from the northern end of the distribution to be theoretically capable of submergence into deep water through cold isothermal water columns typical of those found in Polar Regions. Cold-acclimated juveniles from the southern end of the distribution also showed greater tolerance to pressure (to 400 atm), relative to warm-acclimated individuals from the same population (to 200 atm).

Thermal acclimation is an important mechanism affecting physiological scope, and past studies have indicated shifts in thermal range as a result of acclimation, which in turn affects performance at a given temperature [51,72]. The present study increases our knowledge of the physiological effects of temperature and pressure on invertebrates and highlights the importance of thermal acclimation in experimentation, giving fresh insight into how evolutionary colonisations via polar isothermal water columns may have been achieved.

Acknowledgments

Thanks are given to the skipper and crew of RV Callista (University of Southampton) and to Erla Björk Örnólfsdóttir and the team at Vör Marine Research Center, Breiðafjörður, Iceland for their help with sample collection. Thanks also go to Adam Reed, Alastair Brown and Andrew Oliphant for help with animal maintenance.

Author Contributions

Conceived and designed the experiments: KES ST. Performed the experiments: KES. Analyzed the data: KES. Contributed reagents/materials/analysis tools: ST. Wrote the paper: KES ST.

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