Biochemical changes throughout early- and middle-stage of embryogenesis in lobsters (*Homarus americanus*) under varying thermal regimes

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Most marine crustacean eggs contain a full complement of nutritional resources that fuel the growth and metabolic processes over the course of their development. In terms of biochemical constituents, lipids and proteins play pivotal and central roles in these processes and, accordingly, have been studied extensively in crustaceans. Given the propensity of some ovigerous (egg-bearing) American lobsters (*Homarus americanus*) to undergo seasonal inshore-to-offshore migrations, thereby exposing their eggs to varying thermal regimes, this study's goal was to assess egg quality over their course of development by documenting changes in total lipids, proteins, and egg size (volume) in lobsters subjected to one of three simulated thermal regimes (inshore, offshore, constant (12°C), N = 5/trt, 15 total) in the laboratory and sampled at five discrete time intervals. Total egg lipids showed a marked decrease over time (r^2_{adj} = 0.85, $P < 0.0001$), early in the fall (average = -26%) and late spring (-62%), compared with stark increases in proteins over the same period (r^2_{adj} = 0.63, $P < 0.0001$, averages = 60%, 34%, fall and spring). Although there were no significant differences in total lipid or protein values (or egg sizes) between eggs exposed to inshore and offshore temperatures ($P > 0.05$), differences occurred in eggs exposed to a constant temperature, and they hatched almost three months sooner than inshore or offshore ones. Seasonal temperature fluctuations also appear to control the rates of biochemical processes in lobster eggs but may be confounded by other variables.

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ABSTRACT

Most marine crustacean eggs contain a full complement of nutritional resources that fuel the growth and metabolic processes over the course of their development. In terms of biochemical constituents, lipids and proteins play pivotal and central roles in these processes and, accordingly, have been studied extensively in crustaceans. Given the propensity of some ovigerous (egg-bearing) American lobsters (*Homarus americanus*) to undergo seasonal inshore-to-offshore migrations, thereby exposing their eggs to varying thermal regimes, this study's goal was to assess egg quality over their course of development by documenting changes in total lipids, proteins, and egg size (volume) in lobsters subjected to one of three simulated thermal regimes (inshore, offshore, constant (12°C), N = 5/trt, 15 total) in the laboratory and sampled at five discrete time intervals. Total egg lipids showed a marked decrease over time ($r^2_{adj} = 0.85$, $P < 0.0001$), early in the fall (average = -26%) and late spring (-62%), compared with stark increases in proteins over the same period ($r^2_{adj} = 0.63$, $P < 0.0001$, averages = 60%, 34%, fall and spring). Although there were no significant differences in total lipid or protein values (or egg sizes) between eggs exposed to inshore and offshore temperatures ($P > 0.05$), differences occurred in eggs exposed to a constant temperature, and they hatched almost three months sooner than inshore or offshore ones. Seasonal temperature fluctuations also appear to control the rates of biochemical processes in lobster eggs but may be confounded by other variables.

INTRODUCTION

Egg development for most marine crustaceans relies heavily on the production and sequestering of nutrients required for the development and maintenance over the entire process of embryogenesis. In terms of biochemical constituents, both lipids and proteins play pivotal and central 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 responsible for the overall metabolism of growing crustacean embryos. Remarkably, these constituents have been reported to account for upwards of 60% of the total energy expenditure for growth (*Holland, 1978*; *Amsler & George, 1984*). By contrast, the role of proteins as the basic building blocks of animal tissues are well known (*Holland, 1978*), and function as alternative energy sources under certain conditions (*Schmidt-Nielsen, 1991*; *Heras, Gonzales-Baro & Pollero, 2000*).

Egg development in crustaceans is especially linked to temperature such that incubation periods can be extended (cold temps) or reduced (warm temps). Closely coupled metabolic rates increase with temperature thereby modulating yolk absorption, growth and ultimately, the survival of eggs (*Pandian, 1970*; *Schmidt-Nielsen, 1991*). Development and metamorphosis of planktotrophic larvae, including decapod crustaceans, depends to a great extent on nutrition (*Racotta & Ibarra, 2003*) from both exogenous (from feeding) and endogenous (yolk reserves) sources which are important metrics during early postembryonic development (*Sasaki, McDowell-Capuzzo & Biesiot, 1986*; *Clarke, Brown & Holmes, 1990*). Together, the relationship between the primary biochemical components in crustacean eggs and their

associated 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*). Because the American lobster fishery garners such tremendous economic influence, fisheries scientists and managers focus much of their attention on many aspects of stock assessment including the fecundity, spawning stock biomass, and abundance of egg-bearing (ovigerous) females that are historically protected from being landed (*ASMFC, 2015*). The life history of *H. americanus* includes a complex suite of embryonic, pelagic (larval), and benthic (juvenile and adult) developmental stages (see review in *Lawton & Lavalli, 1995*), most notably, their yolk-laden eggs that are extruded and carried for 9-11 months over the full course of their development (*Talbot & Helluy, 1995*); temperature is a key factor that determines the length of time the eggs are carried (*Templeman, 1940*; *Aiken & Waddy, 1980*). Mature lobster oocytes are large (1.4-1.6 mm diameter upon extrusion) and typically contain large amounts of high-density lipoproteins (> 40%, lipovitellins) that are allocated as yolk material through a complex suite of primary and secondary vitellogenesis (*Nelson, Hedgecock & Borgeson,1988*; *Talbot & Helluy, 1995*).

Besides the often protracted egg development in *H. americanus*, one of the most interesting and sometimes dramatic features of some ovigerous lobsters is their propensity to migrate seasonally over an array of habitat types (including thermal ones) and distances (typically, 5-

10 km, but sometimes much greater) throughout the development of their eggs (see reviews by *Cooper & Uzmann, 1980*; *Lawton & Lavalli, 1995*). The implications of such movement events in ovigerous lobsters has the potential to shape the developmental dynamics of the eggs they carry by subjecting them to differing thermal regimes whose rates of change can be quite different (*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.

Biochemical and energetics considerations in lobster eggs have been well studied and suggest the following key patterns: 1) differing thermal regimes influence the utilization of energy reserves in developing embryos and embryos raised at accelerated temperatures contain residual yolk reserves upon hatch (*Sasaki, McDowell-Capuzzo & Biesiot, 1986*); 2) the energy content of eggs tend to increase with female size (*Attard & Hudon, 1987*); and 3) larval size at hatch is independent of female size (*Ouellet & Plante, 2004*). Despite some contradictory evidence between some of these studies, it is evident that egg resources influence their growth and development.

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, crustacean eggs subjected to

either prolonged warm or cold temperatures can have a deleterious effect on the use of their 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*). Therefore, one way of assessing the effects of temperature on the overall development of lobster eggs is through the proximate analysis of their biochemical components, namely, lipids and proteins.

The goal of this study was to further elucidate the effects of lobster movements over varying thermal regimes (inshore and offshore) during the course of egg development, complementing existing work on egg development and hatch under differing thermal regimes in the laboratory, by quantifying two key biochemical descriptors (lipids and proteins) of egg resource utilization as well as changes in egg size. A constant, slightly elevated temperature was also used to compare egg development under non-fluctuating thermal conditions.

METHODS

Lobster source and egg assessment

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 tanks with shelters. Tanks were exposed to ambient light and sand-filtered

seawater (average temp =15.3 ± 0.5°C), and lobsters were fed a combination of fresh squid and crabs (*Cancer spp.*), twice weekly.

A subset of the eggs in each clutch were viewed under a dissecting scope and staged according to the methods outlined by *Helluy & Beltz (1991)*. These samples also served as covariates for all subsequent statistical analyses. Only lobsters whose eye index was less than 18% were used for this study (*Perkins, 1972*; *Goldstein & Watson 2015b*) in order to encompass as much of the early development process as possible. Lobster carapace lengths (CL) were measured to the nearest 1 mm using digital calipers (Mitutoyo IP 65, Mitutoyo Corp., Japan). A single, circular, laminated disc tag (diameter = 2.0 cm, Floy Tag Inc., Seattle, WA) was fastened to the claw knuckle of each animal for individual identification throughout the duration of the study.

Thermal treatments and sampling

The experimental setup and thermal treatments followed a companion study that served to concurrently quantify lobster egg development and hatch time in the same group of lobsters (see *Goldstein and Watson 2015b*). Briefly, a series of four 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 (Fig. 1). For purposes of this study, inshore locations (shallow and coastal) were considered the same areas where animals were collected (2-5 km from shore, 8-10 m depth), while offshore ones were designated as 12-20 km from shore (20-30 m depth) to simulate those lobsters that might make seasonal, fall migrations offshore (see *Goldstein & Watson 2015a*). Constant temperatures were chosen to simulate a favorable growth temperature similar to eggs observed in *Mackenzie (1988)*. Temperatures in all tanks were logged

automatically every 30-minutes using HOBO pendant loggers (model UA-002-64, Onset Computer, Bourne, MA) and later downloaded into Microsoft Excel using Hoboware software (HOBOware Pro v. 3.0). Temperature profiles from the offshore tank treatment were adjusted semi-regularly to simulate seasonal temperature changes in the field and monitored from historical and real time data published on the Northeastern Regional Association of Coastal Ocean Observing Systems (NERACOOS, http://neracoos.org). A subset of five ovigerous females were sampled at each temperature treatment for a total of 15 lobsters. All lobsters were sampled for eggs at five discrete time periods: twice in the fall and spring (during periods of rapid growth; *Sibert, Ouellet & Brethes, 2004*) and once in the winter.

Lobster eggs (~ 100/sample) were removed from the center of each 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.). Rather than mechanically separate eggs, this technique was chosen for its efficacy. Preliminary studies that were conducted 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*).

For biochemical analyses, egg samples (~ 30/sample) were frozen at -80°C prior to processing and freeze-dried at -40°C for 24 hr (Labconco Freeze Dryer 5, Kansas City, MO). Dried egg samples were then ground down into a fine power using an industrial-grade milling machine (Wiley Mill #4, 40 μm mesh screen, Thomas Scientific, Swedesboro, NJ) and samples were

196 stored in labeled polyethelene scintillation storage vials for subsequent analyses (Fig. 2).

197

198 **Biochemical analyses**

199 Over each sampling interval, a total of three replicate egg samples/female were pooled for lipid
200 and protein values. Total protein levels were determined using a modified Lowry method (*Lowry*
201 *et al.,1951*) using a BioRad protein assay kit with Coomassie Brilliant Blue G-250 (reagent) and
202 bovine serum albumin as a standard (Biorad Laboratories, Hercules, CA). Egg samples were
203 digested in 1N NaOH, filtered and read on a spectrophotometer (Beckman DU-250; $\lambda = 595$).
204 Total lipid was quantified gravimetrically using the general protocol detailed in *Bligh & Dyer*
205 *(1959)*. The procedure was modified in a ratio of 1:2:2.5 chloroform-methanol-water extraction,
206 respectively. Samples were dried for 24 hr. at 37°C and stored in a glass dessicator, before being
207 weighed on an analytic balance (Fig. 2). Detailed protocols for both total lipids and proteins can
208 be found in *Goldstein (2012)*.

209

210 **Egg volumes**

211 For calculating egg volumes, 10-15 eggs were removed at each of the aforementioned five time
212 periods and placed in plastic 2.0 mL storage tubes, preserved in a 4% formalin and sterile
213 seawater solution and stored at 4°C. For each egg, a digital picture was taken under a dissecting
214 microsope (Nikon SMZ-2T, Nikon USA Inc., Melville, NY) using a scope-mounted Nikon
215 Coolpix 995 digital camera. All egg images were imported into an image processing software
216 (Image J v.1.35, see http://rsb.info.nih.gov/ij/) and a digital measuring tool was used to make
217 calculations of each egg's longest length. All calculations were measured to the nearest 0.01 mm
218 (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). 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. There was an overall significant difference in water temperatures between the constant tank treatment and both inshore and offshore ones (*ANOVA;* $F_{2,7} = 10.32$, $P < 0.0001$), 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. 3). 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. 3). 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 are summarized in Tables 1 & 2. Overall egg lipid values showed a marked decrease over time (equation: lipids = 381.76 - 55.00*month, $r^2_{adj} = 0.85$, $P < 0.0001$; Fig. 4), 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. 4), but exhibited large increases in the fall (60.4% inshore, 57.7% offshore, 66.5% constant) and spring (30.1% inshore, 37.1% offshore) and much more modest ones in the winter, typically 10-15%.

Egg volumes

Overall, there was a significant increase in egg volume over time for all eggs over all treatments ($r^2_{adj} = 0.413$, $P < 0.001$). Although 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) differences from month-to-month did exist ($F =$ 2.25, $df = 3$, $P < 0.001$; Fig. 5). Gains in egg volume (for all treatments) accounted for ~ 52% between September and February, although there was a slight decrease (-13.5%) in egg volume for the constant treatment between November and January.

DISCUSSION

The main goal of this study was to document the changes in lipids and proteins in lobster eggs over three disparate thermal regimes and the effect that temperature has on these important biochemical processes. In general, the trends during embryogenesis in *H. americanus* were typical of other decapods: lipid reserves were catabolized while proteins were utilized to make tissues (*Holland, 1978*; *Sasaki, McDowell Capuzzo & Biesiot, 1986*; *Jacobs et al., 2003*; *Brillon, Lambert & Dodson, 2005*). In tandem with these patterns, eggs were also shown to absorb water during development with a resultant increase in egg diameter. Not surprisingly, lobster eggs exposed to an elevated, constant temperature elicited dramatic changes compared with inshore and offshore ones and, as a result, hatched sooner. Furthermore, the methods that were employed in this study were able to replicate those of other studies that tracked similar metrics in lobster eggs over time (*Pandian, 1970*; *Sasaki, McDowell Capuzzo & Biesiot, 1986*; *Sibert, Ouellet & Brethes, 2004*).

This study did not obtain data for biochemical changes that occurred in eggs that were approaching hatch (~ 30 days prior) or the effects of such changes on larval survivorship or condition. As a result, there were no apparent biochemical differences in lobster eggs between inshore and offshore temperature treatments. Despite this, it has been shown that large changes in egg yolk lipids and protein levels occur within the last few weeks of development (*Sibert, Ouellet & Brethes, 2004*), suggesting a large influence in the rate of temperature change between inshore and offshore locations. Concurrent with this are the associated (but different) rates of temperature increase that occur between inshore and offshore waters especially in the late spring

290 and early summer that impact when lobsters hatch (*Goldstein & Watson, 2015a,b*). As a result,
291 this could change how energetic reserves are allocated near the end of development more
292 intensively, compared to the beginning.
293
294 Other studies have shown the influence of such thermal exposures on larval condition (*Sasaki,*
295 *McDowell Capuzzo & Biesiot, 1986*; *Ouellet & Plante, 2004*), and it was very clear that
296 significant changes to lobster egg biochemistry are apparent in the first couple months of
297 development (this study) as well as leading up to the month before hatching (*Sasaki, McDowell*
298 *Capuzzo & Biesiot, 1986*). The effect of temperature on metabolic and developmental rates is
299 expressed through changes in the consumption rates of metabolic reserves that are affected by
300 changing temperatures (*Sasaki, McDowell Capuzzo & Biesiot, 1986*). Thus, the seasonal aspects
301 of fluctuating temperature have a 'real' impact on the rates and course of development in lobster
302 eggs. It is suggested that fluctuating seasonal temperatures help to accelerate egg development
303 during some time frames while depressing it at others, providing temporal windows where
304 hatching generally takes place (*Helluy & Beltz, 1991*; *Waddy & Aiken 1995*; *Goldstein &*
305 *Watson, 2015b*).
306
307 Seasonal movements by ovigerous lobsters provide one potential strategy for exposing their eggs
308 to variable seawater temperatures and locations where the timing of hatch could be favorable.
309 These movements influence overall egg incubation time and may affect how internal egg
310 resources are utilized (*Sasaki, McDowell-Capuzzo & Biesiot, 1986*; *Goldstein & Watson, 2015a*).
311 This was seen most clearly in eggs that were exposed to constant, elevated temperatures. In this
312 case, egg lipid and protein levels changed dramatically and eggs hatched almost three months

313 prior to inshore and offshore egg treatments (Fig. 3). It is presumed that egg hatching in March
314 or April would be detrimental to survival in the plankton due to suboptimal levels in temperature
315 and food across most areas (e.g., match-mismatch hypothesis). Seasonally changing
316 temperatures, including a refractory period of cold seawater temperatures (< 5°C), are important
317 to conserving egg resources for more rapid increases in temperature (> 10°C) that typically occur
318 later on (*Waddy & Aiken, 1995*). These thermal conditions were simulated in both inshore and
319 offshore treatments and resulted in egg development that extended well into the spring and early
320 summer (offshore). Although eggs exposed to a constant temperature, hatched much sooner, they
321 also contained residual yolk reserves upon hatch; this was also documented by *Sasaki,*
322 *McDowell- Capuzzo & Biesiot (1986)*.
323
324 **Lipids and proteins**
325 Most studies conducted on crustacean eggs show that lipids are the major energy reserve
326 (*Holland, 1978*; *Fraser, 1989*; *Clarke, Brown & Holmes, 1990*; *Heras, Gonzales-Baro &*
327 *Pollero, 2000*). Egg yolk lipids were rapidly consumed in all thermal treatments and throughout
328 all months, although much more modestly in winter (Fig. 3; Table 1). This pattern is seen
329 consistently in other crustaceans at similar rates. For example, the egg lipid content of fiddler
330 crab (*Uca rapax)* decreases significantly (78.4%) through embryogenesis, confirming that lipids
331 constitute an important energy source for embryonic development. In addition, lipids are also
332 used as structural components of cell membranes that are being formed as they grow (*Rosa &*
333 *Nunes, 2003*). Thus, the catabolism of lipids is a classic feature of crustacean eggs and many
334 other crustaceans produce eggs with large lipid reserves that are used throughout embryogenesis
335 (*Rosa et al., 2007*). Lipid depletion rates are directly related to incubation temperature, and it has

been observed in other crustaceans that the energy consumption per day, mostly provided by lipids, slightly intensified 3 or 4 days before hatching (esp. with higher temps), could be related to a higher energy production need at this time (*Heras, Gonzales-Baro & Pollero, 2000*). Yolk lipids tend to become catabolized first followed by yolk proteins. These ratios change and can be used to estimate the cost of egg development at differing temperatures (*Sasaki, McDowell-Capuzzo & Biesiot, 1986*). In the field, lipid profiles (e.g., fatty acids) have been used to identify offshore from inshore lobster eggs (*Castell et al., 1995*); therefore, it is possible that these constituents are utilized differently across different geographic regions that correspond to disparate thermal regimes.

For proteins, the consumption rate during embryogenesis may increase as temperature rises (*Conceicao et al., 1998*). Proteins not only function as building blocks for tissue and organs but more so, may act as intermediates in carbohydrate and lipid metabolism (*Schmidt-Nielsen 1991*). Thus, trying to quantify protein levels may be masked by their intricate link to other biochemical components. Over prolonged, cold temperatures or those conditions in which temperatures are too high for even short periods of time, some crustacean embryos may instead utilize proteins as an energy source if lipids are low due to thermally-induced demands (*Conceicao et al., 1998*).

At elevated temperatures (constant), increases in protein levels were clearly detected. At sub-optimal temperatures tissue synthesis tends to be inefficient and more protein might be used as energy instead (*Garcia-Guerrero, Racotta & Villareal, 2003*). Therefore, the duration and rates of differing thermal profiles would most certainly affect these biochemical changes and allocations of resource components over time. How this translates to larval 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 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 compromised larvae compared to larger, multiple ones (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 speculated 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 enhancing or deterring egg quality (Goldstein, unpub. data). 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*). Therefore, the biochemical composition of eggs is directly related to the physiological and nutritional status of the female (*Sasaki, McDowell Capuzzo & Biesiot, 1986*; *Racotta & Ibarra, 2003*), and has an influence on the success of embryonic and larval development (*Holland, 1978*).

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 and other early-life history dynamics.

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Figure 1

Experimental design of lab-based tank system.

Inshore tanks received ambient seawater while offshore and constant tank treatments were manipulated using a series of heaters and chiller units (see *Goldstein & Watson 2015b*, for details). All tanks were maintained on a seasonal photoperiod using programmable timers. Tanks were partitioned to hold individual lobsters (N = 5/tank).

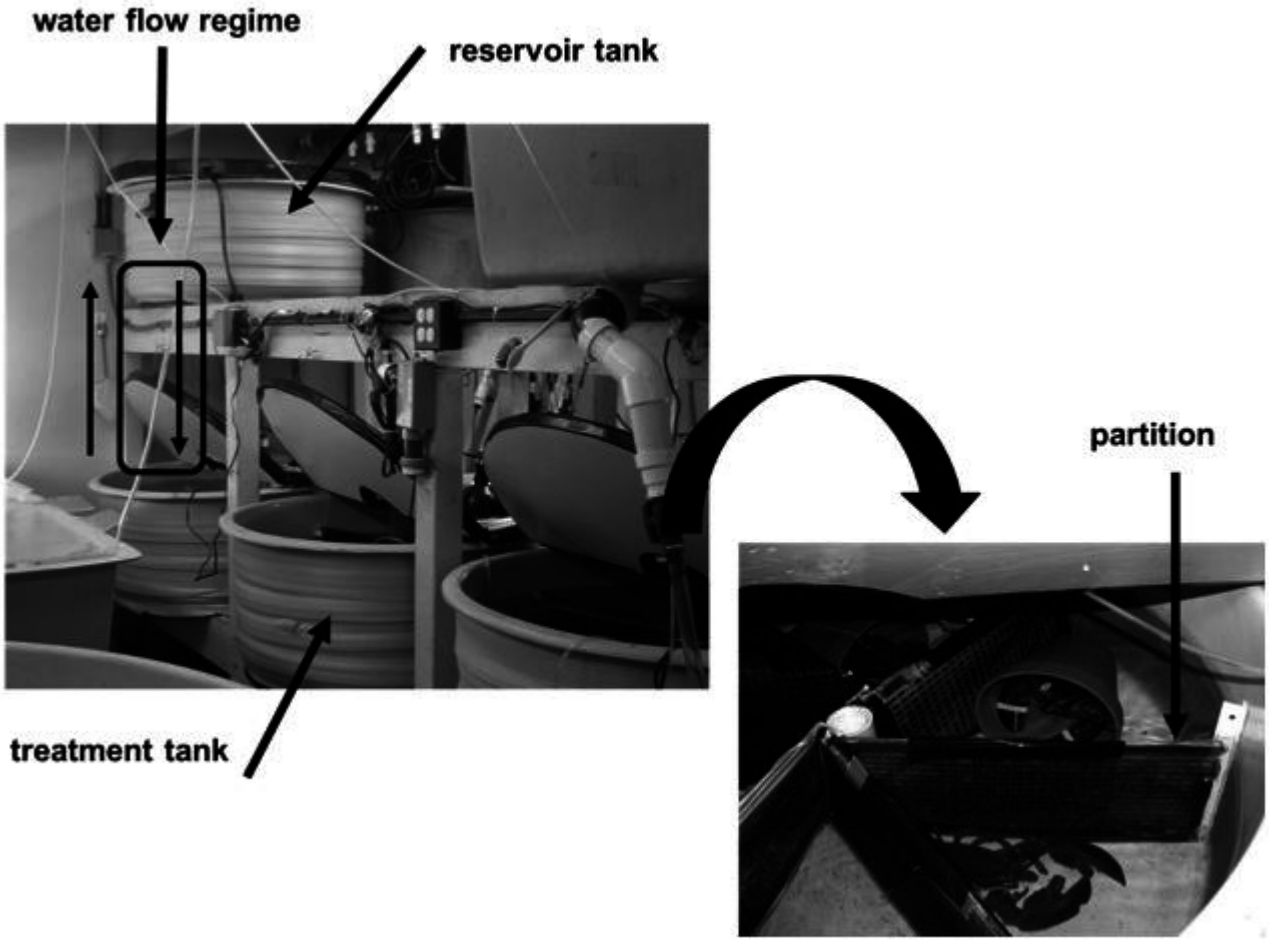


Figure 2

An overview of methods used for some lobster egg analyses.

a.) an image of lobster egg depicting the eyespot and yolk mass b.) freeze-drying egg samples in preparation for biochemical analysis c.) grinding and milling egg samples after freeze-drying and d.) lipid extraction of egg samples using a shaker tray and water bath.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

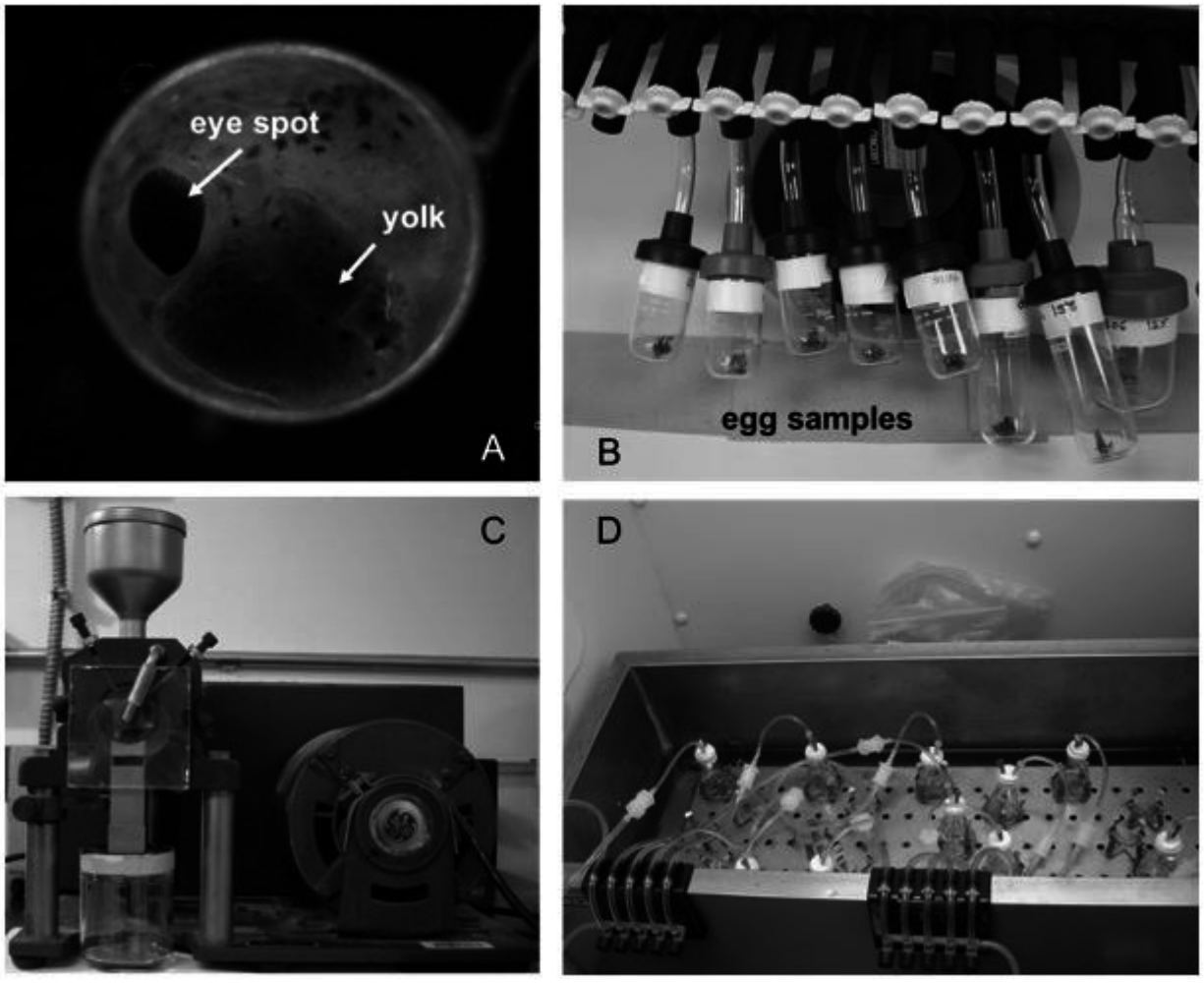
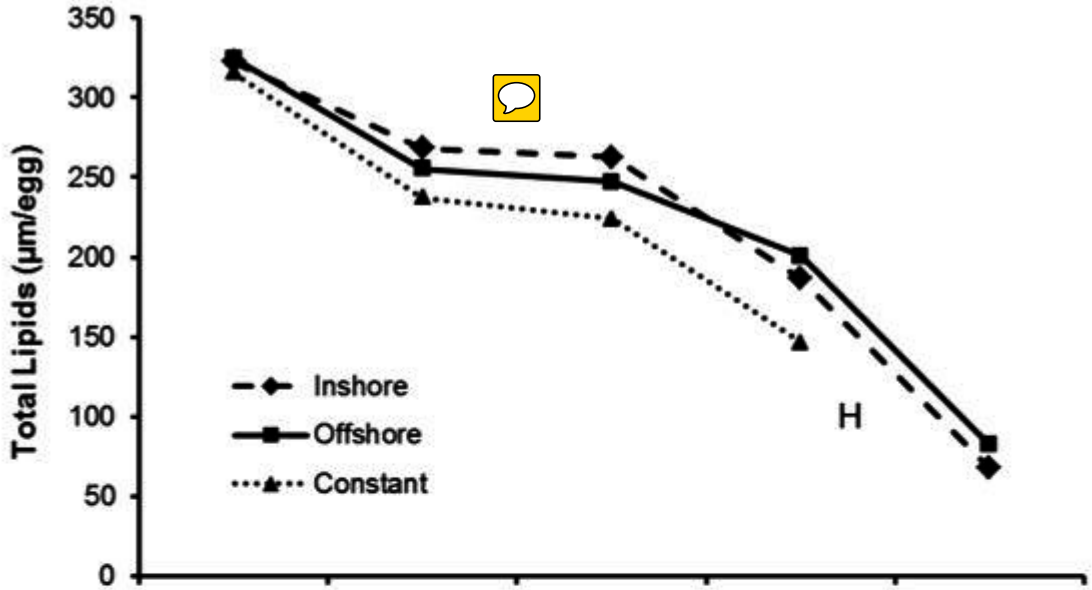


Figure 3

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

Lobsters subjected to inshore and offshore thermal treatments did not hatch their eggs until after May, unlike eggs from the constant treatment, where eggs hatched (H) in April. Points for each treatment represent the means for each treatment group, standard errors are shown in Table 2.


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

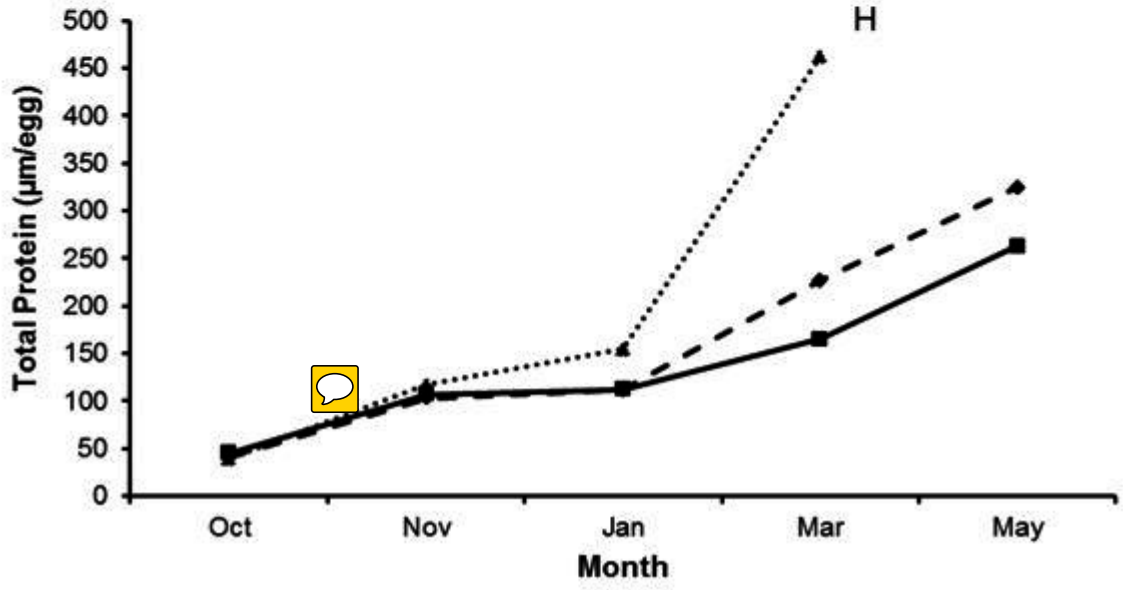

Total Protein (µm/egg)
500
450
400
350
300
250
200
150
100
50
0
H
Oct
Nov
Jan
Mar
May
Month

Figure 4

Relationship between lipids (left) and protein (right) over the course of seven months of egg development for all lobsters sampled (*N* = 5/trt).

Total lobster egg lipid values showed a marked decrease over time (equation: lipids = 381.76 - 55.00*month, r^2_{adj} = 0.85, *P* < 0.0001). 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).

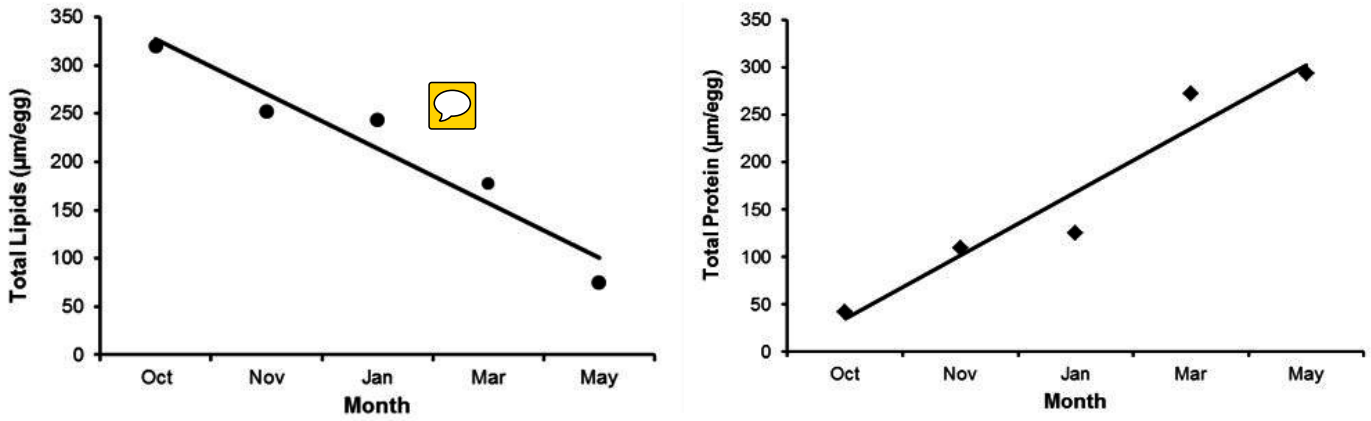


Figure 5

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 ($F = 2.25$, $df = 3$, $P < 0.001$).

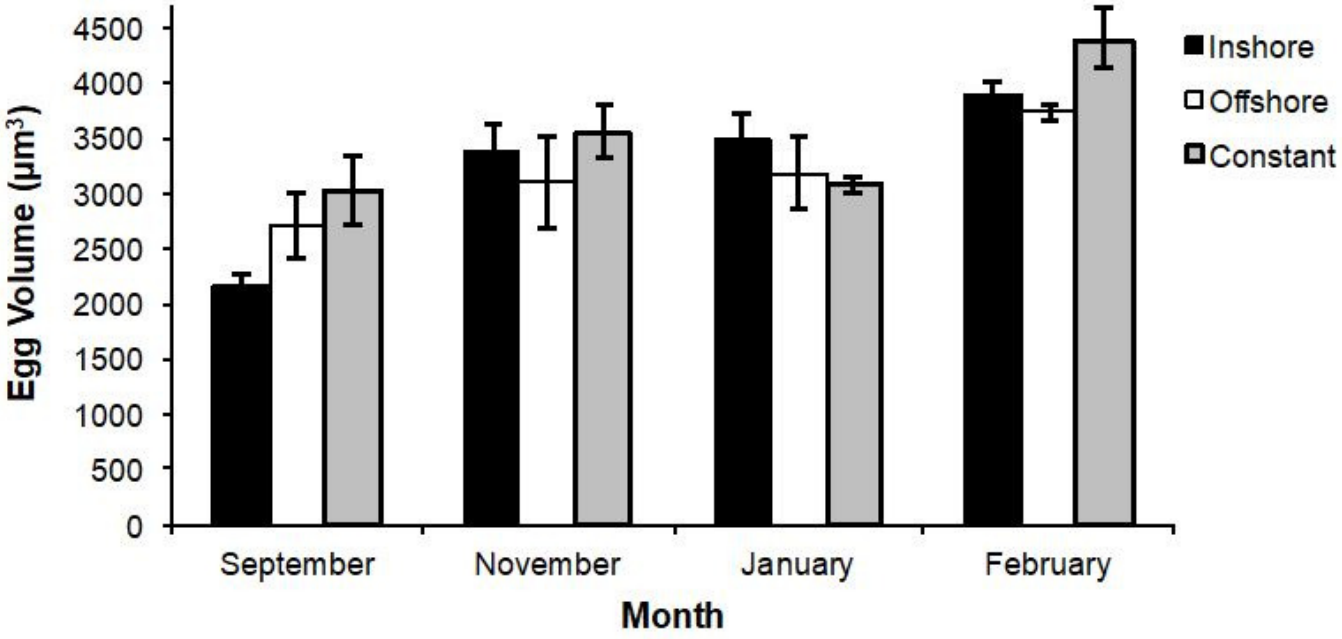


Table 1(on next page)

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.