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

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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. *Buccinum 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.

Keywords

Intracapsular development; thermal tolerance; bioenergetics; plasticity; Buccinidae; Buccinum

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 (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).

66 The effects of temperature on larval and juvenile development is of particular concern and as
67 a result a growing number of studies have recently contributed to this topic (e.g. Anger et al.,
68 2004; Johns, 1981; Sewell and Young, 1999; Stanwell-Smith and Peck, 1998). To date, the
69 majority of studies have examined species that exhibit fully or partially planktonic
70 development (Anger et al., 2004; Johns, 1981; Sewell and Young, 1999; Stanwell-Smith and
71 Peck, 1998; Cancino et al., 2003; Lima and Pechenik, 1985; Pechenik et al., 2003; Roller and
72 Stickle 1989) and only rarely have the effects of temperature on development been described
73 in a species with direct development (Fernandez et al., 2006). Such species have limited
74 dispersal abilities, and typically migrate or radiate at a slower rate (Jabonski, 1986; Thatje,
75 2012), suggesting that species following a direct mode in development may be more 'at risk'
76 from temperature change than those with planktonic development.

77 Species undergoing non-planktonic development often develop within egg capsules which
78 protect against factors such as physical stress, predation, infection and salinity changes
79 (Pechenik, 1983, 1999; Rawlings, 1995, 1999; Strathmann, 1985; Thorson, 1950). Within
80 each capsule, embryos are usually provided with a source of nutrition for development. This
81 is most commonly found in the form of nurse eggs (Chaparro and Paschke, 1990; Ilano et al.,
82 2004; Lahbib et al., 2010; Thorson, 1950), but additional nutrition may also occasionally be
83 gained from intracapsular fluid (Bayne, 1968; Moran, 1999; Pechenik et al., 1984;
84 Stöckmann-Bosbach, 1988) or capsule walls (Ojeda and Chaparro, 2004).

85 The common whelk *Buccinum undatum* is a shallow-water gastropod, which exhibits direct
86 encapsulated development using nurse eggs for nutrition. It is common in the North Atlantic
87 and Arctic oceans, provides locally valuable fisheries across these areas (Hancock, 1967;
88 Morel and Bossy, 2004) and has been suggested as a candidate species for aquaculture
89 (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, 2012a) 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). *Buccinum 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, 2012a). 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

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

118 #### 2.1.2. Farmed in seawater aquarium

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

129 ### 2.2. Egg mass maintenance and investigation

130 Since adult whelks were obtained from the same population, water temperatures at time of
131 collection were similar, and no difference was observed in egg capsule size or number of
132 eggs per capsule between egg masses collected via trawl and those farmed in the aquarium,
133 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 150mm^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 (± 0.01mm), using the following equation taken from Smith and Thatje (2012a);

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

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, 2012a). 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, 2012a), 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 1.8L incubation tank containing aerated, 1µm 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.

157 Every week for the initial 14 weeks and every fortnight for the remaining developmental
158 period, 3 capsules were randomly selected and dissected from each egg mass, the contents
159 were examined and the developmental stage determined. Ontogenetic stage was determined
160 according to Smith and Thatje (2012) and defined as egg, trochophore, early veliger, veliger,
161 pediveliger, pre-hatching juvenile or hatching juvenile. Nurse eggs were consumed through
162 the early veliger stage. For each mass, the outer layer of egg capsules was removed prior to
163 any examination as these were often empty or held a very small number of eggs. From the
164 trochophore stage and throughout nurse egg consumption (Smith and Thatje, 2012a), egg
165 masses were examined daily to determine the duration of short ontogenetic stages. Each egg
166 mass was also examined non-invasively every week. From this, the percentage of the mass at
167 each developmental stage was estimated. When an egg mass had completed early veliger
168 development, a minimum of 10 capsules from each egg mass were opened and the number of
169 developing embryos counted. Each early veliger was stored individually in a pre-weighed
170 (6mm x 4mm) tin capsule and frozen at -80˚C. Samples were freeze-dried over 24 hours and
171 then dry weight was determined (± 1µg). Hatching juveniles at each temperature were
172 sampled and dried and weighed in the same fashion. Each juvenile was then de-calcified
173 using RDC rapid decalcifier (Cellpath, Powys, UK), rinsed in distilled water and then dried
174 and weighed a second time. This allowed total, shell and flesh weights and shell:flesh weight
175 ratios to be determined. Any abnormal individuals were not sampled. Abnormal embryos
176 included those with malformed heads, misshapen bodies or those lacking any mantle or shell
177 development. All samples weighing more than 200 µg were used for later elemental (C and
178 N) analysis. Elemental analysis was carried out using a Fison (Carlo Erba) 1108 Elemental
179 Analyser. The elemental analyser was calibrated using chitin as a standard (% C = 44.71; %
180 N = 6.79). Carbon (C) and Nitrogen (N) percentages were determined during analysis and the
181 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 (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, 2b). 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 – 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, 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 and across all individuals, hatching juvenile weight varied by between 1381 and 4661µg.

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

252 developmental temperatures in percentages of C ($p = 0.997$) or N ($p = 0.998$), or C:N ratios (p
253 $= 0.619$). Significant decreases in C, N and C:N ratio values were observed during
254 development (from early veliger to hatching juvenile) at every temperature investigated. All
255 changes were significant to $p \leq 0.001$ except for changes in C and C:N ratio at 2˚C (both
256 significant to $p \leq 0.01$) and changes in N at 2˚C and C:N at 10˚C (both significant to $p \leq 0.05$).
257 For C and N, percentage depletion increased as developmental temperature increased from
258 17.9% (C) and 12.3% (N) at 2˚C to 32.5% (C) and 29.6% (N) at 14˚C. Reported depletion of
259 C and N, while still significant, was lower at 18˚C than at 14˚C. Rate of depletion in C:N
260 ratio decreased between developmental temperatures of 2 and 10˚C, before increasing again
261 as temperatures increased further.

262 # 4. Discussion

263 ## 4.1. Embryonic development

264 ### 4.1.1. Thermal tolerance during development

265 Within a species' distribution, thermal tolerance ranges are often reported to vary between
266 populations. Such ranges are ultimately dependent on temperature, and thus, shifts may occur
267 with latitude, or in association with ocean currents or other factors affecting local water
268 temperatures. This illustrates a high level of thermal plasticity in response to local
269 temperatures, indicating that population level differences in reproductive adaptations exist.
270 (e.g. Storch et al., 2009; Thatje et al., 2005; Zippay and Hofmann, 2009). Our results indicate
271 this trend to be evident in *B. undatum*. In the present study, complete development was
272 observed between 6 and 18˚C for a population at the southern end of the distribution, from
273 the south coast of the UK. In comparison, populations of the common whelk from the Gulf of
274 St Lawrence, Canada, where the Labrador current causes low annual temperatures, develop in
275 water temperatures of 2 to 3˚C (Martel et al., 1986a), and in Breidafjordur, Iceland, at the

276 northern end of the species distribution, between 3 and 6˚C (Smith and Thatje, 2012b;
277 Authors, unpublished results). Similar fluctuations in thermal tolerance have been noted
278 previously in gastropods (Zippay and Hofmann, 2009) and crustaceans (Storch et al., 2009;
279 Thatje et al., 2005), with different populations having a narrow thermal tolerance range,
280 which scaled with temperature and was specific to the latitude at which it was found. Within
281 a single species population, previous studies have indicated thermal tolerance to often be
282 lower during development than throughout adult life (Dawirs, 1985; deRivera et al., 2007;
283 Gosselin and Chia, 1995; Weiss et al., 2009). For example, hatched juveniles of the gastropod
284 *Nucella emarginata* were negatively impacted by temperatures of 30˚C, while adults of the
285 same species could easily withstand such temperatures (Gosselin and Chia, 1995). In the
286 present study, we found similar results with the thermal tolerance range identified during
287 development (6 to 18˚C) being narrower than annual local water temperatures for the
288 sampled population (4 to 22˚C). Such findings suggest that thermal tolerance during early
289 ontogeny may be fundamental in setting a species' geographic limits.

290 Developmental success (to hatching) was greatest within the natural developmental
291 temperature range of the local populations (6 and 10˚C) and decreased at temperatures
292 outside this. Similar scenarios have been observed on many previous occasions in marine
293 ectotherms including temperate and sub-polar crustaceans (Anger et al., 2003; Johns, 1981)
294 and tropical and polar echinoderms (Sewell and Young, 1999; Stanwell-Smith and Peck,
295 1998). Interestingly, in each of the above studies, optimum success was observed at
296 temperatures towards the middle or top of the thermal range investigated. In comparison, in
297 the present investigation peak survivorship was at temperatures at the bottom of the thermal
298 tolerance window for development and at the lower end of the habitat temperature limits for
299 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, 2012a; 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).

4.1.2. 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).

4.2. 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, 2012a). 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, 2012a; 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, 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 (Authors, 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 (Authors, 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 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, 2012a). 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 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, 2012a) 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 et al., 1999; Chaparro and Paschke, 1990; 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

395 are widely assumed to be of greater quality than smaller siblings, being less prone to factors
396 like predation, starvation and physical stress (e.g. Gosselin and Rehak, 2007; Lloyd and
397 Gosselin, 2007; Przeslawski, 2004, 2011; Rivest, 1983; Spight, 1976; Thorson, 1950).

398 ### 4.3. Bioenergetics through early development

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

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

417 ## 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.

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Legends to figures

Figure 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.

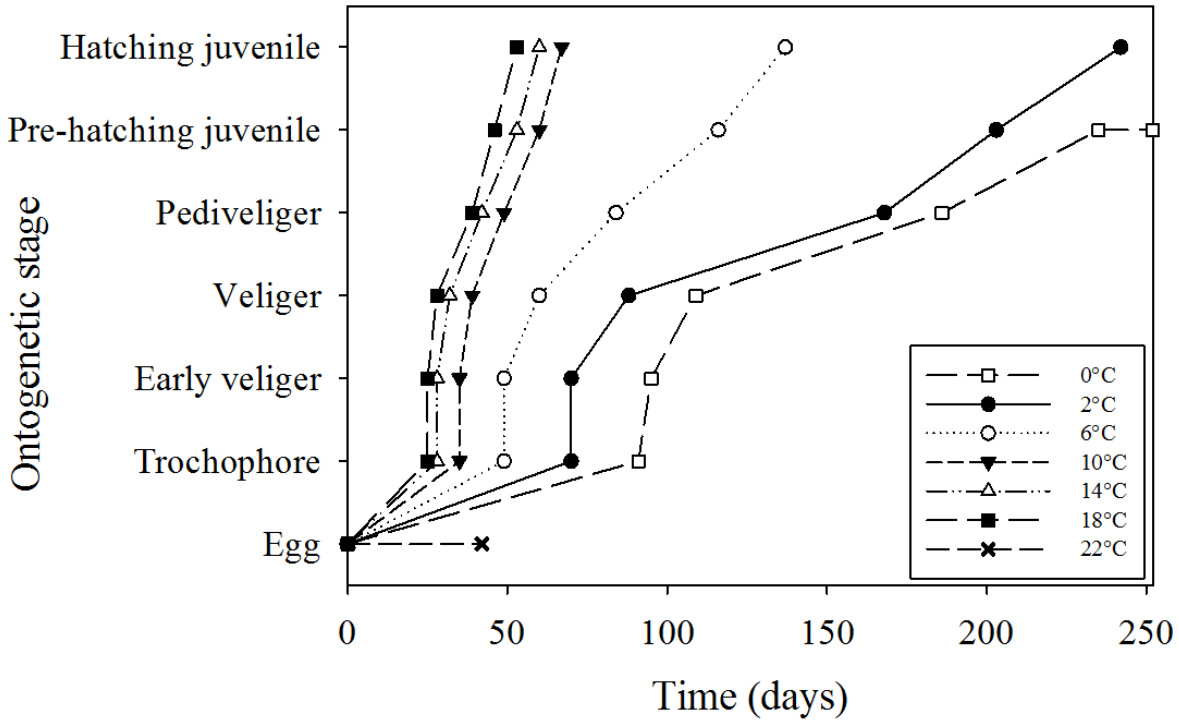
Figure 2: (a) Number of early veligers per capsule, (b) early veliger weight (post nurse egg consumption) and (c) juvenile weights for *Buccinum undatum.* For (a) and (b), filled circled represent samples collected from the Solent (50°47' N, 001°15' W), off the south coast of the UK, and developed at 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 n.

Figure 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 n values and are identical for all 3 plots.

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.

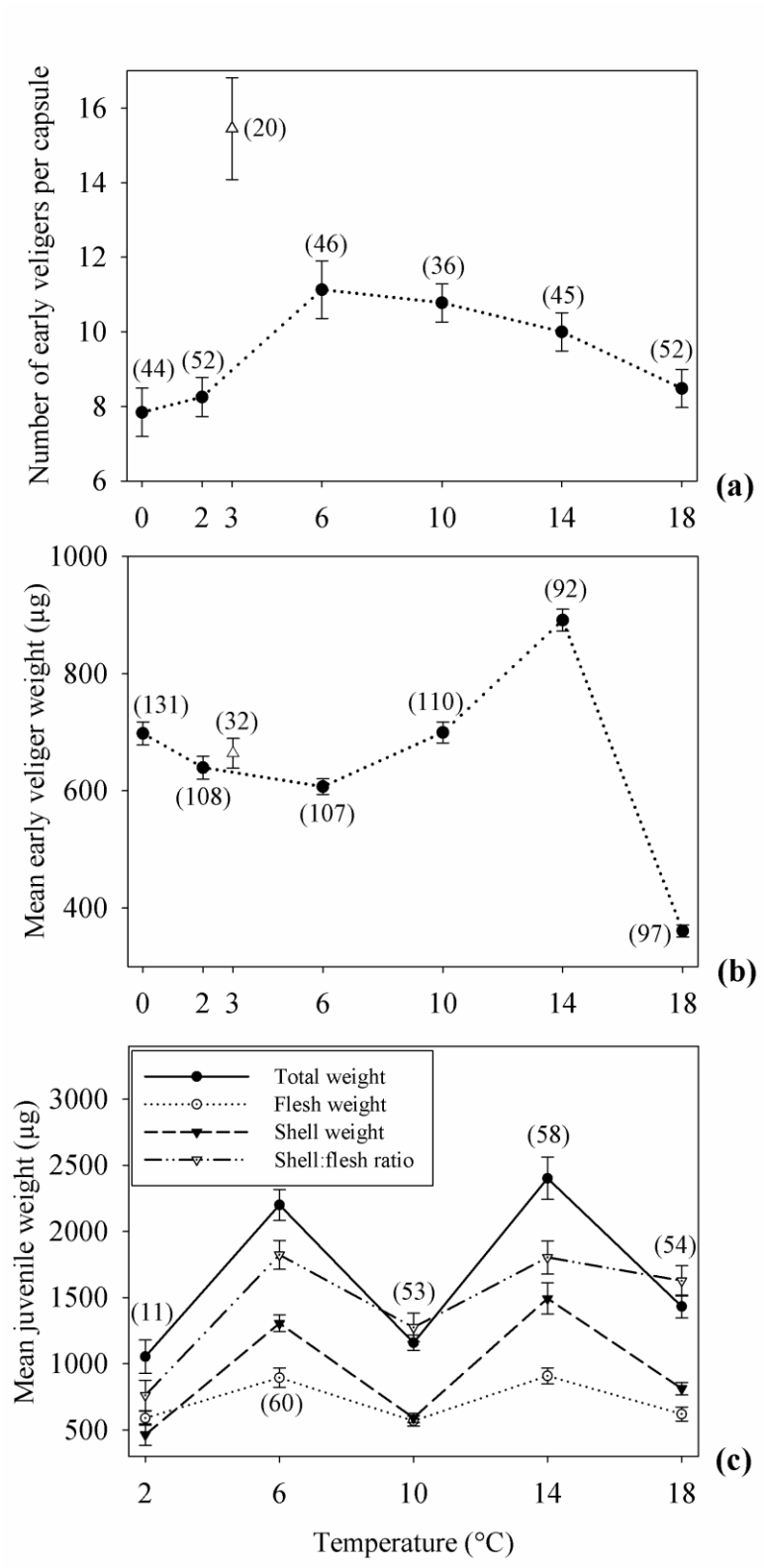
		Time at developmental stage in days – whole egg mass (mean number of days spent at stage)						
	Temperature	0°C	2°C	6°C	10°C	14°C	18°C	22°C
Developmental stage	Egg	0 to 105 (91)	0 to 77 (70)	0 to 56 (49)	0 to 42 (33)	0 to 35 (28)	0 to 28 (24)	0 to 42[a] (n/a)
	Trochophore	63 to 112 (4)	56 to 84 (3)	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.



610

611 Figure 1



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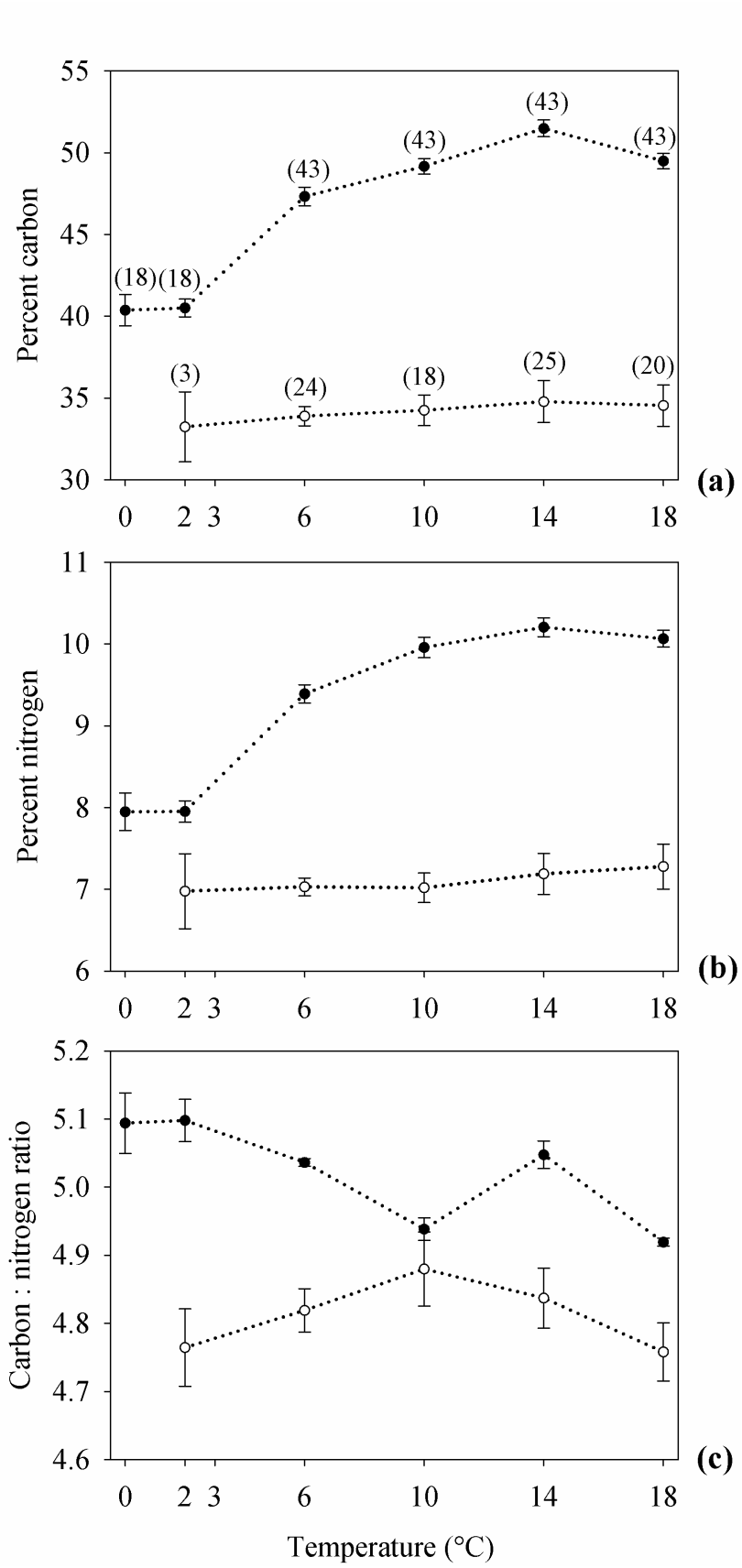
613 Figure 2



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615 Figure 3