A peer-reviewed version of this preprint was published in PeerJ on 16 May 2019.

View the peer-reviewed version (peerj.com/articles/6952), which is the preferred citable publication unless you specifically need to cite this preprint.

Goldstein JS, Watson III WH. 2019. Biochemical changes throughout early- and middle-stages of embryogenesis in lobsters (*Homarus americanus*) under different thermal regimes. PeerJ 7:e6952 https://doi.org/10.7717/peerj.6952

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

Jason S Goldstein Corresp., [1, 2] , **Winsor H Watson** [2]

[1] Maine Coastal Ecology Center, Wells National Estuarine Research Reserve, Wells, ME, USA

[2] Department of Biological Sciences and, University of New Hampshire School of Marine Sciences and Ocean Engineering, Durham, NH, USA

Corresponding Author: Jason S Goldstein
Email address: jsgoldstein2@gmail.com

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.

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

Jason S. Goldstein[1,2*] and Winsor H. Watson III[2]

[1]Wells National Estuarine Research Reserve, The Maine Coastal Ecology Center, 342 Laudholm Farm Road, Wells, ME, 04090 USA. <jgoldstein@wellsnerr.org>

[2]Department of Biological Sciences and School of Marine Sciences and Ocean Engineering, University of New Hampshire, 46 College Road, Durham, NH 03824 USA.

[*]corresponding author

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

81 associated variability are considered central to the early-life history patterns for these organisms
82 (*Vance, 1973*; *Jaeckle, 1995*).
83
84 This is especially true for American lobsters, *Homarus americanus* H. Milne-Edwards (1837)
85 characterized as large, highly mobile decapods whose habitats include coastal and continental
86 shelf waters, bays and estuaries from Labrador, Canada to Cape Hatteras, U.S. (*Fogarty, 1995*).
87 Because the American lobster fishery garners such tremendous economic influence, fisheries
88 scientists and managers focus much of their attention on many aspects of stock assessment
89 including the fecundity, spawning stock biomass, and abundance of egg-bearing (ovigerous)
90 females that are historically protected from being landed (*ASMFC, 2015*). The life history of *H.*
91 *americanus* includes a complex suite of embryonic, pelagic (larval), and benthic (juvenile and
92 adult) developmental stages (see review in *Lawton & Lavalli, 1995*), most notably, their yolk-
93 laden eggs that are extruded and carried for 9-11 months over the full course of their
94 development (*Talbot & Helluy, 1995*); temperature is a key factor that determines the length of
95 time the eggs are carried (*Templeman, 1940*; *Aiken & Waddy, 1980*). Mature lobster oocytes are
96 large (1.4-1.6 mm diameter upon extrusion) and typically contain large amounts of high-density
97 lipoproteins (> 40%, lipovitellins) that are allocated as yolk material through a complex suite of
98 primary and secondary vitellogenesis (*Nelson, Hedgecock & Borgeson,1988*; *Talbot & Helluy,*
99 *1995*).
100
101 Besides the often protracted egg development in *H. americanus*, one of the most interesting
102 and sometimes dramatic features of some ovigerous lobsters is their propensity to migrate
103 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

150 seawater (average temp =15.3 ± 0.5°C), and lobsters were fed a combination of fresh squid and
151 crabs (*Cancer spp.*), twice weekly.
152
153 A subset of the eggs in each clutch were viewed under a dissecting scope and staged according to
154 the methods outlined by *Helluy & Beltz (1991)*. These samples also served as covariates for all
155 subsequent statistical analyses. Only lobsters whose eye index was less than 18% were used for
156 this study (*Perkins, 1972*; *Goldstein & Watson 2015b*) in order to encompass as much of the
157 early development process as possible. Lobster carapace lengths (CL) were measured to the
158 nearest 1 mm using digital calipers (Mitutoyo IP 65, Mitutoyo Corp., Japan). A single, circular,
159 laminated disc tag (diameter = 2.0 cm, Floy Tag Inc., Seattle, WA) was fastened to the claw
160 knuckle of each animal for individual identification throughout the duration of the study.
161
162 **Thermal treatments and sampling**
163 The experimental setup and thermal treatments followed a companion study that served to
164 concurrently quantify lobster egg development and hatch time in the same group of lobsters (see
165 *Goldstein and Watson 2015b*). Briefly, a series of four 0.91 m diameter (600 L) tanks (2 tanks/
166 treatment) were used to simulate either inshore, offshore, or constant (12 ± 0.4°C) temperature
167 regimes on a year-round basis (Fig. 1). For purposes of this study, inshore locations (shallow and
168 coastal) were considered the same areas where animals were collected (2-5 km from shore, 8-10
169 m depth), while offshore ones were designated as 12-20 km from shore (20-30 m depth) to
170 simulate those lobsters that might make seasonal, fall migrations offshore (see *Goldstein &*
171 *Watson 2015a*). Constant temperatures were chosen to simulate a favorable growth temperature
172 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.

242 **Lipid and protein content**

243 Total egg lipid levels from inshore and offshore thermal regimes were very different from their
244 constant temperature counterpart (*SPANOVA;* $F_{2,44} = 10.3$, $P = 0.0002$) and also differed by
245 month ($F_{4,44} = 302.9$, $P < 0.0001$; Fig. 3). Likewise, total protein levels in lobster eggs between
246 inshore and offshore thermal regimes also differed from eggs exposed to constant temperatures
247 (*SPANOVA;* $F_{2,44} = 67.17$, $P = 0.0002$) as well as by month ($F_{4,44} = 350.3$, $P < 0.0001$, Fig. 3).
248 The interactive effect of temperature and month was significant for both lipid ($F_{7,44} = 2.27$, P
249 <0.045) and protein levels ($F_{7,44} = 46.5$, $P < 0.0001$) and are summarized in Tables 1 & 2.
250 Overall egg lipid values showed a marked decrease over time (equation: lipids = 381.76 -
251 55.00*month, $r^2_{adj} = 0.85$, $P < 0.0001$; Fig. 4), falling most dramatically early in the fall (-16.8%
252 inshore, -21.4% offshore, -24.8% constant) and late spring (-63.7% inshore, -59.0% offshore).
253 By contrast, total lobster egg protein values increased over the same time frame (equation:
254 proteins = -35.53 + 69.11*month, $r^2_{adj} = 0.63$, $P < 0.0001$; Fig. 4), but exhibited large increases
255 in the fall (60.4% inshore, 57.7% offshore, 66.5% constant) and spring (30.1% inshore, 37.1%
256 offshore) and much more modest ones in the winter, typically 10-15%.

257
258
259 **Egg volumes**

260 Overall, there was a significant increase in egg volume over time for all eggs over all treatments
261 ($r^2_{adj} = 0.413$, $P < 0.001$). Although there were no significant changes with respect to egg volume
262 by treatment ($F = 0.73$, $df = 2$, $P = 0.513$) (overall means: inshore = 3226 ±163 μm^3, offshore =
263 3254 ± 167 μm^3, constant = 3476 ± 152 μm^3) differences from month-to-month did exist ($F =$
264 2.25, $df = 3$, $P < 0.001$; Fig. 5). Gains in egg volume (for all treatments) accounted for ~ 52%
265 between September and February, although there was a slight decrease (-13.5%) in egg volume
266 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.

ACKNOWLEDGEMENTS

A special thanks to Nancy Whitehouse of the UNH Dairy Nutritional Research Center whose expertise and advice on biochemical analyses and statistics were very helpful to the completion

of this project in addition to the use and training of diagnostic lab equipment. Project interns, Sarah Havener, May Grose and Michelle Provencier, were also very helpful with many aspects of this study and their help is much appreciated. Nate Rennels and Noel Carlson of the UNH Coastal Marine Lab for their help in maintaining lobsters and the seawater system. A special appreciation to NH lobstermen Alan Vangile (F/V Special K) and Michael Pawluk (F/V Gretchen D) for the boat hours to trap and collect egg-bearing lobsters for this study.

REFERENCES

Aiken DE, Waddy SL. 1980. Reproductive Biology. In: Cobb JS, Phillips BF, eds. *The Biology and Management of Lobsters*. New York: Academic Press. 275–276.

Amsler MO, George RY. 1984. Seasonal variation in the biochemical composition of the embryos of *Callinectes sapidus*. Rathbun. *Journal of Crustacean Biology* **4**:546–553.

Atlantic States Marine Fisheries Commission (ASMFC). 2015. Stock Assessment Report NA10NMF4740016. 493p.

Attard J, Hudon C. 1987. Embryonic development and energetic investment in egg production in relation to size of female lobster (*Homarus americanus).Canadian Journal of Fisheries and Aquatic Sciences* **44**:1157–1163.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**:911–917.

Brillon S, Lambert Y, Dodson J. 2005. Egg survival, embryonic development, and larval characteristics of northern shrimp (*Pandalus borealis*) females subject to different temperature and feeding conditions. *Marine Biology* **147**:895–911.

Campbell A, Stasko AB. 1986. Movements of lobsters (*Homarus americanus*) tagged in the Bay of Fundy, Canada. *Marine Biology* **92**:393–404.

Castell JD, Boston LD, Miller RJ, Kenchington T. 1995. The potential identification of the geographic origin of lobster eggs from various wild stocks based on fatty acid composition. *Canadian Journal of Fisheries and Aquatic Sciences* **52**:1135–1140.

Charmantier G, Aiken DE. 1987. Osmotic regulation in late embryos and prelarvae of the American lobster *Homarus americanus* H. Milne-Edwards 1837 (Crustacea, Decapoda). *Journal of Experimental Marine Biology and Ecology* **109**:101–108.

Clarke A. 1992. Egg size and egg composition in polar shrimps (Caridea: Decapoda). *Journal of Experimental Marine Biology and Ecology* **16**:188–203.

Clarke A, Brown J, Holmes L. 1990. The biochemical composition of eggs from *Macrobrachium rosenbergii* in relation to embryonic development. *Comparative Biochemistry and Physiology* **96(B)**:505–511.

Conceicao L, Ozorio R, Suurd E, Verreth J. 1998. Amino acid profile and amino acid utlilization in larval African catfish *Clarias gariepinus*, effects of ontogeny and temperature. *Fish Physiology and Biochemistry* **19**:43–57.

Cooper R, Uzmann J. 1980. Ecology of Juvenile and Adult Homarus. In: Cobb JS, Phillips BF, eds. *The Biology and Management of Lobsters*. New York: Academic Press, 97–142.

Cowan DF, Watson WH III, Solow AR, Mountcastle AM. 2006. Thermal histories of brooding lobsters, *Homarus americanus*, in the Gulf of Maine. *Journal of Marine Biology* **179**:70–78.

Dehn PF, Aiken DE, Waddy SL. 1983. Aspects of vitellogenesis in the lobster *Homarus americanus*. *Canadian Technical Report of Fisheries and Aquatic Sciences* **161**:1–28.

Fogarty MJ. 1995. Populations, Fisheries, and Management. In: Factor JR, ed. *Biology of the lobster* Homarus americanus. San Diego: Academic Press, 111–137.

Fraser A. 1989. Triacylglicerol content as a condition index for fish, bivalve and crustacean larvae. *Canadian Journal of Fisheries and Aquatic Science* **46**:1868–1872.

Garcia-Guerrero M, Racotta I, Villareal H. 2003. Effect of temperature on lipids, proteins, and carbohydrates levels during development from egg extrusion to juvenile stage of *Cherax quadricarinatus* (Decapoda: Parastacidae). *Comparative Biochemistry and Physiology Part A* **135**:147–154.

Garcia-Guerrero M, Hendrickx ME. 2004. Embryology of decapod crustaceans I. Embryonic development of the mangrove crabs *Goniopsis pulchra* and *Aratus pisonii* (Decapoda: Brachyura). *Journal of Crustacean Biology* **24**:666–672.

Gardner C. 2001. Composition of eggs in relation to embryonic development and female size in giant crabs (*Pseudocarcinus gigas* Lamarck). *Marine and Freshwater Research* **52**:333–338.

Goldstein JS. 2012. The impact of seasonal movements by ovigerous American lobsters (*Homarus americanus*) on egg development and larval release. Ph.D. dissertation, University of New Hampshire, Durham, NH.

Goldstein JS, Watson WH III. 2015a. Quantifying the influence of natural inshore and offshore thermal regimes on egg development in the North American lobster, *Homarus americanus*. *Biological Bulletin* **228**:1-12.

Goldstein JS, Watson WH III. 2015b. Seasonal movements of American lobsters in southern Gulf of Maine coastal waters: Patterns, environmental triggers, and implications for larval release. *Marine Ecology Progress Series* **524**:197-211.

Helluy S, Beltz BS. 1991. Embryonic development of the American lobster *Homarus americanus*: Quantitative staging and characterization of an embryonic molt cycle. *Biological Bulletin* **180**:355–371.

Heras H, Gonzales-Baro M, Pollero R. 2000. Lipid and fatty acid composition and energy portioning during the embryo development in the shrimp *Macrobrachium borellii*. *Lipids* **35**:645–651.

Holland D. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: D. C. Malins (Ed.). Biochemical and Biophysical Perspectives in Marine Biology. Academic Press, Seattle, Washington, 85–123.

Jaeckle WB. 1995. Variation in the size, energy content and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward L, ed. *Ecology of Marine Invertebrate Larvae*. Boca Raton: CRC Press, 49–77.

Jacobs JR, Biesiot PM, Perry HM, Trigg C. 2003. Biochemical composition of embryonic crabs *Callinectes sapidus* Rathbun 1896 (Crustacea: Decapoda) from the Gulf of Mexico. *Bulletin of Marine Science* **72**:311–324.

Johnson KJ, Goldstein JS, Watson WH III. 2011. Two methods for determining the fertility status of early-stage American lobster, *Homarus americanus*, eggs. *Journal of Crustacean Biology* **31**:693–700.

Lawton P, Lavalli KL. 1995. Postlarval, juvenile, adolescent, and adult ecology. In: Factor JR, ed. *Biology of the lobster Homarus americanus*. San Diego: Academic Press, 47–81.

Lowry OH, Rosebrough NJ, Lewis Farr A, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**:265–274.

MacKenzie BR. 1988. Assessment of temperature effect on interrelationships between stage durations, mortality, and growth in laboratory-reared *Homarus americanus* Milne Edwards larvae. *Journal of Experimental Marine Biology and Ecology* **116**:87–98.

Manush SM, Pal K, Das T, Mukherjee S. 2006. The influence of temperatures ranging from 25 to 36 C on developmental rates, morphogenesis and survival of freshwater prawn (*Macrobrachium rosenbergii*) embryos. *Aquaculture* **256**:529–536.

Nelson K, Hedgecock D, Borgeson W. 1988. Factors influencing egg extrusion in the American lobster (*Homarus americanus*). *Canadian Journal of Fisheries and Aquatic Sciences* **45**:797–804.

Ouellet P, Plante F. 2004. An investigation of the sources of variability in American lobster (*Homarus americanus*) eggs and larvae: Female size and reproductive status, and interannual and interpopulation comparisons. *Journal of Crustacean Biology* **24**:481–495.

Pandian TJ. 1970. Yolk utilization and hatching in the Canadian lobster *Homarus americanus*. *Marine Biology* **7**:249–254.

Perkins H. 1972. Developmental rates at various temperatures of embryos of the northern lobster (*Homarus americanus* Milne Edwards). *Fisheries Bulletin* **70**:95–99.

Petersen S, Anger K. 1997. Chemical and physiological changes during the embryonic development of the spider crab *Hyas arenus* (Decapoda: Majidae). *Comparative Biochemistry and Physiology* **117B**:299–306.

Pinheiro M, Hattori G. 2003. Embryology of the mangrove crab *Ucides cordatus* (Brachyura: Ocypodidae). *Journal of Crustacean Biology* **23**:729–737.

Racotta IS, Ibarra M. 2003. Shrimp larval quality in relation to broodstock condition. *Aquaculture* **227**:107–130.

Rosa R, Nunes ML. 2003. Tissue biochemical composition in relation to the reproductive cycle of deep-sea decapod *Aristeus antennatus* in the south Portugese coast. *Journal of the Marine Biological Association of the United Kingdom* **83**:963–970.

Rosa R, Calado L, Narciso L, Nunes ML. 2007. Embryogenesis of decapod crustaceans with different life history traits, feeding ecologies, and habitats: a fatty acid approach. *Marine Biology* **151**:935–947.

Sainte-Marie B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab, *Chionoecetes opilio*, in the northwest Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences* **50**:2147–2156.

Sasaki GC, McDowell Capuzzo J, Biesiot P. 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*. *Canadian Journal of*

598 *Fisheries and Aquatic Sciences* **43**:2311–2319.
599
600 **Schmidt-Nielsen K. 1991.** Animal Physiology: Adaptation and Environment, 4th Ed. Cambridge
601 University Press. Cambridge, UK.
602
603 **Sibert V, Ouellet P, Brethes J-C. 2004.** Changes in yolk total proteins and lipid components
604 and embryonic growth rates during lobster (*Homarus americanus*) egg development
605 under a simulated temperature cycle. *Marine Biology* **144**:1075–1086.
606
607 **Talbot P, Helluy S. 1995.** Reproduction and Embryonic Development. In: Factor JR, ed.
608 *Biology of the lobster* Homarus americanus. San Diego: Academic Press, 177–216.
609
610 **Templeman W. 1940.** Embryonic development rates and egg laying of Canadian lobsters.
611 *Journal of the Fisheries Research Board of Canada* **5**:71–83.
612 **Vance R. 1973.** Reproductive strategies in marine benthic invertebrates. *American Naturalist*
613 **107**:339–352.
614
615 **Waddy SL, Aiken DE. 1995.** Temperature regulation of reproduction in female American
616 lobsters (*Homarus americanus*). *ICES Marine Science Symposia* **199**:54–60.
617
618 **Watson WH III, Vetrovs A, Howell WH. 1999.** Lobster movements in an estuary. *Journal of*
619 *Marine Biology* **134**:65–75.
620
621

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).

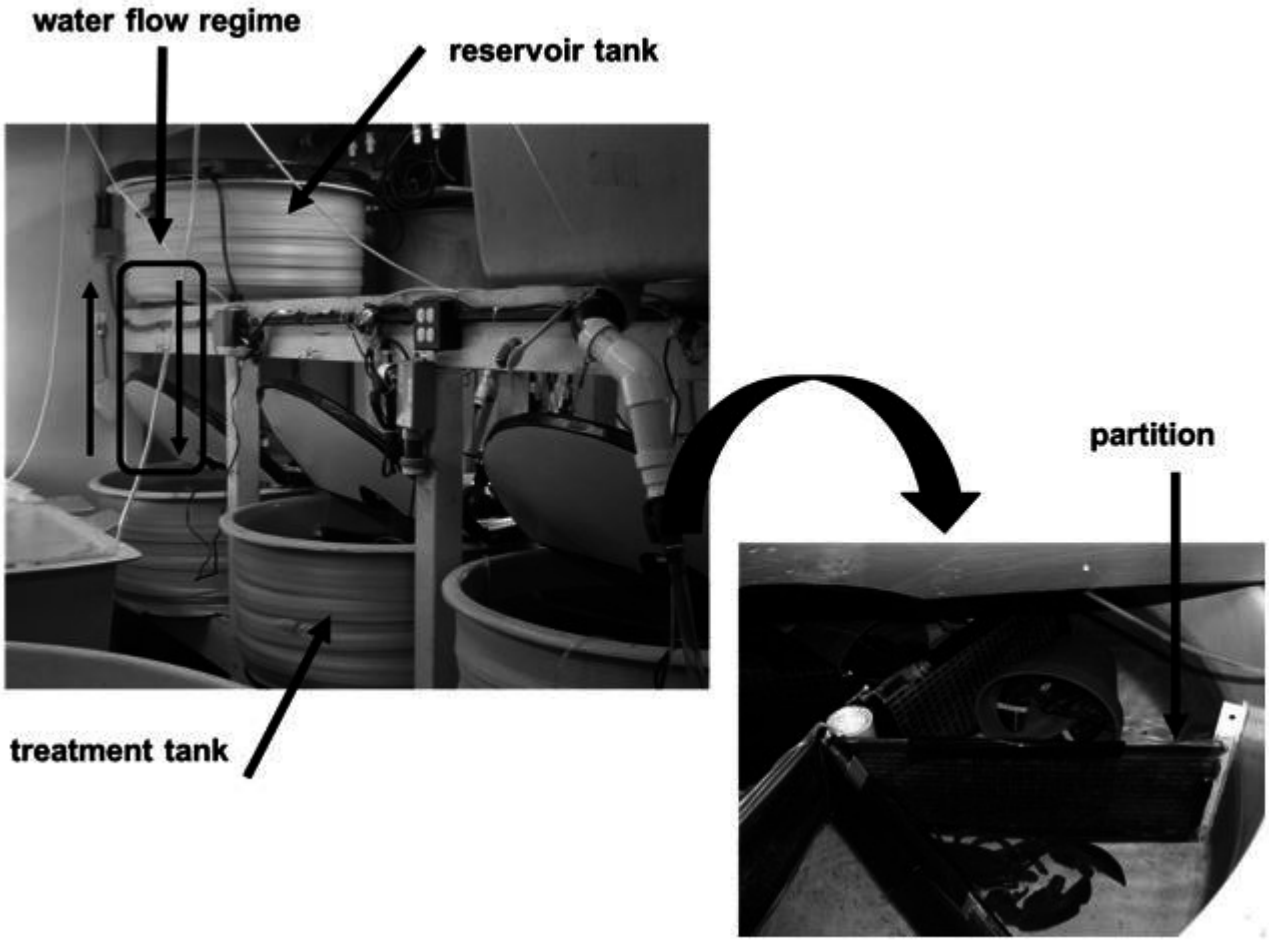


Figure 2

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.*

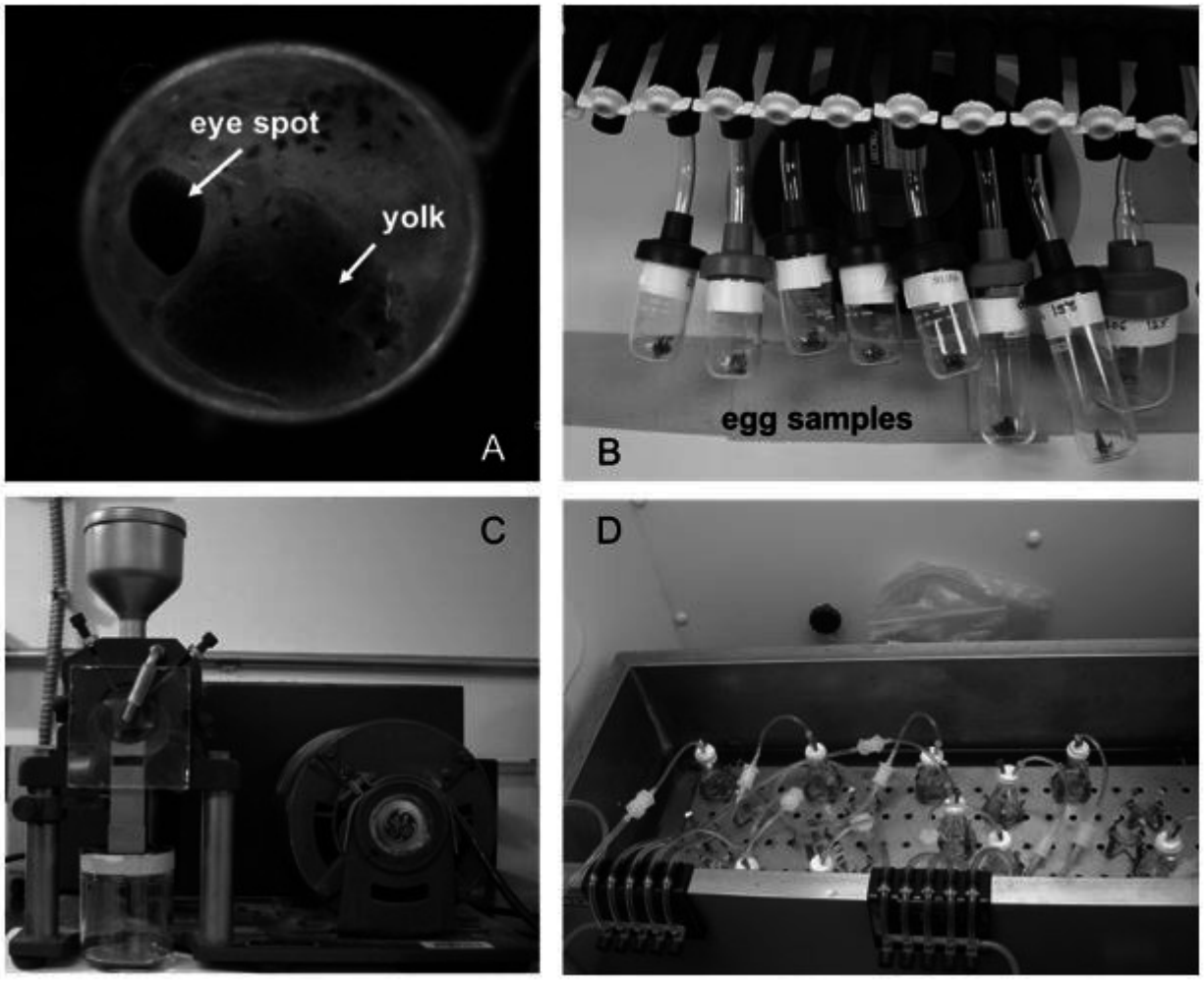
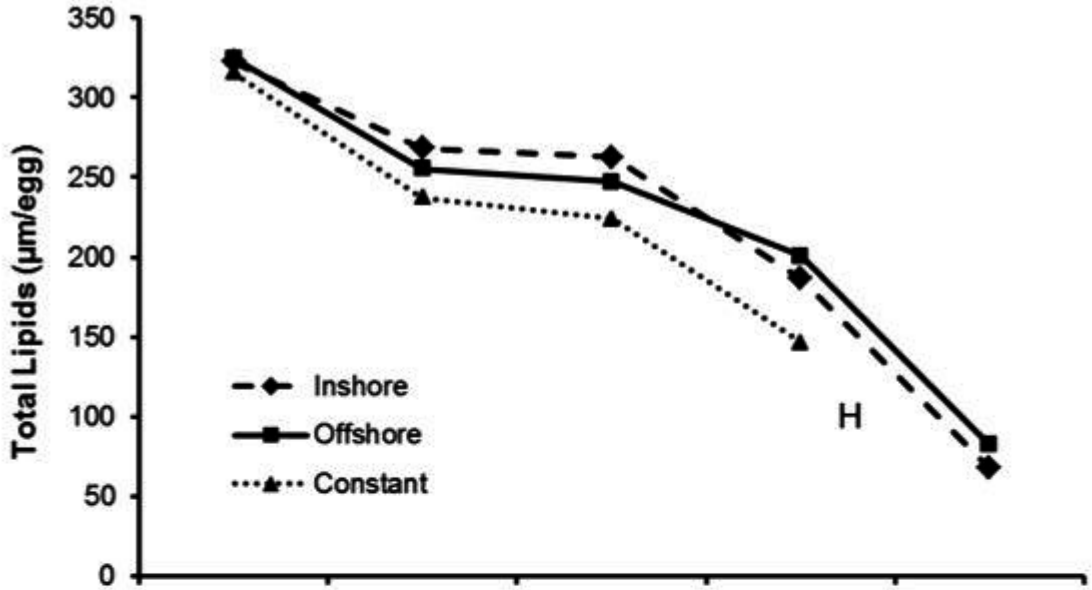


Figure 3

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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26741v1 | CC BY 4.0 Open Access | rec: 20 Mar 2018, publ: 20 Mar 2018


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

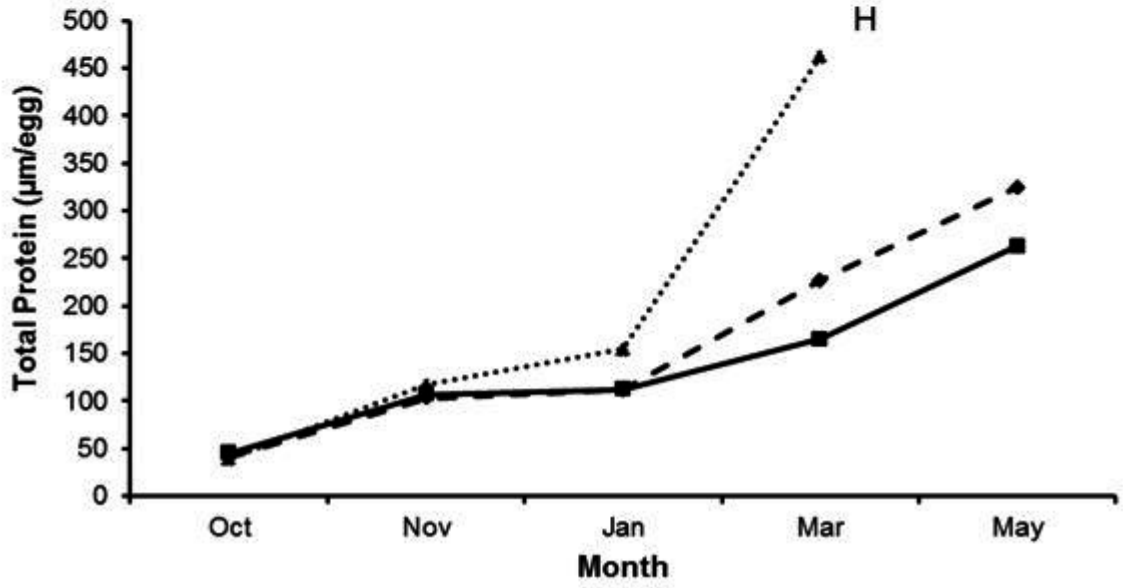

Total Protein (µm/egg)
500
450
400
350
300
250
200
150
100
50
0
H
Oct
Nov
Jan
Mar
May
Month

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).

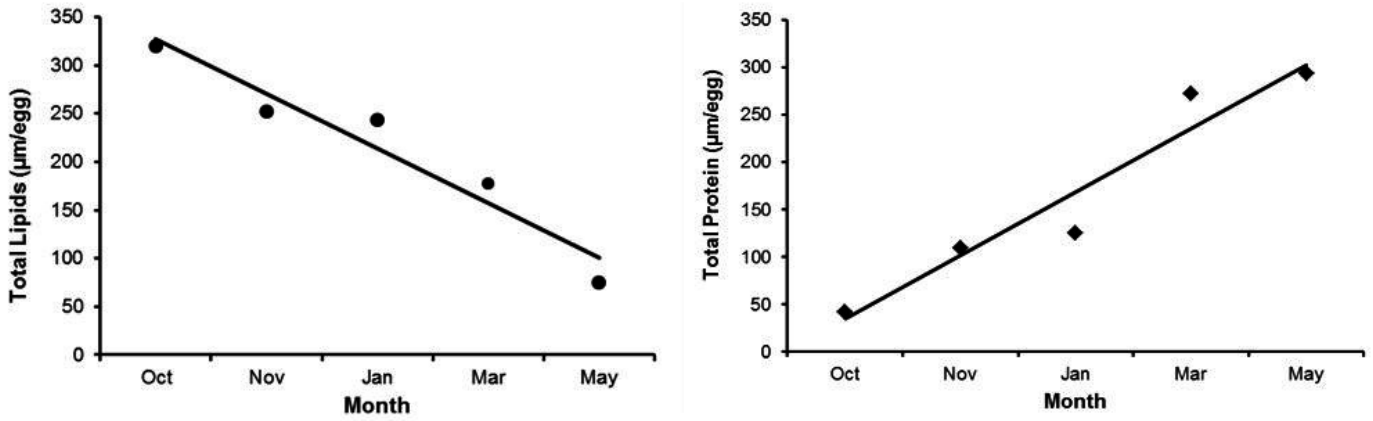


Figure 5

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

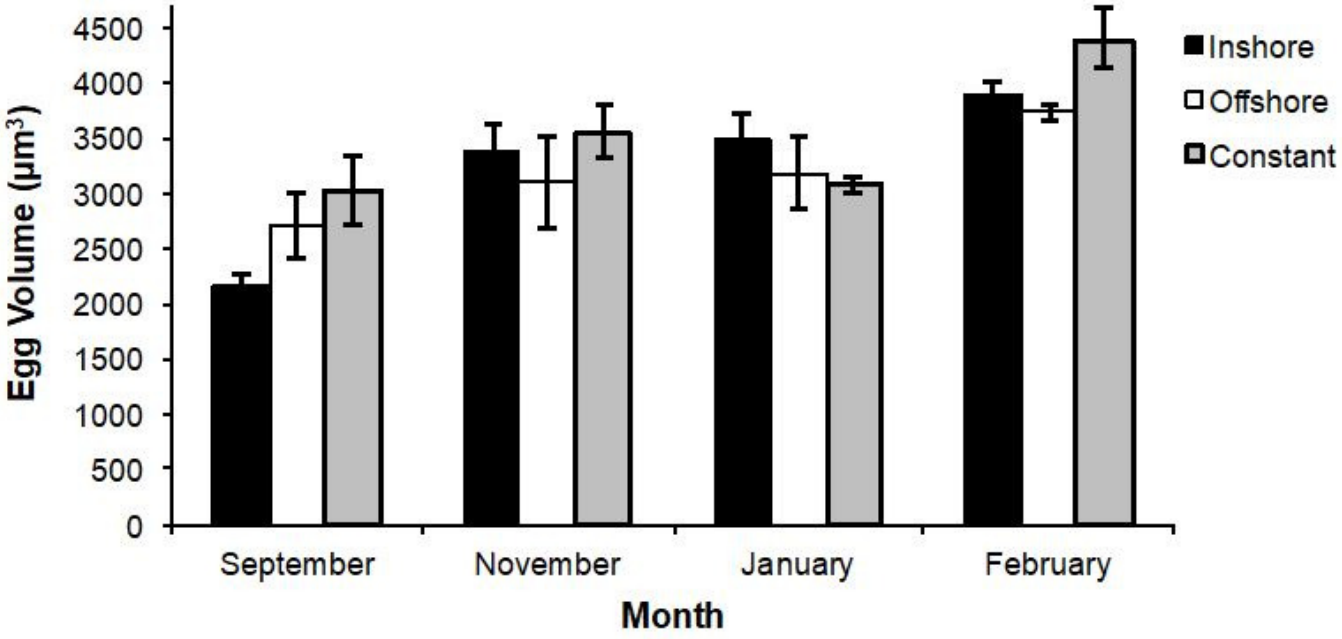


Table 1(on next page)

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 ($\alpha = 0.05$)

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.