Biochemical changes throughout early- and middle-stages of embryogenesis in lobsters (*Homarus americanus*) under different thermal regime

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Most marine crustacean eggs contain the full complement of nutritional resources required to fuel their growth and development. Given the propensity of many ovigerous (egg-bearing) American lobsters (*Homarus americanus*) to undergo seasonal inshore-to-offshore migrations, thereby exposing their eggs to varying thermal regimes, the goal of this study was to determine the impact of water temperature on egg quality over their course of development. This was accomplished by documenting changes in total lipids, proteins, and size (volume) of eggs subjected to one of three simulated thermal regimes: inshore, offshore, and constant (12°C). Total egg lipids showed a marked decrease over time (r^2_{adj} = 0.85, $P < 0.0001$), while protein levels increased over the same period (r^2_{adj} = 0.63, $P < 0.0001$). Although there were no significant differences in total lipids, proteins or egg sizes between eggs exposed to inshore and offshore temperatures ($P > 0.05$), they differed from values for eggs exposed to a constant temperature, which also hatched almost three months sooner. This is most likely due to the fact that eggs held at a constant 12°C did not experience a period of slow development during the colder months from November to March that are important for synchronizing egg hatch and may be compromised by elevated seawater temperatures.

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ABSTRACT

Most marine crustacean eggs contain the full complement of nutritional resources required to fuel their growth and development. Given the propensity of many ovigerous (egg-bearing) American lobsters (*Homarus americanus*) to undergo seasonal inshore-to-offshore migrations, thereby exposing their eggs to varying thermal regimes, the goal of this study was to determine the impact of water temperature on egg quality over their course of development. This was accomplished by documenting changes in total lipids, proteins, and size (volume) of eggs subjected to one of three simulated thermal regimes: inshore, offshore, and constant (12°C). Total egg lipids showed a marked decrease over time ($r^2_{adj} = 0.85$, $P < 0.0001$), while protein levels increased over the same period ($r^2_{adj} = 0.63$, $P < 0.0001$). Although there were no significant differences in total lipids, proteins or egg sizes between eggs exposed to inshore and offshore temperatures ($P > 0.05$), they differed from values for eggs exposed to a constant temperature, which also hatched almost three months sooner. This is most likely due to the fact that eggs held at a constant 12°C did not experience a period of slow development during the colder months from November to March that are important for synchronizing egg hatch and may be compromised by elevated seawater temperatures.

INTRODUCTION

Egg development for most marine crustaceans relies heavily on the production and sequestering of nutrients required for their development and maintenance over the entire process of embryogenesis. In terms of biochemical constituents, both lipids and proteins play pivotal roles throughout development, and as a result, have been studied extensively in both crustaceans and fishes alike (*Fraser, 1989*; *Jaeckle, 1995*; *Rosa et al., 2007*). Lipids comprise the structural integrity of most cells and are required for much of the metabolism of crustacean embryos; accounting for upwards of 60% of the total energy expenditure for growth (*Holland, 1978*; *Amsler & George, 1984*). By contrast, proteins as the basic building blocks of animal tissues (*Holland, 1978*), and can also function as alternative energy sources under certain conditions (*Schmidt-Nielsen, 1991*; *Heras, Gonzales-Baro & Pollero, 2000*).

Egg development in crustaceans is also very sensitive to thermal conditions, and incubation periods can be extended by cold water temperatures, or reduced during warmer conditions, thus influencing timing in hatch (*Goldstein & Watson, 2015b*). Furthermore, as metabolic rates increase at higher temperatures nutrients are used up at a faster rate and this can influence egg survival (*Pandian, 1970*; *Schmidt-Nielsen, 1991*). This can also have an impact on the development and metamorphosis of larvae because they derive their nutrition from both exogenous (feeding) and endogenous (yolk reserves) sources (*Sasaki, McDowell Capuzzo & Biesiot, 1986)*. Therefore, the relationship between the primary biochemical components in crustacean eggs and their variability are considered central to the early-life history patterns for these organisms (*Vance, 1973*; *Jaeckle, 1995*).

This is especially true for American lobsters, *Homarus americanus* H. Milne-Edwards (1837) characterized as large, highly mobile decapods whose habitats include coastal and continental shelf waters, bays, and estuaries from Labrador, Canada to Cape Hatteras, U.S. (*Fogarty, 1995*). Lobster eggs are typically extruded and fertilized in the late summer and fall, and then carried on the underside of the female's abdomen for 9-11 months before they hatch the following spring/summer. During this time, they are exposed to seasonal fluctuations in water temperature that can have a pronounced impact on their development (*Goldstein & Watson, 2015a*). Moreover, many ovigerous females undertake inshore/offshore seasonal migrations that can either enhance, or reduce, the magnitude of the seasonal temperature fluctuations their eggs experience (*Campbell & Stasko, 1986*; *Cowan et al., 2006*; *Goldstein & Watson, 2015a*). For example, ovigerous lobsters subjected to inshore thermal regimes in the lab exhibited more rapid egg development and hatched sooner than their offshore counterparts (*Goldstein & Watson, 2015b*). Therefore, the seasonal movements of ovigerous lobsters to thermally disparate waters may be strategies to both enhance egg development and the survival of larvae in the plankton. Although optimal temperatures for lobster egg growth are not fully known, naturally fluctuating temperatures result in disparate growth patterns and subsequently, differing hatch times (*Sibert, Ouellet & Brethes, 2004*; *Goldstein & Watson, 2015b*). In general, either prolonged warm or cold temperatures can have a deleterious effect on the use of the egg yolk reserves (*Garcia-Guerrero, Racotta & Villareal, 2003*; *Manush et al., 2006*), and it has been suggested that prolonged cold temperatures (< 4°C) negatively affect egg development in *H. americanus* (*Waddy & Aiken, 1995*).

It has also been suggested that the ability of lobsters to move between temperature regimes may be advantageous to egg development (*Gendron et al., 2009*). The goal of this study was to determine the effects of temperature on the protein and lipid reserves of *Homarus americanus.* Specifically, we sought to test the hypothesis that seasonal migrations of ovigerous females (and the disparate temperatures they are exposed to) have a significant, positive impact on egg development and the biochemical reserves that are passed onto their larvae.

METHODS

Lobster source and egg assessment

A total of 15 egg-bearing (ovigerous) lobsters were legally collected (New Hampshire Fish & Game permit, RSA 214:29) in late August and early September (2006) along the New Hampshire (NH) seacoast near Rye, NH and Gunboat Shoals (43°.0274 N; 70°.6938 W) by permitted commercial lobstermen using standard baited traps. Lobsters were transported to the University of New Hampshire (UNH) Coastal Marine Laboratory in Newcastle, NH and initially held in a large 1,200 L fiberglass tank with shelters. The holding tank was sourced by ambient sand-filtered seawater (average temp =15.3 ± 0.5°C) and was subjected to ambient light. Lobsters in the tank were fed a combination of fresh squid and crabs (*Cancer spp.*), twice per week.

Lobster carapace lengths (CL) were measured to the nearest 1 mm using digital calipers (Mitutoyo IP 65, Mitutoyo Corp., Japan) (size range: 84-96 mm CL) and a laminated disc tag (diameter = 2.0 cm, Floy Tag Inc., Seattle, WA) was fastened to propus of each animal with a single ziptie for identification throughout the duration of the study. A subset of eggs from each

lobster clutch (n=15-20) were viewed under a dissecting scope on September-5 and staged according to the methods outlined by *Helluy & Beltz (1991)*. Only lobster embryos with eye indices less than 18% (*Perkins, 1972*; *Goldstein & Watson, 2015b*) were used for this study to encompass as much of the early development process as possible.

Thermal treatments and sampling

The experimental setup and thermal treatments are described in detail in *Goldstein and Watson (2015b*). Briefly, six 0.91 m diameter (600 L) tanks (2 tanks/ treatment) were used to simulate either inshore, offshore, or constant (12 ± 0.4°C) temperature regimes on a year-round basis. Lobsters in each tank were kept isolated using mesh dividers and each animal received a shelter and was subjected to ambient photoperiod. For purposes of this study thermal regimes were simulated to either match inshore locations (shallow and coastal, 2-5 km from shore, 8-10 m depth) or the offshore habitats (12-20 km from shore, 20-30 m depth) to which lobster migrate in the fall and overwinter (see *Goldstein & Watson, 2015a*). Constant temperatures were chosen to simulate a favorable (and predictable) growth temperature similar to eggs observed in *Mackenzie (1988)*. Temperatures in all tanks were logged every 30-minutes using HOBO pendant loggers (model UA-002-64, Onset Computer, Bourne, MA) and later downloaded using Hoboware software (HOBOware Pro v. 3.0). Inshore temperatures mimicked the ambient seawater that was circulated through the CML seawater system while the temperatures in the offshore tanks were adjusted biweekly to simulate offshore temperatures based on historical and real time data published on the Northeastern Regional Association of Coastal Ocean Observing Systems (NERACOOS, http://neracoos.org). A total of five ovigerous females were exposed to each of the three temperature treatments and their eggs were sampled at five discrete time periods: twice

in the fall (Oct-15 & Nov-15) during initial growth, once in the winter (Jan-15), when eggs remain in a relative stasis, and twice in the spring (Mar-15 & May-25), during periods of rapid growth (*Sibert, Ouellet & Brethes, 2004; Goldstein & Watson, 2015b*).

At each sampling interval, lobster eggs (~100/sample) were removed from the center of each lobster clutch with a pair of fine forceps and placed in labeled plastic sample trays. All egg samples were rinsed and gently agitated with a 0.5% sodium hypochlorite and distilled water solution for ~ 1 min., after which they were rinsed with 100% distilled water and blotted dry to remove the cement matrix holding the eggs together (P. Talbot pers. comm.). Previous studies indicated that this chemical separation technique was non-invasive and did not compromise the biochemical integrity of the egg due to their complex and thickened membranes (*Johnson, Goldstein & Watson, 2011*). Each egg sample was divided into 30 egg aliquots then freeze-dried at -40°C for 24 hr. (Labconco Freeze Dryer 5, Kansas City, MO) and served as a stock for subsequent analyses. Samples were then ground down into a fine power using an industrial-grade milling machine (Wiley Mill #4, 40µm mesh screen, Thomas Scientific, Swedesboro, NJ) and samples were stored in labeled polyethelene scintillation storage vials for subsequent analyses.

Biochemical analyses

For each of the five sampling intervals, we determined total lipids and proteins for each lobster in triplicate (n=30 x 3) egg samples/female using the methods described in *Goldstein (2012)*. In brief, total protein levels were determined using a modified Lowry method (*Lowry et al., 1951*) using a BioRad protein assay kit with Coomassie Brilliant Blue G-250 (reagent standard) and bovine serum albumin as a standard (Biorad Laboratories, Hercules, CA). Egg samples were

digested in 1N NaOH, filtered, and read on a spectrophotometer (Beckman DU-250; $\lambda = 595$). Total lipid was quantified gravimetrically using the general protocol detailed in *Bligh & Dyer (1959)*. The procedure was modified to use a ratio of 1:2:2.5 chloroform-methanol-water extraction, respectively. Samples were dried for 24 hr at 37°C and stored in a glass dessicator, before being weighed on an analytic balance.

Egg volumes

For calculating egg volumes, 10-15 fresh eggs were removed in the middle of each of four sampling months (Sep, Nov, Jan, and Feb) from each lobster and placed in plastic 2.0 mL storage tubes, preserved in a 4% formalin and sterile seawater solution and stored at 4°C. Although we had intended to sample and calculate egg volumes in accordance with the same sampling regimes as our biochemical assays, for logistical reasons we were not able to do so. For each egg, a digital picture was taken using a Nikon Coolpix 995 digital camera mounted to a dissecting microsope (Nikon SMZ-2T, Nikon USA Inc., Melville, NY). All egg images were imported into an image processing software (Image J v.1.35, see http://rsb.info.nih.gov/ij/) and a digital measuring tool was used to make calculations of each egg's longest axis. All calculations were measured to the nearest 0.1 mm (then converted to μm) and values for each sample were averaged (± se). Egg volumes were then calculated using the formula: $V = 4/3*(\pi r^3)$, where r is the radius for spheroid-shaped embryos (see *Garcıa-Guerrero & Hendrickx, 2004*).

Data analysis

Analysis of variance (ANOVA) was used to investigate potential differences in egg protein and lipid content between the three thermal regimes (fixed factor 1) at each of the five sampling

intervals (fixed factor 2). A 3x5 full factorial design was used and analyzed as a split-plot (SP) ANOVA (whole-plot = temperature, sub-plot = month, $df_{total} = 15$) using a PROC MIXED model in SAS v. 9.3 (SAS Institute Inc., Cary, NC). For all ANOVA models, we tested the assumptions of normality, independence, and homogeneity of variance and used a Kuskal Wallis H-test where these assumptions were not supported. Differences between groups were compared using the PDIFF function in SAS. Regression analyses were carried out using JMP v. 9.3 (SAS Institute Inc., Cary, NC) statistical software. All means are expressed ± se.

RESULTS

Water temperatures

Seawater temperatures over the course of this study (October-May) averaged 7.1 ± 0.24°C (range = 2.1-11.2) for inshore laboratory simulations, compared with 6.0 ± 0.19°C (range = 2.8-10.1) for the offshore thermal regime, and 12.2 ± 0.21°C for the constant treatment tank (Fig. 1). There was an overall significant difference in water temperatures between the constant tank treatment and both inshore and offshore ones (*ANOVA, Kruskal-Wallis H-Test;* $F_{2,7} = 10.32$, $P < 0.0001$; Fig. 1) but not between inshore and offshore.

Lipid and protein content

Total egg lipid levels from inshore and offshore thermal regimes were very different from their constant temperature counterpart (*SPANOVA;* $F_{2,44} = 10.3$, $P = 0.0002$) and also differed by month ($F_{4,44} = 302.9$, $P < 0.0001$; Fig. 2). Likewise, total protein levels in lobster eggs between

inshore and offshore thermal regimes also differed from eggs exposed to constant temperatures (*SPANOVA;* $F_{2,44} = 67.17$, $P = 0.0002$) as well as by month ($F_{4,44} = 350.3$, $P < 0.0001$, Fig. 2). The interactive effect of temperature and month was significant for both lipid ($F_{7,44} = 2.27$, $P < 0.045$) and protein levels ($F_{7,44} = 46.5$, $P < 0.0001$) and these results are summarized in Tables 1 & 2. Overall egg lipid values showed a decrease over time (equation: lipids = 381.76 - 55.00*month, $r^2_{adj} = 0.85$, $P < 0.0001$; Fig. 2), falling most dramatically early in the fall (-16.8% inshore, -21.4% offshore, -24.8% constant) and late spring (-63.7% inshore, -59.0% offshore). By contrast, total lobster egg protein values increased over the same time-frame (equation: proteins = -35.53 + 69.11*month, $r^2_{adj} = 0.63$, $P < 0.0001$; Fig. 2), and exhibited the largest increases in the fall (60.4% inshore, 57.7% offshore, 66.5% constant) and spring (30.1% inshore, 37.1% offshore).

Egg volumes

Overall, there was a significant increase in egg volume over time for all eggs over all treatments ($r^2_{adj} = 0.413$, $P < 0.001$). However, there were no significant changes with respect to egg volume by treatment ($F = 0.73$, $df = 2$, $P = 0.513$) (overall means: inshore = 3226 ±163 μm^3, offshore = 3254 ± 167 μm^3, constant = 3476 ± 152 μm^3), even though there were differences from month-to-month ($F = 2.25$, $df = 3$, $P < 0.001$; Fig. 3).

DISCUSSION

The main goal of this study was to document the changes in lipids and proteins in lobster eggs as they developed during exposure to three different thermal regimes by designing field-simulated

238 temperature regimes in the laboratory. It is important to note that biochemical differences in the
239 field may manifest themselves differently compared with lab-based empirical data. In a
240 companion study (*Goldstein & Watson, 2015a*), we saw no differences in lobster egg
241 development rate when comparing lab- and field-based data. We would therefore expect that
242 biochemical attributes in eggs would also not be appreciably different between lab and field
243 treatments, although it would be interesting to verify this. In general, we found that the trends
244 during embryogenesis in *H. americanus* were typical of other decapods: lipid reserves were
245 catabolized while proteins were utilized to make tissues (*Holland, 1978*; *Sasaki, McDowell*
246 *Capuzzo & Biesiot, 1986*; *Jacobs et al., 2003*; *Brillon, Lambert & Dodson, 2005*). In tandem
247 with these patterns, eggs were also shown to increase in egg diameter. Not surprisingly, lobster
248 eggs exposed to an elevated, constant temperature developed faster and thus used up their energy
249 reserves much sooner than eggs subjected to natural thermal regimes, which included decreases
250 in water temperatures from November to March.
251
252 This study did not obtain data for biochemical changes that occurred in eggs that were
253 approaching hatch (~ 30 days prior) nor the effects of such changes on larval survivorship or
254 condition. As a result, there were no apparent biochemical differences in lobster eggs between
255 inshore and offshore temperature treatments. Despite this, it has been shown that large changes
256 in egg yolk lipids and protein levels occur within the last few weeks of development (*Sibert,*
257 *Ouellet & Brethes, 2004*), when it is hypothesized that water temperatures are most variable,
258 suggesting a large influence in the rate of temperature change between inshore and offshore
259 locations. Concurrent with this are the associated (but different) rates of temperature increase
260 that occur between inshore and offshore waters especially in the late spring and early summer

that impact when lobsters hatch (*Goldstein & Watson, 2015a,b*). As a result, this could change how energetic reserves are allocated near the end of development more intensively, compared to the beginning.

Other studies have shown the influence of such thermal exposures on larval condition (*Sasaki, McDowell Capuzzo & Biesiot, 1986*; *Ouellet & Plante, 2004*), and it was very clear that significant changes to lobster egg biochemistry are apparent in the first couple months of development (this study) as well as leading up to the month before hatching (*Sasaki, McDowell Capuzzo & Biesiot, 1986*). The effect of temperature on metabolic and developmental rates is expressed through changes in the consumption rates of metabolic reserves that are affected by changing temperatures (*Sasaki, McDowell Capuzzo & Biesiot, 1986*). Thus, the seasonal aspects of fluctuating temperature have a 'real' impact on the rates and course of development in lobster eggs. It is suggested that fluctuating seasonal temperatures help to accelerate egg development during some time frames while depressing it at others, providing temporal windows where hatching generally takes place (*Helluy & Beltz, 1991*; *Waddy & Aiken 1995*; *Goldstein & Watson, 2015b*).

Seasonal movements by ovigerous lobsters provide one potential strategy for exposing their eggs to variable seawater temperatures and locations where the timing of hatch could be favorable (*Gendron & Ouellet, 2009*). These movements influence overall egg incubation time and may affect how internal egg resources are utilized (*Sasaki, McDowell-Capuzzo & Biesiot, 1986*; *Goldstein & Watson, 2015a*). This was seen most clearly in eggs that were exposed to constant, elevated temperatures albeit, this would unlikely ever happen because natural temperatures

almost always show some fluctuation. We do show however that low, ambient temperatures in the winter slow down development, but this is not surprising. Seasonally changing temperatures, including a refractory period of cold seawater temperatures (< 5°C), are important to conserving egg resources for more rapid increases in temperature (> 10°C) that typically occur later on (*Waddy & Aiken, 1995*). These thermal conditions were simulated in both inshore and offshore treatments and resulted in egg development that extended well into the spring and early summer (offshore). Although eggs exposed to a constant temperature, hatched much sooner, they also contained residual yolk reserves upon hatch; this was also documented by *Sasaki, McDowell-Capuzzo & Biesiot (1986)*.

Lipids and proteins

Many studies conducted on crustacean eggs show that lipids are the major energy reserve (*Holland, 1978*; *Fraser, 1989*; *Clarke, Brown & Holmes, 1990*; *Heras, Gonzales-Baro & Pollero, 2000*). Although egg yolk lipids were consumed at disparate rates in our thermal treatments and throughout all months, lipids were consumed much more modestly in winter (Fig. 2; Table 1). This pattern is seen consistently in other crustaceans at similar rates. For example, the egg lipid content of fiddler crab (*Uca rapax)* decreases significantly (78.4%) through embryogenesis, confirming that lipids constitute an important energy source for embryonic development. In addition, lipids are also used as structural components of cell membranes that are being formed as they grow (*Rosa & Nunes, 2003*). Thus, the catabolism of lipids is a classic feature of crustacean eggs and many other crustaceans produce eggs with large lipid reserves that are used throughout embryogenesis (*Rosa et al., 2007*). Lipid depletion rates are directly related to incubation temperature, and it has been observed in other crustaceans that the energy

307 consumption per day, mostly provided by lipids, slightly intensified 3 or 4 days before hatching
308 (esp. with higher temps), could be related to a higher energy production need at this time (*Heras,*
309 *Gonzales-Baro & Pollero, 2000*). Yolk lipids tend to become catabolized first followed by yolk
310 proteins. These ratios change and can be used to estimate the cost of egg development at
311 differing temperatures (*Sasaki, McDowell- Capuzzo & Biesiot, 1986*). In the field, lipid profiles
312 (*e.g.,* fatty acids) have been used to identify offshore from inshore lobster eggs (*Castell et al.,*
313 *1995*); therefore, it is possible that these constituents are utilized differently across different
314 geographic regions that correspond to disparate thermal regimes.
315
316 For proteins, the consumption rate during embryogenesis may increase as temperature rises
317 (*Conceicao et al., 1998*). Proteins not only function as building blocks for tissue and organs but
318 more so, may act as intermediates in carbohydrate and lipid metabolism (*Schmidt-Nielsen 1991*).
319 Thus, trying to quantify protein levels may be masked by their intricate link to other biochemical
320 components. Over prolonged, cold temperatures or those conditions in which temperatures are
321 too high for even short periods of time, some crustacean embryos may instead utilize proteins as
322 an energy source if lipids are low due to thermally-induced demands (*Conceicao et al., 1998*).
323
324 At elevated temperatures (constant), increases in protein levels were clearly detected and at these
325 'sub-optimal' temperatures, tissue synthesis tends to be inefficient and more protein might be
326 used as energy instead (*e.g., Garcia-Guerrero, Racotta & Villareal, 2003*). Therefore, the
327 duration and rates of differing thermal profiles would most certainly affect these biochemical
328 changes and allocations of resource components over time. How this translates to larval
329 survivorship remains poorly understood. However, *Sasaki, McDowell Capuzzo & Biesiot (1986)*

showed that up until Stage IV (post-larval), lobsters depended upon stored capacities of lipids and that proper temperature synchronization in these residual lipids maybe favorable to settlement processes.

Egg volume

The increase of water in the eggs (egg volume) as seen in this study and others is directly related to water uptake during new cell formation in the embryo and has been noted to increase by more than 50% over the course of development (*Pandian, 1970*). Increases in egg volume are primarily due to water uptake by the embryo as well as from the retention of metabolic water resulting from respiration (*Pandian, 1970*; *Petersen & Anger, 1997*). The associated osmotic changes that ensue during egg development can be an important component to hatching and have also been implicated in mechanically aiding the breakage of the chorion near the time of hatch (*Pandian, 1970*). Slight changes in lobster egg volume have been previously explained as a function of a plastic response to variations in salinity (*Charmantier & Aiken, 1987*), and for later eggs, a consequence of physiological factors during development (*Pinheiro & Hattori, 2003*). In these instances, the movements or residency of lobsters in certain locations where seawater salinities can vary dramatically during certain times of the year (*e.g.,* estuaries; *Watson, Vetrovs & Howell, 1999*) may have an impact on aspects of development or hatch, especially near the latter part of egg development (*Charmantier & Aiken, 1987*).

Female size and condition

In this study we did not specifically address the influence of maternal size or nutritional condition on egg quality in *H. americanus*. However, other related studies have showed that

caloric energy content per egg increases with female size (*Attard & Hudon, 1987*). *Sibert, Ouellet & Brethes (2004)* described this relationship by creating a growth index model for egg development and found that bigger eggs used yolk lipids more efficiently and sustained faster embryonic growth compared with smaller eggs. In addition, *Ouellet & Plante (2004)* reported that first-time (primiparous) spawners produced smaller larvae compared to larger, multiparous lobsters (although larval size was independent of female size). Results from these key studies point out the need to more clearly investigate these factors in more depth. Since female size and reproductive history may play a role in the allocation of metabolic egg reserves.

Large invertebrate eggs often have greater organic content than small eggs (*Clarke, Brown & Holmes, 1990*; *Clarke, 1992*) but egg size is not always an accurate predictor of organic content in decapods. *Jacobs et al. (2003)* for example, found that the larger size of blue crab (*Callinectes sapidus*) embryos in the spring is due, for the most part, to increased water uptake and the concomitant increase in inorganic salts (ash) commonly seen in crustacean embryos (*Pandian 1970*). An effect of female size on egg reserve allocation has been reported in other decapods including snow crab (*Chionoecetes opilio*), giant crab (*Pseudocarcius gigas*), and lobster (*Homarus americanus*) (*Attard & Hudon, 1987*; *Sainte-Marie, 1993*; *Gardner, 2001*). In lobsters it has been postulated that the effect of female size may mean that larger females make a greater contribution towards egg reserves (*Attard & Hudon, 1987*); however, the added effect of temperature on egg 'quality' may, in some cases override this effect and more work is needed to address this. In addition to female size are potential effects that maternal nutrition has on modulating egg quality (*Goldstein & Shields, 2018*). The lecithotrophic nature of lobster eggs is determined largely through the sequestering of maternal nutrients throughout the processes of

primary and secondary vitellogenesis during oocyte formation, the latter of which is highly dependent on the female's organic energy reserves (*e.g.,* lipoprotein; *Dehn, Aiken & Waddy, 1983*).

CONCLUSIONS

Although the changes in biochemical components (lipids and proteins) in developing lobster eggs were not dramatically different from inshore and offshore thermal regimes, there is still the potential for variations in the energetics of embryogenesis influenced by the seasonal movements of some lobsters to and from these two disparate locations. Thus, as seasonal thermal cycles fluctuate or potentially shift (*i.e.*, climate change), the timing of egg hatch and associated egg quality may modulate further biochemical changes to lobster eggs and have implications for hatch, larval competency, and condition as well as other early-life history dynamics.

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Figure 1

Summarized temperature data from three thermal treatments.

Weekly temperature averages compiled for three thermal treatments: inshore, offshore, and constant) from October-June, 2006-2007. See Methods section for details. There was an overall significant difference in water temperatures between the constant tank treatment and both inshore and offshore ones (*ANOVA, Kruskal-Wallis H-Test;* $F_{2,7}$ =10.32, $P < 0.0001$) but not between inshore and offshore.

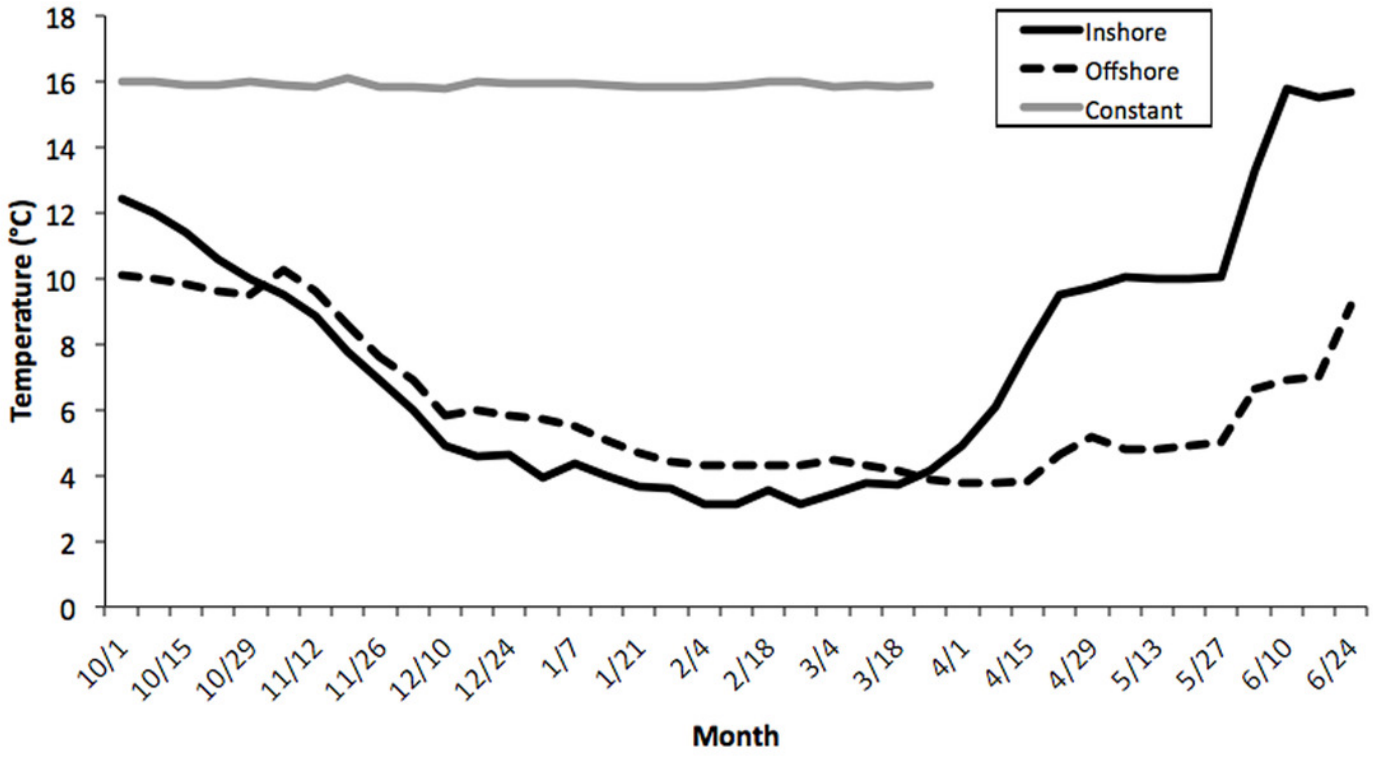
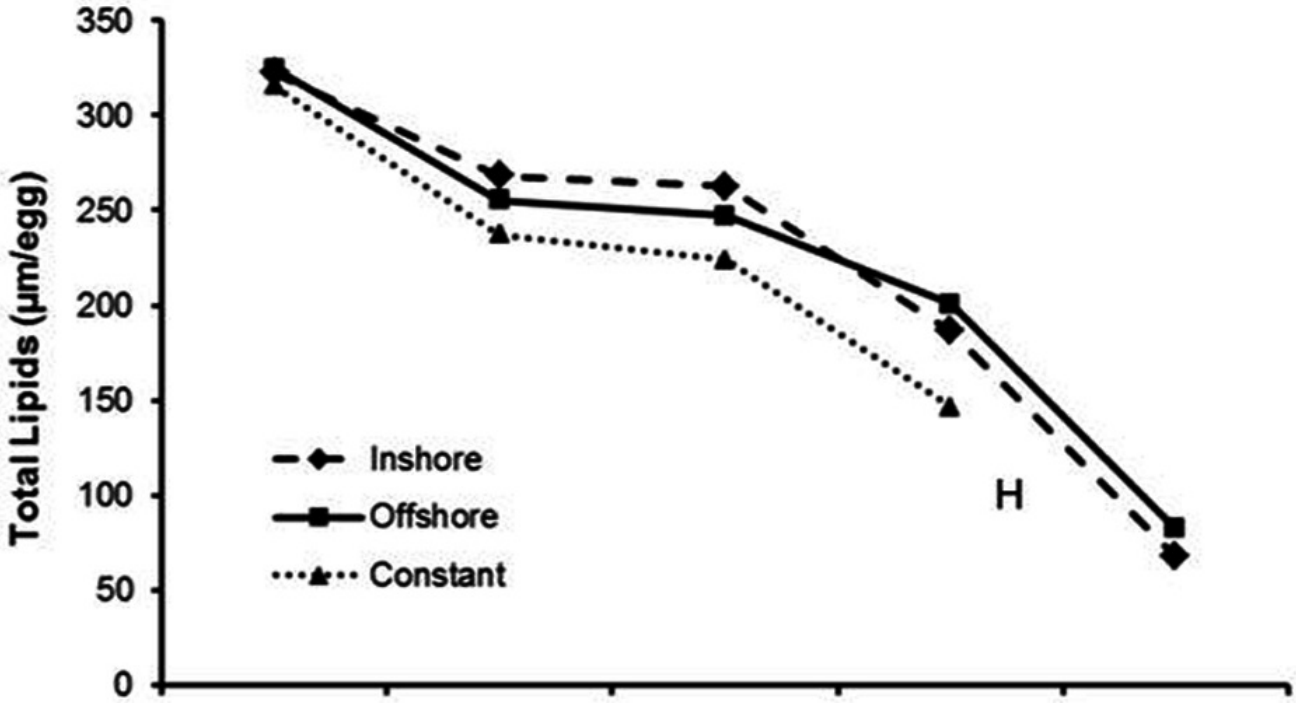


Figure 2

Change in lipids (top) and protein (bottom) levels through the course of seven months of egg development for all lobsters sampled (n = 5/trt).


Total Lipids (µm/egg)
350
300
250
200
150
100
50
0
Inshore
Offshore
Constant
H

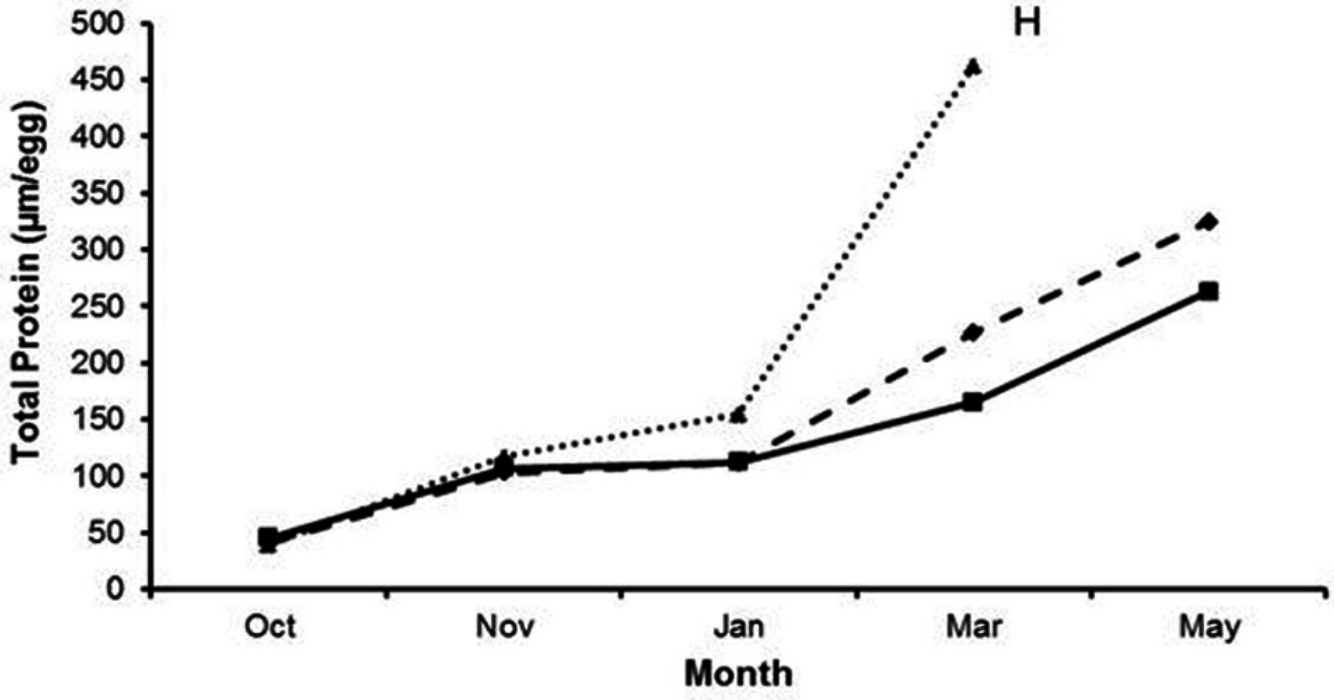

Total Protein (µm/egg)
500
450
400
350
300
250
200
150
100
50
0
H
Oct
Nov
Jan
Mar
May
Month

Figure 3

A summary of means (± se) for changes in lobster egg volumes (given in μm^3) over a six month period.

There were no significant differences in egg volume by treatment (Tukey's HSD; $q = 2.40$, $P > 0.05$), but differences did exist from month-to-month ($F = 2.25$, $df = 3$, $P < 0.001$).

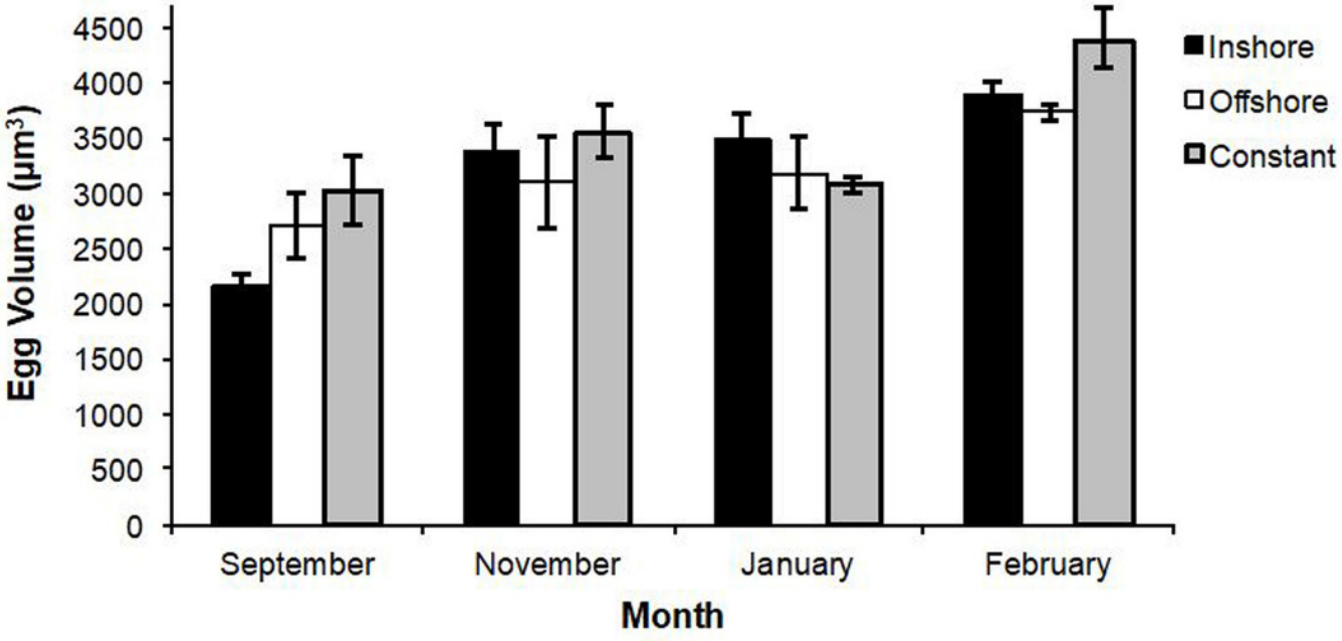


Table 1(on next page)

A summary of means (± se) for lobster egg total lipids and total proteins over five months.

Post-hoc differences (from SAS) for both variables are given below; groups with different superscripts denote treatment differences ($P < 0.001$).

	Lipids				
	October	November	January	March	May
Inshore	322.2 ± 7.5	268.2 ± 9.6	262.6 ± 12.2	186.4 ± 7.3	67.6 ± 3.6
Offshore	324.6 ± 7.4	255.2 ± 11.7	247.0 ± 12.5	200.8 ± 3.8	82.4 ± 7.3
Constant	315.7 ± 8.7	237.3 ± 4.8	224.0 ± 7.2	146.4 ± 12	

	Proteins				
	October	November	January	March	May
Inshore	322.2 ± 7.5	268.2 ± 9.6	262.6 ± 12.2	186.4 ± 7.3	67.6 ± 3.6
Offshore	324.6 ± 7.4	255.2 ± 11.7	247.0 ± 12.5	200.8 ± 3.8	82.4 ± 7.3
Constant	315.7 ± 8.7	237.3 ± 4.8	224.0 ± 7.2	146.4 ± 12	

	Post-hoc PDIFF Results (α = 0.05)		
Treatment group:	Constant[a]	Inshore[b]	Offshore[b]

Table 1 A summary of means **(**± se) for lobster egg total lipids and total proteins over five months. Post-hoc differences (from SAS) for both variables are given below; groups with different superscripts denote treatment differences ($P < 0.001$).

Table 2(on next page)

Pairwise comparisons between temperature treatment and month for both lipids and protein values.

Shaded *P*-values (< 0.05) denote significant differences between temperatures for a specific month.

Treatment	October	November	January	March	May
inshore * offshore	0.85	0.30	0.21	0.25	0.24
inshore * constant	0.89	0.03	0.002	0.002	-
constant * offshore	0.72	0.22	0.04	< 0.0001	-

Table 2 Pairwise comparisons between temperature treatment and month for both lipids and protein values. Shaded *P*-values (< 0.05) denote significant differences between temperatures for a specific month.