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.

2 **Biochemical changes throughout early- and middle-stages of embryogenesis in lobsters**

3 **(*Homarus americanus*) under varying thermal regimes**

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34 **ABSTRACT**

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37 growth and metabolic processes over the course of their development. In terms of biochemical

38 constituents, lipids and proteins play pivotal and central roles in these processes and,

39 accordingly, have been studied extensively in crustaceans. Given the propensity of some

40 ovigerous (egg-bearing) American lobsters (*Homarus americanus*) to undergo seasonal inshore-

41 to-offshore migrations, thereby exposing their eggs to varying thermal regimes, this study's goal

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45 five discrete time intervals. Total egg lipids showed a marked decrease over time (r^2_{adj} = 0.85, P

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47 increases in proteins over the same period (r^2_{adj} = 0.63, P < 0.0001, averages = 60%, 34%, fall

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51 sooner than inshore or offshore ones. Seasonal temperature fluctuations also appear to control the

52 rates of biochemical processes in lobster eggs but may be confounded by other variables.

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56 **INTRODUCTION**
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59 Egg development for most marine crustaceans relies heavily on the production and sequestering

60 of nutrients required for the development and maintenance over the entire process of

61 embryogenesis. In terms of biochemical constituents, both lipids and proteins play pivotal and

62 central roles throughout development, and, as a result, have been studied extensively in both

63 crustaceans and fishes alike (*Fraser, 1989*; *Jaeckle, 1995*; *Rosa et al., 2007*). Lipids comprise the

64 structural integrity of most cells and are responsible for the overall metabolism of growing

65 crustacean embryos. Remarkably, these constituents have been reported to account for upwards

66 of 60% of the total energy expenditure for growth (*Holland, 1978*; *Amsler & George, 1984*). By

67 contrast, the role of proteins as the basic building blocks of animal tissues are well known

68 (*Holland, 1978*), and function as alternative energy sources under certain conditions (*Schmidt-*

69 *Nielsen, 1991*; *Heras, Gonzales-Baro & Pollero, 2000*).

70

71 Egg development in crustaceans is especially linked to temperature such that incubation periods

72 can be extended (cold temps) or reduced (warm temps). Closely coupled metabolic rates increase

73 with temperature thereby modulating yolk absorption, growth and ultimately, the survival of

74 eggs (*Pandian, 1970*; *Schmidt-Nielsen, 1991*). Development and metamorphosis of

75 planktotrophic larvae, including decapod crustaceans, depends to a great extent on nutrition

76 (*Racotta & Ibarra, 2003*) from both exogenous (from feeding) and endogenous (yolk reserves)

77 sources which are important metrics during early postembryonic development (*Sasaki,*

78 *McDowell-Capuzzo & Biesiot, 1986*; *Clarke, Brown & Holmes, 1990*). Together, the relationship

79 between the primary biochemical components in crustacean eggs and their

80

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-

104 10 km, but sometimes much greater) throughout the development of their eggs (see reviews

105 by *Cooper & Uzmann, 1980*; *Lawton & Lavalli, 1995*). The implications of such movement

106 events in ovigerous lobsters has the potential to shape the developmental dynamics of the

107 eggs they carry by subjecting them to differing thermal regimes whose rates of change can be

108 quite different (*Campbell & Stasko, 1986*; *Cowan et al., 2006*; *Goldstein & Watson, 2015a*).

109 For example, ovigerous lobsters subjected to inshore thermal regimes in the lab exhibited

110 more rapid egg development and hatched sooner than their offshore counterparts (*Goldstein*

111 *& Watson, 2015b*). Therefore, the seasonal movements of ovigerous lobsters to thermally

112 disparate waters may be strategies to both enhance egg development and the survival of

113 larvae in the plankton.

114

115 Biochemical and energetics considerations in lobster eggs have been well studied and suggest the

116 following key patterns: 1) differing thermal regimes influence the utilization of energy reserves

117 in developing embryos and embryos raised at accelerated temperatures contain residual yolk

118 reserves upon hatch (*Sasaki, McDowell-Capuzzo & Biesiot, 1986*); 2) the energy content of eggs

119 tend to increase with female size (*Attard & Hudon, 1987*); and 3) larval size at hatch is

120 independent of female size (*Ouellet & Plante, 2004*). Despite some contradictory evidence

121 between some of these studies, it is evident that egg resources influence their growth and

122 development.

123

124 Although optimal temperatures for lobster egg growth are not fully known, naturally fluctuating

125 temperatures result in disparate growth patterns and subsequently, differing hatch times (*Sibert,*

126 *Ouellet & Brethes, 2004*; *Goldstein & Watson 2015b*). In general, crustacean eggs subjected to

127   either prolonged warm or cold temperatures can have a deleterious effect on the use of their yolk

128   reserves (*Garcia-Guerrero, Racotta & Villareal, 2003*; *Manush et al., 2006*), and it has been

129   suggested that prolonged cold temperatures (< 4°C) negatively affect egg development in *H.*

130   *americanus* (*Waddy & Aiken, 1995*). Therefore, one way of assessing the effects of temperature

131   on the overall development of lobster eggs is through the proximate analysis of their biochemical

132   components, namely, lipids and proteins.

133

134   The goal of this study was to further elucidate the effects of lobster movements over varying

135   thermal regimes (inshore and offshore) during the course of egg development, complementing

136   existing work on egg development and hatch under differing thermal regimes in the laboratory,

137   by quantifying two key biochemical descriptors (lipids and proteins) of egg resource utilization

138   as well as changes in egg size. A constant, slightly elevated temperature was also used to

139   compare egg development under non-fluctuating thermal conditions.

140

141   **METHODS**

142

143   **Lobster source and egg assessment**

144   Egg-bearing (ovigerous) lobsters were legally collected (New Hampshire Fish & Game permit,

145   RSA 214:29) in late August and early September (2006) along the New Hampshire (NH)

146   seacoast near Rye, NH and Gunboat Shoals (43°.0274 N; 70°.6938 W) by permitted commercial

147   lobstermen using standard baited traps. Lobsters were transported to the University of New

148   Hampshire (UNH) Coastal Marine Laboratory in Newcastle, NH and initially held in a large

149   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

173   automatically every 30-minutes using HOBO pendant loggers (model UA-002-64, Onset

174   Computer, Bourne, MA) and later downloaded into Microsoft Excel using Hoboware software

175   (HOBOware Pro v. 3.0). Temperature profiles from the offshore tank treatment were adjusted

176   semi-regularly to simulate seasonal temperature changes in the field and monitored from

177   historical and real time data published on the Northeastern Regional Association of Coastal

178   Ocean Observing Systems (NERACOOS, http://neracoos.org). A subset of five ovigerous

179   females were sampled at each temperature treatment for a total of 15 lobsters. All lobsters were

180   sampled for eggs at five discrete time periods: twice in the fall and spring (during periods of

181   rapid growth; *Sibert, Ouellet & Brethes, 2004*) and once in the winter.

182

183   Lobster eggs (~ 100/sample) were removed from the center of each clutch with a pair of fine

184   forceps and placed in labeled plastic sample trays. All egg samples were rinsed and gently

185   agitated with a 0.5% sodium hypochlorite and distilled water solution for ~ 1 min., after which

186   they were rinsed with 100% distilled water and blotted dry to remove the cement matrix holding

187   the eggs together (P. Talbot pers. comm.). Rather than mechanically separate eggs, this technique

188   was chosen for its efficacy. Preliminary studies that were conducted indicated that this chemical

189   separation technique was non-invasive and did not compromise the biochemical integrity of the

190   egg due to their complex and thickened membranes (*Johnson, Goldstein & Watson, 2011*).

191

192   For biochemical analyses, egg samples (~ 30/sample) were frozen at -80°C prior to processing

193   and freeze-dried at -40°C for 24 hr (Labconco Freeze Dryer 5, Kansas City, MO). Dried egg

194   samples were then ground down into a fine power using an industrial-grade milling machine

195   (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

219   calculated using the formula: $V = 4/3*(\pi\ r^3)$, where r is the radius for spheroid-shaped embryos

220   (see *García-Guerrero & Hendrickx, 2004*).

221

222   **Data analysis**

223   Analysis of variance (ANOVA) was used to investigate potential differences in egg protein and

224   lipid content between the three thermal regimes (fixed factor 1) at each of the five sampling

225   intervals (fixed factor 2). A 3x5 full factorial design was used and analyzed as a split-plot (SP)

226   ANOVA (whole-plot = temperature, sub-plot = month, df_{total} = 15) using a PROC MIXED model

227   in SAS v. 9.3 (SAS Institute Inc., Cary, NC). Differences between groups were compared using

228   the PDIFF function in SAS. Regression analyses were carried out using JMP v. 9.3 (SAS

229   Institute Inc., Cary, NC) statistical software. All means are expressed ± se.

230

231   **RESULTS**

232

233   **Water temperatures**

234   Seawater temperatures over the course of this study (October-May) averaged 7.1 ± 0.24°C (range

235   = 2.1-11.2) for inshore laboratory simulations, compared with 6.0 ± 0.19°C (range = 2.8-10.1)

236   for the offshore thermal regime, and 12.2 ± 0.21°C for the constant treatment tank. There was an

237   overall significant difference in water temperatures between the constant tank treatment and both

238   inshore and offshore ones (*ANOVA; $F_{2,7}$ =10.32, P < 0.0001*) but not between inshore and

239   offshore.

240

241

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.

267   **DISCUSSION**

268

269   The main goal of this study was to document the changes in lipids and proteins in lobster eggs

270   over three disparate thermal regimes and the effect that temperature has on these important

271   biochemical processes. In general, the trends during embryogenesis in *H. americanus* were

272   typical of other decapods: lipid reserves were catabolized while proteins were utilized to make

273   tissues (*Holland, 1978*; *Sasaki, McDowell Capuzzo & Biesiot, 1986*; *Jacobs et al., 2003*; *Brillon,*

274   *Lambert & Dodson, 2005*). In tandem with these patterns, eggs were also shown to absorb water

275   during development with a resultant increase in egg diameter. Not surprisingly, lobster eggs

276   exposed to an elevated, constant temperature elicited dramatic changes compared with inshore

277   and offshore ones and, as a result, hatched sooner. Furthermore, the methods that were employed

278   in this study were able to replicate those of other studies that tracked similar metrics in lobster

279   eggs over time (*Pandian, 1970*; *Sasaki, McDowell Capuzzo & Biesiot, 1986*; *Sibert, Ouellet &*

280   *Brethes, 2004*).

281

282   This study did not obtain data for biochemical changes that occurred in eggs that were

283   approaching hatch (~ 30 days prior) or the effects of such changes on larval survivorship or

284   condition. As a result, there were no apparent biochemical differences in lobster eggs between

285   inshore and offshore temperature treatments. Despite this, it has been shown that large changes

286   in egg yolk lipids and protein levels occur within the last few weeks of development (*Sibert,*

287   *Ouellet & Brethes, 2004*), suggesting a large influence in the rate of temperature change between

288   inshore and offshore locations. Concurrent with this are the associated (but different) rates of

289   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

336   been observed in other crustaceans that the energy consumption per day, mostly provided by

337   lipids, slightly intensified 3 or 4 days before hatching (esp. with higher temps), could be related

338   to a higher energy production need at this time (*Heras, Gonzales-Baro & Pollero, 2000*). Yolk

339   lipids tend to become catabolized first followed by yolk proteins. These ratios change and can be

340   used to estimate the cost of egg development at differing temperatures (*Sasaki, McDowell-*

341   *Capuzzo & Biesiot, 1986*). In the field, lipid profiles (e.g., fatty acids) have been used to identify

342   offshore from inshore lobster eggs (*Castell et al., 1995*); therefore, it is possible that these

343   constituents are utilized differently across different geographic regions that correspond to

344   disparate thermal regimes.

345

346   For proteins, the consumption rate during embryogenesis may increase as temperature rises

347   (*Conceicao et al., 1998*). Proteins not only function as building blocks for tissue and organs but

348   more so, may act as intermediates in carbohydrate and lipid metabolism (*Schmidt-Nielsen 1991*).

349   Thus, trying to quantify protein levels may be masked by their intricate link to other biochemical

350   components. Over prolonged, cold temperatures or those conditions in which temperatures are

351   too high for even short periods of time, some crustacean embryos may instead utilize proteins as

352   an energy source if lipids are low due to thermally-induced demands (*Conceicao et al., 1998*).

353

354   At elevated temperatures (constant), increases in protein levels were clearly detected. At sub-

355   optimal temperatures tissue synthesis tends to be inefficient and more protein might be used as

356   energy instead (*Garcia-Guerrero, Racotta & Villareal, 2003*). Therefore, the duration and rates

357   of differing thermal profiles would most certainly affect these biochemical changes and

358   allocations of resource components over time. How this translates to larval survivorship remains

359 poorly understood. However, *Sasaki, McDowell Capuzzo & Biesiot (1986)* showed that up until

360 Stage IV (post-larval), lobsters depended upon stored capacities of lipids and that these residual

361 lipids maybe favorable to settlement processes.

362

363 **Egg volume**

364 The increase of water in the eggs (egg volume) as seen in this study and others is directly related

365 to water uptake during new cell formation in the embryo and has been noted to increase by more

366 than 50% over the course of development (*Pandian, 1970*). Increases in egg volume are

367 primarily due to water uptake by the embryo as well as from the retention of metabolic water

368 resulting from respiration (*Pandian, 1970*; *Petersen &Anger, 1997*). The associated osmotic

369 changes that ensue during egg development can be an important component to hatching and have

370 also been implicated in mechanically aiding the breakage of the chorion near the time of hatch

371 (*Pandian, 1970*). Slight changes in lobster egg volume have been previously explained as a

372 function of a plastic response to variations in salinity (*Charmantier & Aiken, 1987*), and for later

373 eggs, a consequence of physiological factors during development (*Pinheiro & Hattori, 2003*). In

374 these instances, the movements or residency of lobsters in certain locations where seawater

375 salinities can vary dramatically during certain times of the year (e.g., estuaries; *Watson, Vetrovs*

376 *& Howell, 1999*) may have an impact on aspects of development or hatch, especially near the

377 latter part of egg development (*Charmantier & Aiken, 1987*).

378

379 **Female size and condition**

380 In this study we did not specifically address the influence of maternal size or nutritional

381 condition on egg quality in *H. americanus*. However, other related studies have showed that

382 caloric energy content per egg increases with female size (*Attard & Hudon, 1987*). *Sibert,*

383 *Ouellet & Brethes (2004)* described this relationship by creating a growth index model for egg

384 development and found that bigger eggs used yolk lipids more efficiently and sustained faster

385 embryonic growth compared with smaller eggs. In addition, *Ouellet & Plante (2004)* reported

386 that first-time (primiparous) spawners produced compromised larvae compared to larger,

387 multiple ones (although larval size was independent of female size). Results from these key

388 studies point out the need to more clearly investigate these factors in more depth. Since female

389 size and reproductive history may play a role in the allocation of metabolic egg reserves.

390

391 Large invertebrate eggs often have greater organic content than small eggs (*Clarke, Brown &*

392 *Holmes, 1990*; *Clarke, 1992*) but egg size is not always an accurate predictor of organic content

393 in decapods. *Jacobs et al. (2003)* for example, found that the larger size of blue crab (*Callinectes*

394 *sapidus*) embryos in the spring is due, for the most part, to increased water uptake and the

395 concomitant increase in inorganic salts (ash) commonly seen in crustacean embryos (*Pandian*

396 *1970*). An effect of female size on egg reserve allocation has been reported in other decapods

397 including snow crab (*Chionoecetes opilio*), giant crab (*Pseudocarcius gigas*) and lobster

398 (*Homarus americanus*) (*Attard & Hudon, 1987*; *Sainte-Marie, 1993*; *Gardner, 2001*). In lobsters

399 it has been speculated that the effect of female size may mean that larger females make a greater

400 contribution towards egg reserves (*Attard & Hudon, 1987*); however, the added effect of

401 temperature on egg 'quality' may, in some cases override this effect and more work is needed to

402 address this.

403

404   In addition to female size are potential effects that maternal nutrition has on enhancing or

405   deterring egg quality (Goldstein, unpub. data). The lecithotrophic nature of lobster eggs is

406   determined largely through the sequestering of maternal nutrients throughout the processes of

407   primary and secondary vitellogenesis during oocyte formation, the latter of which is highly

408   dependent on the female's organic energy reserves (e.g., lipoprotein; *Dehn, Aiken & Waddy,*

409   *1983*). Therefore, the biochemical composition of eggs is directly related to the physiological

410   and nutritional status of the female (*Sasaki, McDowell Capuzzo & Biesiot, 1986*; *Racotta &*

411   *Ibarra, 2003*), and has an influence on the success of embryonic and larval development

412   (*Holland, 1978*).

413

414   **CONCLUSIONS**
415
416

417   Although the changes in biochemical components (lipids and proteins) in developing lobster

418   eggs were not dramatically different from inshore and offshore thermal regimes, there is still the

419   potential for variations in the energetics of embryogenesis influenced by the seasonal movements

420   of some lobsters to and from these two disparate locations. Thus, as seasonal thermal cycles

421   fluctuate or potentially shift (i.e., climate change) the timing of egg hatch and associated egg

422   quality may modulate further biochemical changes to lobster eggs and have implications for

423   hatch and other early-life history dynamics.

424
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435

436
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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).

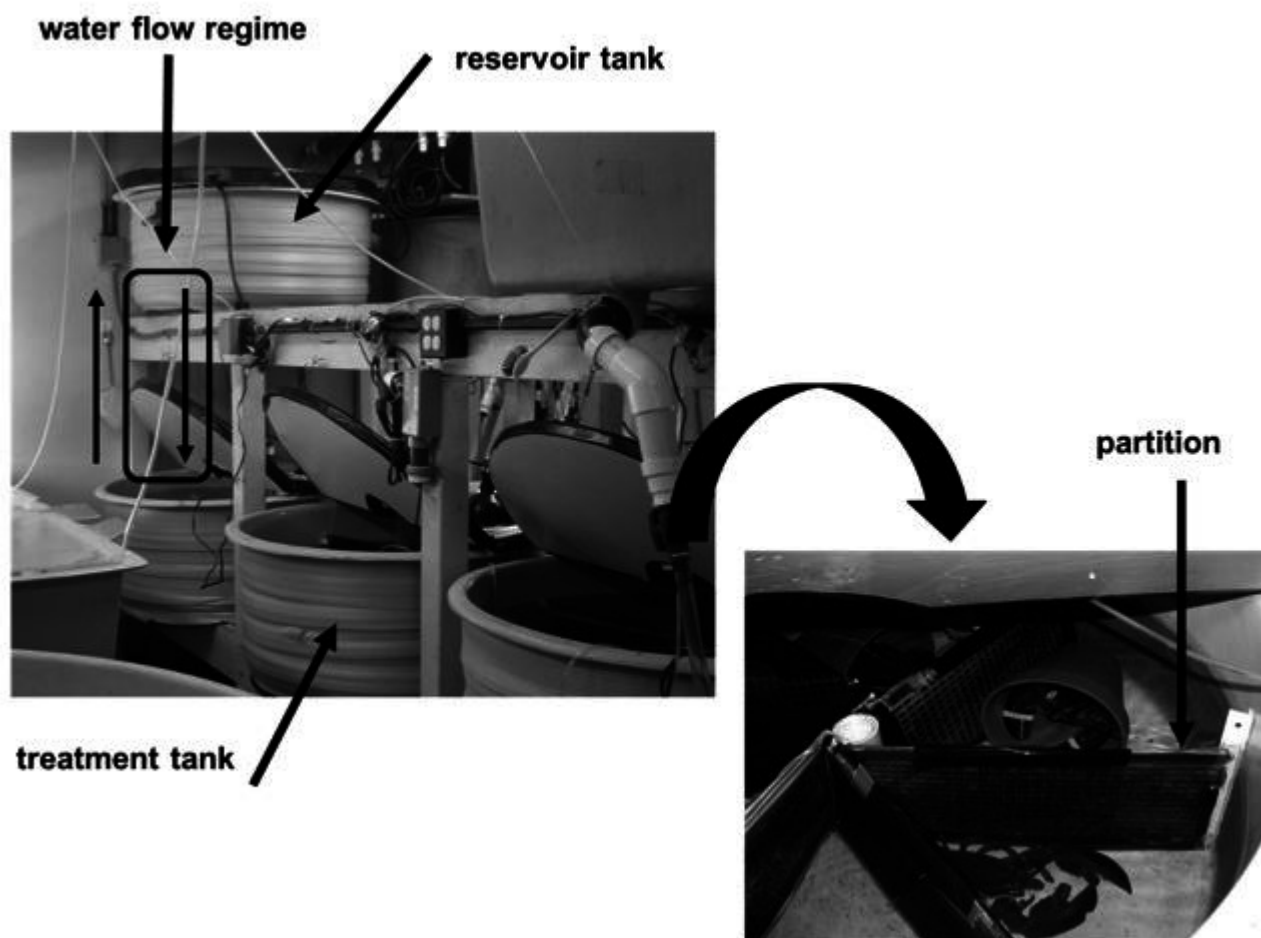



Figure 2

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.

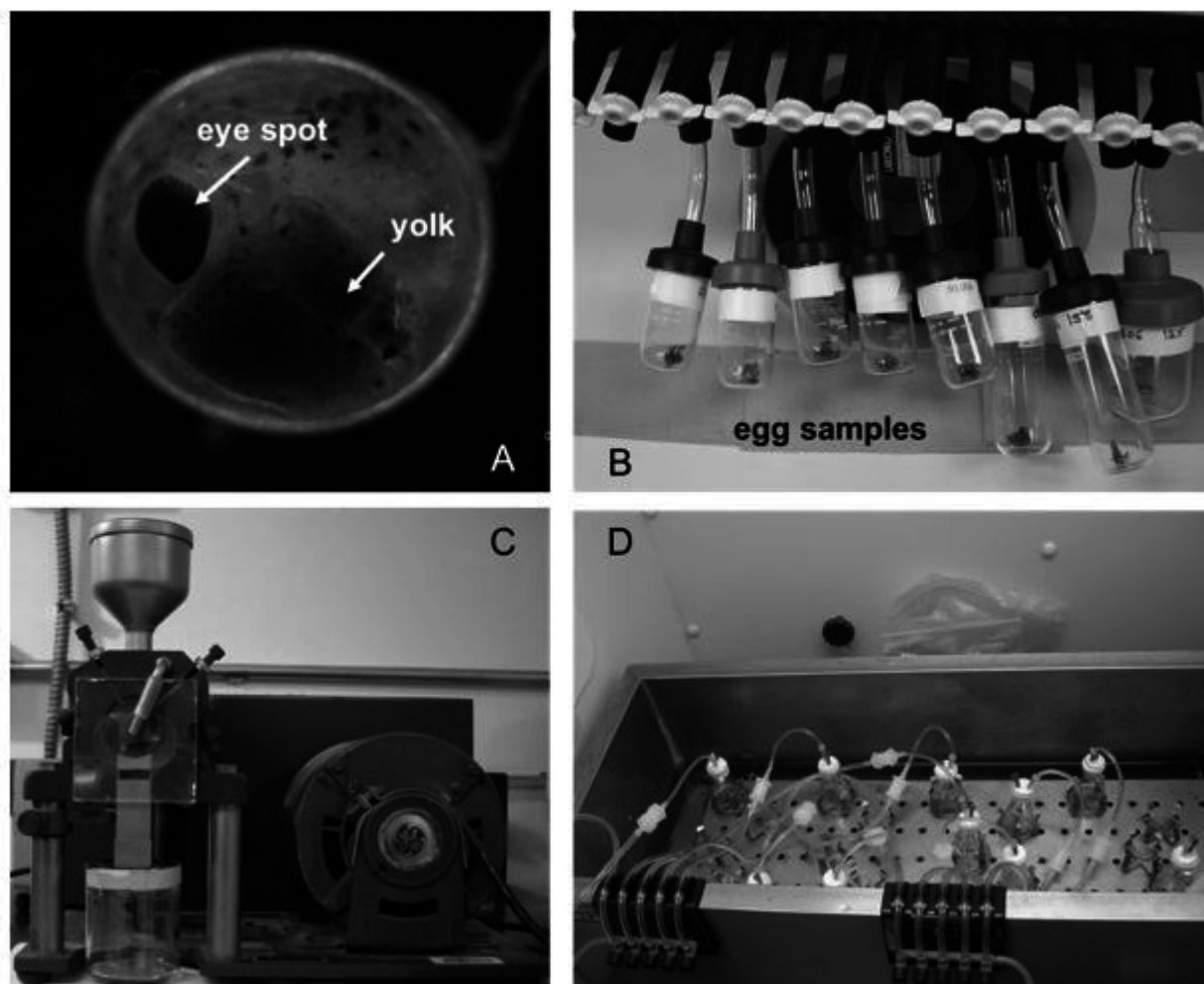



Figure 3

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.

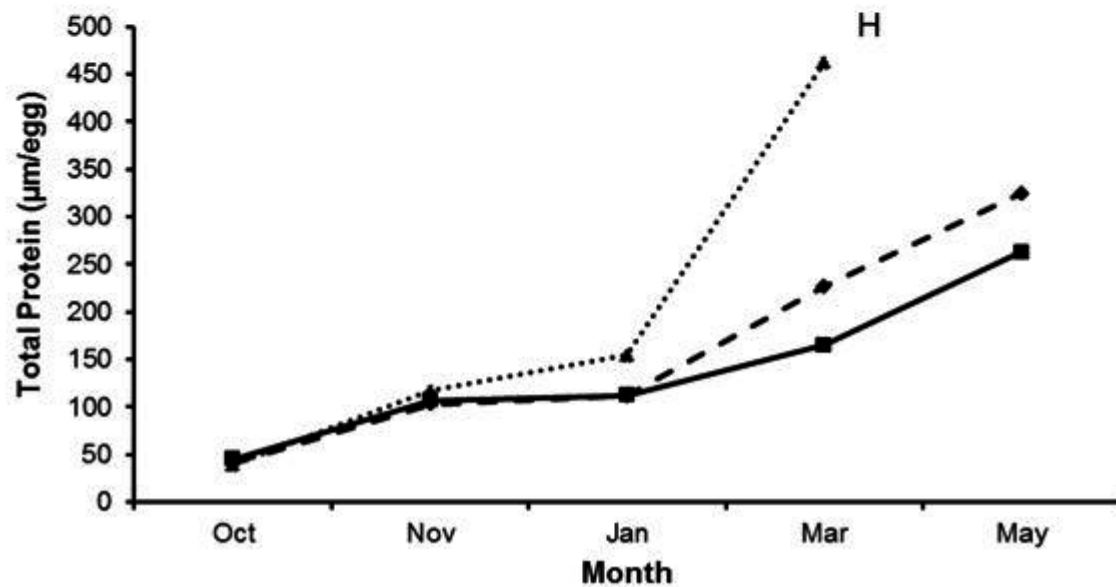
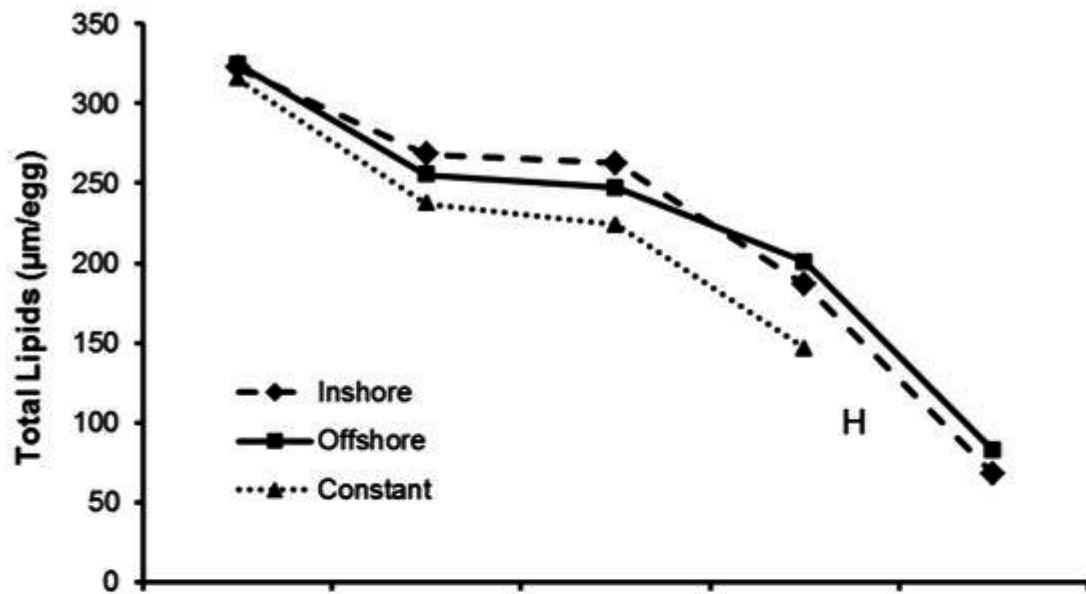



Figure 4

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).

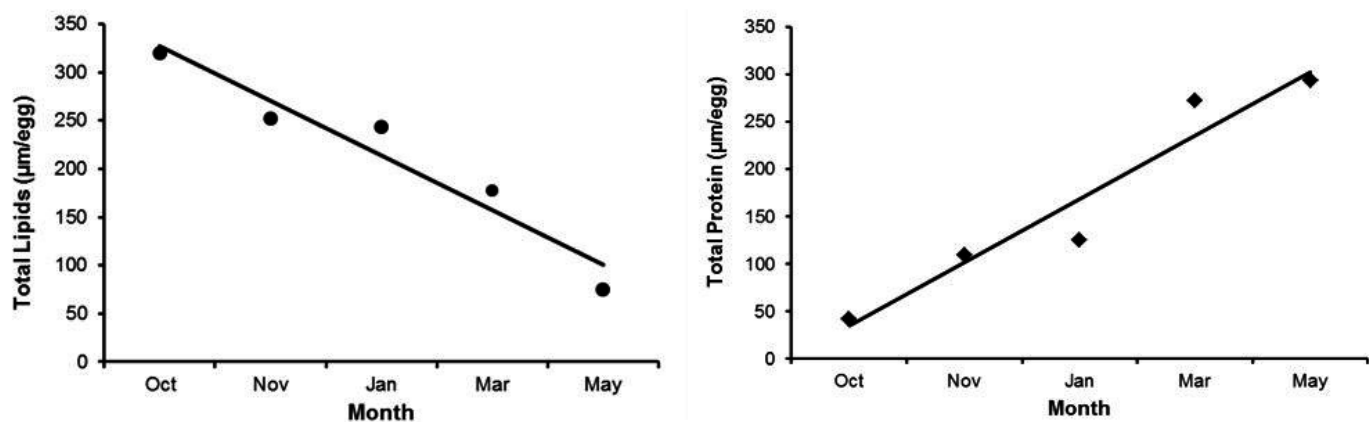
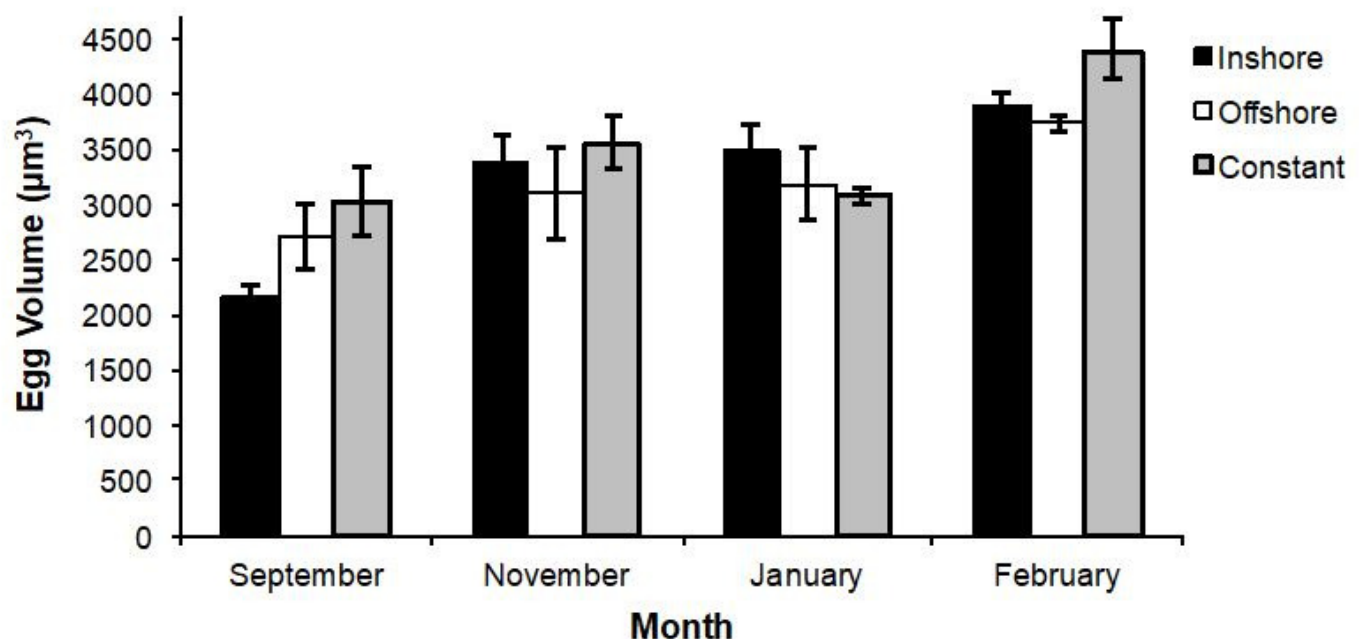



Figure 5

A summary of means (± se) for changes in lobster egg volumes (given in μm³) 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).

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 ($\alpha = 0.05$)

6 Treatment group: Constant[a] Inshore[b] Offshore[b]

9 **Table 1** A summary of means (± se) for lobster egg total lipids and total proteins over five

10 months. Post-hoc differences (from SAS) for both variables are given below; groups with

11 different superscripts denote treatment differences ($P < 0.001$).

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	-

4
5
6 **Table 2** Pairwise comparisons between temperature treatment and month for both lipids and

7 protein values. Shaded P-values (< 0.05) denote significant differences between temperatures for

8 a specific month.