Contribution of variability in embryo development rate and status at hatch to the protracted hatch period of female American lobster *Homarus americanus*

by

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ABSTRACT

Female American lobsters typically hatch their embryos over protracted hatch periods of 15-32 days. To investigate the mechanisms underlying this intra-brood variation in hatch time, I reared individual embryos from June to September 2017 at a constant temperature of 9°C, took photos of them at the beginning of the study and prior to hatch, and measured their eye size as a proxy of their development status. A multiple linear regression model explained 74% of the variability in hatch day, and indicated that embryos that hatcher earlier in the summer are (i) more developed come spring (44% of explained variance), (ii) less advanced in development (28%), and (iii) develop at a faster rate (27%). This study suggests that the process of hatching is not strictly associated with a particular role of the brooding female, but rather is perhaps mostly a function of processes or attributes of individual embryos.

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PEI: Perkins eye index

Introduction

It was recognized over a century ago (Hjort 1914) that year class strength and population structure of marine fishes are dependent on the success of early life stages, in particular their survival during the transition from yolk-dependent to feeding larvae; if mortality of these early life stages is high, that particular year class will have low numbers. This importance of early life stages for population structure is also true for marine invertebrates (Olson and Olson 1989). The three main processes likely responsible for variation in the success of these early life stages are predation, starvation, and strong currents carrying larvae to unsuitable habitats (Thorson 1950).

Food availability has long been considered a key factor in the mortality of marine invertebrate planktonic larvae, of whom a key life stage is spent in the water column. Food availability affects larvae both directly through starvation and indirectly by increasing the amount of time larvae stay in the water column, which increases the likelihood of predation and unfavourable dispersal (Thorson 1950).

The importance of food availability to the success of early life stages, as first fully recognized by Hjort (1914) and Thorson (1950), was the basis of Cushing's (1990) match-mismatch hypothesis, which proposes that feeding success of larvae is a major contributor to inter-annual variability in recruitment of marine fish populations. This hypothesis proposes, more specifically, that for successful early survival, the breeding phenology of the predator (e.g. emergence of larvae) must be timed with the availability of the prey (Cushing 1990; Durant *et al.* 2007). Cushing proposed his match-mismatch hypothesis to explain i – why emergence of herring larvae occurred at similar times as

the spring and autumn plankton blooms (Cushing 1967), and ii – why herring, plaice, sockeye salmon and cod spawned over a relatively small period of time, with the average peak of spawn only varying by a week (Cushing 1969). This hypothesis has also been used to explain patterns in hatch in marine invertebrates. For example, the presence of phytoplankton has been shown to act as a stimulant for spawning in green sea urchins *Strongylocentrotus droebachiensis*, and blue mussels *Mytilus edulis* (Starr *et al.* 1990).

Marine organisms with pelagic larvae have evolved different strategies for "matching" the presence of their larvae in the water column with that of their food. For example, time of hatch in the bivalve *Macoma balthica* has been shown to be dependent on the amount of prey (i.e., flagellates) present in the environment (Bos *et al.* 2006), and time of emergence in Northern Shrimp *Pandalus borealis* has been shown to be dependent on physical oceanographic conditions that synchronize larval emergence with primary and secondary plankton blooms (Ouellet *et al.* 2007). For some marine invertebrates, this ability to match with food needs to be very precise, such as in the Cook Inlet king crab *Paralithodes camtschatica,* of which larvae that experienced an extended (84 hours) period of starvation were unable to catch their prey (i.e., copepods) once the prey were made present in the environment (Paul and Paul 1980).

One strategy evolved by many organisms to increase fitness in a variable and unpredictable environment is "bet-hedging", which involves producing offspring with variable attributes (e.g., size at hatch) to increase long-term fitness (Seger and Brockmann 1987). The "fitness benefit" of bet-hedging in a variable environment was first shown in the modeling of an annual seed, with adults having to "choose" between growing and producing seeds, or instead remaining dormant and delaying germination

(Cohen 1966). The growth and reproduction of annual plants is confined to one season, by delaying germination they are preventing 100% failure of reproduction that may occur if all the offspring are faced with unfavourable conditions (Cohen 1966). By varying phenotypic attributes in offspring, specifically in an varying/unpredictable environment, the adult is ensuring that at least some of the offspring are likely to survive the environmental conditions they are met with, instead of only selecting for one attribute, which may only be successful in a specific environmental condition. The bet-hedging strategy most often seen in adult marine invertebrate is producing offspring of a same brood that vary considerably in size (Marshall *et al.* 2008). One evolutionary trade-off potentially associated with producing varying size offspring in the marine pelagic environment is that larger individuals typically have better feeding capabilities and spend less time in the water column than their smaller counterparts, but has an overall benefit of decreasing the variation in among-generation fitness (Marshall and Keough 2007; Seger and Brockmann 1987). Another potential bet-hedging strategy is to extend hatch over a prolonged period, to increase the likelihood of some larvae encountering favourable pelagic conditions (Philippi and Seger 1989; Simovich and Hathaway 1997). This strategy has been proposed for four different crab species in the genus *Lithodes*, where hatch durations range from 29 days for blue king crab *Paralithodes platypus*, 32 days for red king crabs *P. camtschaticus*, 34 days for golden king crab *Lithodes aequispinus* and 31 to 35 days for the southern king crab *L. santolla* (Paul and Paul 2001; Stevens 2006; Stevens and Swiney 2007; Thatje *et al.* 2003). It is believed that if these crabs are unable to detect or "predict" through environmental cues when spring blooms occur, and hence when the offspring's prey will be present, hatching larvae over

a protracted period increases the likelihood that some are met with suitable conditions (Stevens 2006).

The American lobster fishery is Canada's number one seafood export, while also contributing significantly to the socioeconomics of many fishing communities throughout Atlantic Canada (Fisheries and Oceans Canada 2017). Despite its importance, we have limited understanding of important biological and demographic processes for the species, including stock-recruit relationships. There is no current understanding of the relationship between time of hatch and availability of prey in American lobster, including how and the extent to which these processes are well synchronized. The main diet of American lobster larvae is copepods and diatoms (Ennis 1995), whose presence and density are dependent on fluctuations in temperature and phytoplankton blooms (Winder and Cloern 2010). "Matching" is likely extremely important for American lobster larvae, as stage I larvae that are starved in the lab have been shown to be incapable of molting to stage II; beyond ≈7 days of starvation, the larvae reached a "point of no return", beyond which they could not develop further even if food became abundant (Abrunhosa and Kittaka 1997).

Female American lobster have been shown to have a protracted hatch period in nature (Ennis 1975; Tlusty *et al.* 2008) and in the lab (Attard 1987; Scarratt 1964; Wilder 1953). In Atlantic Canada they have been observed to hatch their larvae in the summer, typically between June and September (Campbell 1989; Scarratt 1964; Templeman 1940), with 15 to 32 days elapsing before all larvae emerge from a clutch (Ennis 1975). This protracted hatch period may be a bet-hedging strategy, meant to

increase a female's fitness by increasing the likelihood that at least some of the larvae are synchronized with good conditions for survival.

The mechanisms underlying the hatch time and the release of larvae in American lobsters are unclear. The embryo's membrane eventually breaks independently of any action by the female, due to internal build-up of pressure (Davis 1964), although release of the larvae into the water has been attributed to the physical "fanning" of a females pleopods (Ennis 1975). Until embryos are raised in isolation, detached from the female, it will be difficult to determine how much of the variation in hatch time is due to the female versus the embryos. If the female truly does not contribute to the physical rupture of the egg membrane at hatch, then the protracted hatch period must be a function of processes occurring at the embryo level, and may for example be due to differences in embryo develop rate or development status at hatch.

In this study, individual detached American lobster embryos from different females and regions were followed in the lab from June 2017 to hatch in September 2017, to increase our understanding of the mechanisms underlying the protracted hatch period of lobster larvae. Multiple linear regression models were used to estimate the contribution of three different processes to variation in hatch time among individual embryos: i- embryo development rate in fall-winter-early spring prior to sampling, based on the "development status" of embryos when we sampled them in spring 2017, ii- embryo development rate in spring-early summer prior to hatch, based on developmental rate during our lab experiment prior to hatch, and iii- embryo "development status" at hatch, based on development status prior to hatch during our lab experiment.

Methods

Embryo collection

At-sea sampling during the lobster fishing season was completed to collect embryos from egg-bearing female lobsters. Embryos were collected between June 8 and June 20, 2017, from six locations in eastern Canada: Dingwall and Canso in Nova Scotia, Malpeque and Tracadie in Prince Edward Island, and St. Martins and Dipper Harbour in New Brunswick (Figure 1). I performed the sampling in Dingwall, and the remaining locations were sampled by members of local lobster fishing associations that are members of the Lobster Node, a large collaborative research project on lobster stock structure and connectivity (Rochette *et al.* 2017). At each location, embryos were sampled from the first 25 embryo-bearing females caught that had embryos with visible eyes; selecting females with "eyed embryos" ensured that these would be hatching in the coming summer months, rather than in the following year. Six clumps comprising five to ten embryos were haphazardly chosen from various locations on the female's abdomen, and placed in sea water in a cooler for transportation to the lab; previous work has found no consistent variation in development status of embryos taken from different locations of a female's brood (R. Rochette, personal communication). We received all embryos in the lab within 24 hours of collection and immediately placed them in trays within temperature-controlled tanks until the beginning of the study, which occurred between 6 and 18 days following the arrival of embryos in the lab.

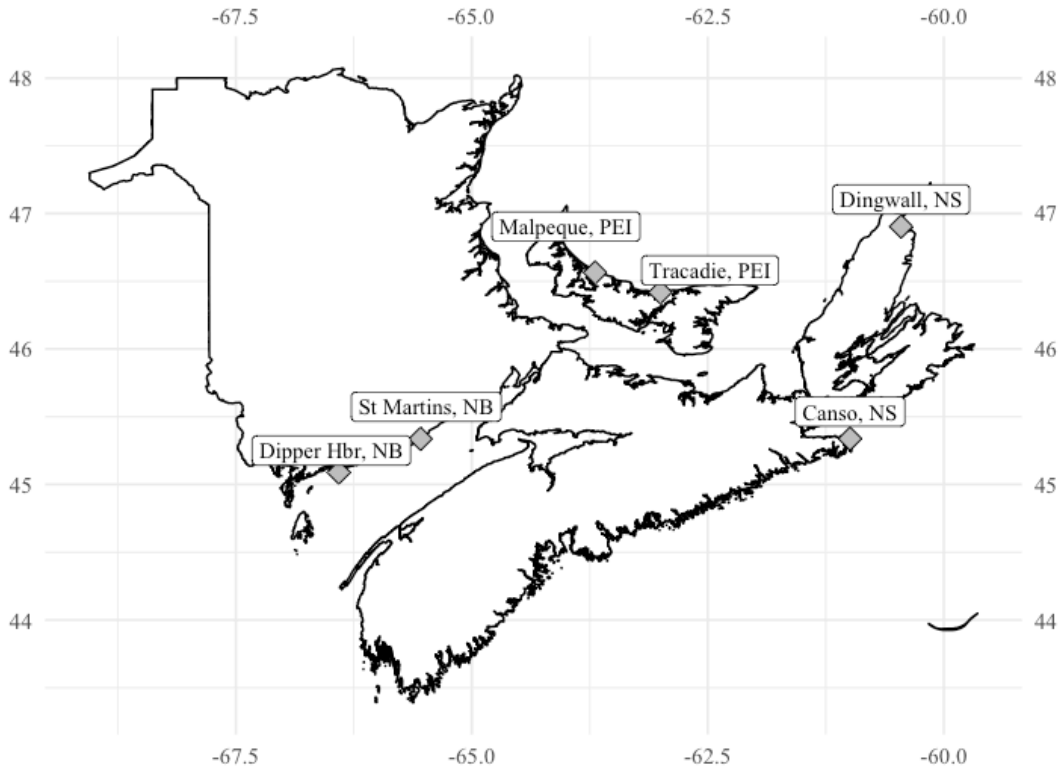

Figure 1. Six study locations in Atlantic Canada where at-sea sampling was performed during the lobster fishing season in June 2017 to collect eyed embryos from female American lobster.

Lab set-up

Embryos from 12 females from each of the 6 locations were selected for this study. A larger number of females and embryos were sampled at sea to allow for sub-sampling in the lab, and ensure similar stage of development among embryos from different females and locations at the beginning of the experiment. Eight embryos from each of the 72 females were chosen based on their preliminary eye size (350-400 μm), which we used as a proxy for embryo development (Perkins 1972), and distributed amongst 12 trays and four 30 L tanks. Each of the 12 trays had 24 wells, divided in half using 1 mm mesh in order to keep embryos separate to allow for individual tracking. Each tank had three of the twelve trays, and each tray contained 48 eggs, two eggs per female, with four females from each location in one tray. The position of embryos from different females and locations in the tray was rotated between the 12 trays, to minimize the potential impact of tray position on embryo development.

Each tank had a HOBO Pro V2 Data Logger (U22-00) to monitor temperature, and temperature as well as salinity in each tank were also monitored every other day using a YSI Professional Plus handheld multi-parameter meter. For the initial 25 days of the experiment, tanks were set at a targeted temperature of 8°C, with a mean ± SD of 8.02 ± 0.09°C (Table 1). The tank temperatures were then raised to a targeted 9°C with a mean ± SD of 9.09 ± 0.32°C for the remaining 63 days of the experiment (Table 1) to promote hatching. Temperatures were controlled using an Aquabiotech enviro-monitron and a dual heating-cooling unit; the lab was maintained at 10°C to aid in maintaining the water temperature inside each tank at its target point.

Table 1. Variation in temperature (°C) among and within the four experimental tanks over the initial 24 days (Period A: June 26th to July 20th) and the last 63 days (Period B: July 20th to September 20th) of the experiment.

Period	Tank	Target	Average	SD	Range
A	1	8	7.90	0.01	(7.87, 8.10)
	2	8	8.09	0.01	(8.07, 8.12)
	3	8	8.12	0.02	(8.10, 8.25)
	4	8	7.97	0.02	(7.95, 8.05)
B	1	9	8.99	0.32	(8.84, 10.74)
	2	9	9.09	0.09	(8.92, 9.81)
	3	9	9.17	0.37	(8.94, 11.13)
	4	9	9.09	0.39	(8.79, 10.98)

Data Collection

Photos of individual embryos, from the saggital view, were taken using a Lecia M212 5 microscope and AMscope MD1900 camera, on a weekly (± 1 day) basis until hatch. The separation of embryos allowed photos of the same embryo to be taken and tracked through development. As embryos began hatching, they were checked daily instead of weekly to gain a better estimate of hatch time, which was determined as the presence of the stage I larva. For the purpose of this study, only the first photo and the final photo prior to hatch were used to investigate variation in development rate, due to time constraints. Following the 13-week observation period, individual embryos' "progression photos" were compared to correct for errors in labelling and/or mix-up of embryos during the study.

The eye perimeter of each embryos was traced using a Wacom CINTIQ DTH 1300 drawing tablet, and subsequently measured using ImageJ (Rasband 1997-2016). Embryo development was assessed on the basis of the Perkins Eye Index (PEI) (Perkins 1972), which is the average of the maximum length and maximum width of the eye, and is a proxy used for monitoring the development of American lobster embryos (Perkins 1972). Development Rate was calculated using the difference between the Final PEI and the Initial PEI, and dividing this value by the number of weeks (± 1 day) that elapsed between these measurements.

Data Analysis

Five predictors were considered in modelling the variation in time of hatch: Initial PEI, Final PEI, Development Rate, Location, and Female. Initial PEI, Final PEI

and Development Rate were continuous scale variables, Location was a fixed-effect categorical variable with 6 levels, and Female was a random-effect categorical variable with 12 levels nested within Location (because each female is specific to one location).

To analyze this data, I used statistical hypothesis testing using alpha of 0.05, and estimation of best-fitted model using AIC. First, to estimate the significance of the 5 predictors variables in explaining observed variation in time of hatch I used a mixed linear ANOVA model using JMP (SAS Institute Inc. 1989-2007). Secondly, a total of 21 models (7 mixed linear, 14 linear models), with varying combinations of the original five predictors and their interactions, were created in RStudio (RStudio Team 2015) and Akaike Information Criterion (AIC) values were calculated to determine the best-fitted model. Standardized coefficients (coefficient/standard error) were calculated to determine the relative contribution of each predictor to variation in time of hatch, while accounting for the different scales of these parameters.

To test the linear model's assumptions, I made quantile-quantile normal plots (QQ Plot), plots of residuals against response, and plots of residuals against predictors. Due to 10 data points (3%) being outside of the 95% confidence interval of the QQ Plot, I calculated Cooks Distance and Welsch and Kuh measures to identify potential outliers. To determine whether these outliers had a marked impact on the inferences, I compared the results (including standardized coefficients) of the best fitted model obtained by the AIC to those of robust linear regressions, and linear regressions with all outliers (Cooks Distance = 0.011, DFFITS = 0.23) removed. We used linear modelling to ensure that there was no significant relationship between number of embryos that survived per female and the observed variation in hatch duration.

Results

Variation of hatch time among females

Of the original 576 detached eggs from 72 females used in this study, 458 (80%) successfully hatched, within an 11-week period. The weekly photos we took of each embryo enabled us to confirm the identity of 374 (65%) of the embryos that survived across 68 (94%) females, which were used for the analyses in this study. The remaining embryos either died (20%), or we were not confident that their photographic time series was the same embryo throughout the study period (15%), and thus were not used for these analyses. We terminated the study after 13 weeks with 28 (5%) embryos remaining, due to time constraints. The hatch duration for the 68 females ranged from 3 to 52 days, and the number of embryos that successfully hatched per female ranged from 3-8. The relation between mean time of hatch and the number of eggs each female successfully hatched (of the 8 that started the experiment) was not significant ($p=0.29$).

Mixed linear model

Overall, embryos hatched over a 67-day period, from July 10 to September 27, 2017. Hatch time was significantly ($p<0.001$) affected by Initial PEI, Final PEI, and Development Rate, but was not significantly affected by Location ($p=0.87$) and Female ($p=0.27$) nested within Location (Table 2).

Table 2. Results of mixed-model ANOVA for modelling variation in time of hatch. Five predictors were considered: Initial PEI, Final PEI, Development Rate, Location, and Female. Initial PEI, Final PEI and Development Rate were continuous-scale variables, Location was a fixed-effect categorical variable with 6 levels, and Female was a random-effect categorical variable with 12 levels nested within Location (each female is specific to one location).

Source	Sums of Squares	Mean Squares	Degrees of Freedom	F ratio	P-value
Initial PEI	10901.60	10901.60	1	364.42	< 0.001
Final PEI	5346.20	5740.05	1	178.71	< 0.001
Development Rate	5740.05	5740.05	1	191.88	< 0.001
Location	74.26	11.85	5	0.35	0.8771
Female(Location)	2068.89	33.37	62	1.12	0.2731
Error	9064.26	29.91	303		

Model selection

Based on the AIC results (Table 3), the best-fitted model (AIC=2343) contained all three continuous explanatory variables but neither of the categorical variables (female and location). The equation of this best model was:

Time of hatch = 44.47 – 0.42 Initial PEI + 0.40 Final PEI – 1.76 Development Rate

Time of hatch was negatively dependent on Initial PEI and Development Rate, as embryos with a larger initial eye size and a faster development rate hatched earlier. Time of hatch was positively dependent on Final PEI, as embryos with a larger eye size prior to hatch hatched later. This model explained 74.64% of the variation in time of hatch, with Initial PEI contributing the most to the explained variation (44.30%), followed by Final PEI (28.38%) and Rate PEI (27.33%) (Table 4).

Despite our attempts to standardize the Initial PEI of embryos used in the study, these were found to differ significantly among locations. For some locations, less than 12 females' eggs were found in the original range, causing some deviations from the target range. Also, preliminary measurements were performed two weeks before the Initial PEI photos were taken, thus allowing for development and increases in variation in eye size. However, individual linear models performed on each location to reduce range in Initial PEI in analyses supported the global model's conclusions concerning the relative contribution of each continuous scale variable to the explained variability in hatch time (Table 4).

When these models were run without identified outliers, the same three factors, Initial PEI, Final PEI and Rate PEI were determined to be significant, and relative proportions of variation were similar.

Table 3. AIC values for linear model selection with the following candiadate terms to predict time of hatch (ToH): Initial PEI (I), Final PEI (F), Development Rate (DR), Location (L), and Female nested in location F(L). The 10 models with lowest AIC values, and the null model are shown. The best fitted model is determined by the lowest AIC value (*).

Model	**AIC**
ToH = I + F + DR	2343 *
ToH = I + F + DR + L	2350
ToH = I + F + DR + L + F(L)	2387
ToH = I + F + L	2605
ToH = I + F	2606
ToH = I + F + L + F(L)	2613
ToH = I + R + L + F(L)	2614
ToH = I + R	2620
ToH = I + R + L	2624
ToH = I + L + F(L)	2635
ToH = mean	3881

Table 4. Relative contribution of linear models predicting time of hatch based on Initial PEI, Final PEI, and Development Rate of lobster embryos. The global model represents results with all six locations grouped together in the model, followed by models for each separate location. Standardized coefficients (coefficient/standard error) were calculated to account for different scales between the variables. Percent contribution is representative of proportion of explained variation in the model.

	Standardized Coefficients			**Percent Contribution**			
	Initial PEI	**Final PEI**	**Rate**	**Initial PEI**	**Final PEI**	**Rate**	**Overall R^2**
Global Model	31.66	20.28	19.53	44.30	28.38	27.33	74.64
Tracadie, PEI	9.48	5.58	5.89	45.27	26.62	28.10	68.21
Malpeque, PEI	21.35	13.74	11.13	46.19	29.73	24.08	87.32
Dingwall, NS	8.29	5.72	5.99	41.44	28.57	29.99	55.99
Canso, NS	8.62	7.12	7.20	37.56	31.06	31.38	71.76
Dipper, NB	12.95	10.43	9.26	39.66	31.95	28.38	70.75
St Martins, NB	13.19	8.45	9.33	42.60	27.28	30.12	82.71
Average				42.12	29.20	28.68	

Discussion

Variation in time of hatch

In this study, American lobster embryos were reared individually, detached from the females, to determine whether the female seems to play a role in the protracted hatch period that has been observed both in nature (Ennis 1975; Tlusty *et al.* 2008) and in the lab (Attard 1987; Scarratt 1964; Wilder 1953). Prior to this study the development of American lobster embryos had not been studied in individual embryos detached from the female, making it impossible to confirm that female behavior is essential to hatch. Results of this study indicate that embryos actually can and do successfully hatch if isolated from the female the spring preceding their hatch. It is possible, though, that the mortality of embryos we observed (20%) is greater than would have occurred if the embryos were still attached to the female.

The hatch duration of individual females seen in this study ranged between 3 to 52 days, which is comparable to what has been documented for embryos hatching from a female's brood in the lab, i.e., 15 to 32 days (Ennis 1975). In particular, there is no evidence that embryos raised individually have a narrower hatch period than embryos attached to a female, especially considering that only a small number of embryos were observed from each female in this study (n=8), compared to the upwards of 10,000 eggs that would typically be on a female's abdomen (Ennis 1995). These results further support the hypothesis that the process of hatching is not strictly associated with a particular role or action of the brooding female, but rather is at least partly and perhaps mostly a function of processes or attributes of individual embryos.

Variation in hatch time of individual embryos is likely the result of genetic differences among embryos. Embryos are genetically different individuals, and it is not unexpected that they would show variation in phenotypic attributes related to development. It is unclear, however, whether there is selection at the level of the female for variation in these attributes (i.e., bet hedging, see below), or whether this variation is simply the result of meiotic processes (i.e., random assortment and crossing over) leading to the fusion of variable male and female gametes, with little selection on the genes in question. In addition to genetic effects, non-genetic maternal effects may also contribute to this variation, for example it may be that females are not giving every egg the same quantity/quality of yolk. The sole source of nutrients for American lobster embryos is the reserves contained within the yolk provided by the female (Sasaki *et al.* 1986), and the potential variability in amounts and quality of yolk may result in some embryos developing faster than others. Variation in the caloric content of lobster embryos has been noted before, and it has been suggested that this variation could contribute to developmental differences among embryos (Attard 1987; Sasaki *et al.* 1986; Wickins *et al.* 1995), but this hypothesis has never been explicitly tested.

Although females do not appear to play a required role in the hatch of lobster embryos, they nevertheless probably assist the release of larvae into the water column via "fanning" of their pleopods (Ennis 1975). No study has attempted to quantify the contribution of females versus individual embryos in causing time of hatch in American lobsters. In other crustaceans, the contribution of embryos and females to hatch is highly debated, and different studies have come to different conclusions; in some species embryos are unable to hatch when detached from the female beyond a certain time

(Saigusa 1992), and in other species larvae successfully hatch when detached (Branford 1978; Ennis 1973), such as was observed in this study. It is possible that role of the female in causing hatch is related to the hatching strategy, with females that hatch synchronously contributing more to hatch than females that hatch asynchronously (Saigusa 1992).

Contribution of location to time of hatch

Timing of hatch did not vary significantly among locations. However, it must be realized that this is not indicative of geographic variation in hatch time in nature, as all embryos used in this study were selected to be at a similar initial development status and they were all raised at the same temperature, which would not have been the case if the eggs had remained in their respective locations. What this does mean, however, is that embryos from the same location that are exposed to the same conditions exhibit a protracted hatch period and the mechanisms controlling time of hatch of these embryos are the same, as the three factors investigated (Initial PEI, Final PEI, Development Rate) remained significant predictors, and their relative contributions to hatch time were similar.

Contribution of development rate and development status to time of hatch

Development of the lobster embryos can be divided into three periods: the initial late-summer to early-fall period of rapid development following spawning, winter diapause during which development is nearly or completely halted, and spring-early summer development that is rapid and culminates in hatch (Gendron and Ouellet 2009). In this study, Initial PEI and Development Rate were both use to quantify development

rate, but over different portions of the embryos' development period; Initial PEI represents the rate of development over the late-summer to fall period, and Development Rate represents the rate of development over the spring to early-summer development prior to development. We estimated that 44% of the explained variation in hatch time (75% of total variance) was related to the initial eye size, or the development status at the beginning of the spring-early summer development period. Embryos that had a larger eye size at the beginning of the development period hatched earlier than those with a smaller eye size, suggesting that embryos that are more developed come spring, hatch earlier in the summer. This finding also implies that development rate of embryos during the summer and fall post-spawn has a significant incidence on their day of hatch the following summer. It should be noted, however, that there was some development, upwards of 6-7 weeks, that did occur in early spring prior to our initial eye measurements, thus some of thus some of the contribution of initial eye size to hatch time may have to do with spring development rate as well. These results clearly show that the importance of development rate for determining time of hatch still remains, although the importance of which development period is most important is somewhat unclear.

Final PEI and Development Rate during the experimental period also contributed significantly, and similarly, to the explained variation in time of hatch. Final PEI contributed 28% of the explained variation in time of hatch, and embryos with larger eye sizes prior to hatch, hatched later than the embryos with smaller eyes. This suggests that the embryos that hatch later in the summer are at a more advanced stage of development.

Development Rate during the experimental period accounted for 27% of the explained variation in hatch time, with embryos that developed at a faster rate hatching earlier. This result is intuitive, and indicates that once effects of initial and final eye size are taken into account, faster developing embryos hatch earlier than more slowly developing embryos. Although it was not quantified in this study, as comparisons were made with only the first and final photo, development rate was not constant throughout development, with minimal changes in eye size occurring between some weeks, and marked change in eye size in others.

For quantifying development in American lobster, the PEI is consistently used as a proxy (Gendron and Ouellet 2009; Helluy and Beltz 1991; Perkins 1972). Our results displaying the relationship between these PEI values and time of hatch are consistent with our understanding that the progression in eye size is indicative of development status, and provide evidence that PEI is a useful proxy for estimating development status of an American lobster embryo.

Implications for match-mismatch

The protracted hatch period documented in this (3-52 days) and previous (15-32 days (Ennis 1975)) studies is similar to the 29-day hatch duration observed in the Blue King Crab *Paralithodes platypus* (Stevens 2006). For the king crab it has been speculated that the protracted hatch period was a bet-hedging strategy in a highly variable and unpredictable environment, resulting from the crab being unable to directly detect the presence of phytoplankton via environmental or chemical cue (Stevens 2006). By bet-hedging in this manner it is unlikely that all of a female King Crab's offspring

will hatch at the optimal time, but if the female is unable to accurately determine when the prey of its offspring are present in the water column, then hatching over a protracted period should increase the likelihood of at least some of the offspring being met with suitable conditions (Stevens 2006). This strategy i.e. bet hedging, may also be behind the protracted hatch period that is observed in American lobster. To test whether American lobster females and/or embryos can sense the presence of prey, and coordinate hatch accordingly, females and/or embryos could be raised in seawater containing phytoplankton, as well as control tanks containing filtered sea water, to determine if the range in hatch time varies between the two environments.

Summary

This study was the first to track the development and quantify hatch time of American lobster embryos detached from the female. I was able to confirm that embryos can be raised up to hatch during the spring-summer development period when detached from the female, and that the protracted hatch period observed in earlier studies persists in detached embryos, ranging from 3 to 52 days. By using eye size as an index for development, and through multiple linear regression modelling, I determined that embryos that hatch earlier in the summer i-developed more during the initial summer-fall development period, ii-are at a less advanced stage of development at time of hatch, and iii-developed at faster rates during the final spring-summer development period. Based on the relative contribution of Initial PEI for predicting time of hatch I can also suggest that embryo development rate is placing embryos at different development statuses in the spring and affects time of hatch, along with development status at hatch.

The evolutionary purpose behind this observed protracted hatch period in American lobster is still unknown, however future studies on the potential use of bet-hedging strategies and match-mismatch may aid in furthering our understanding.

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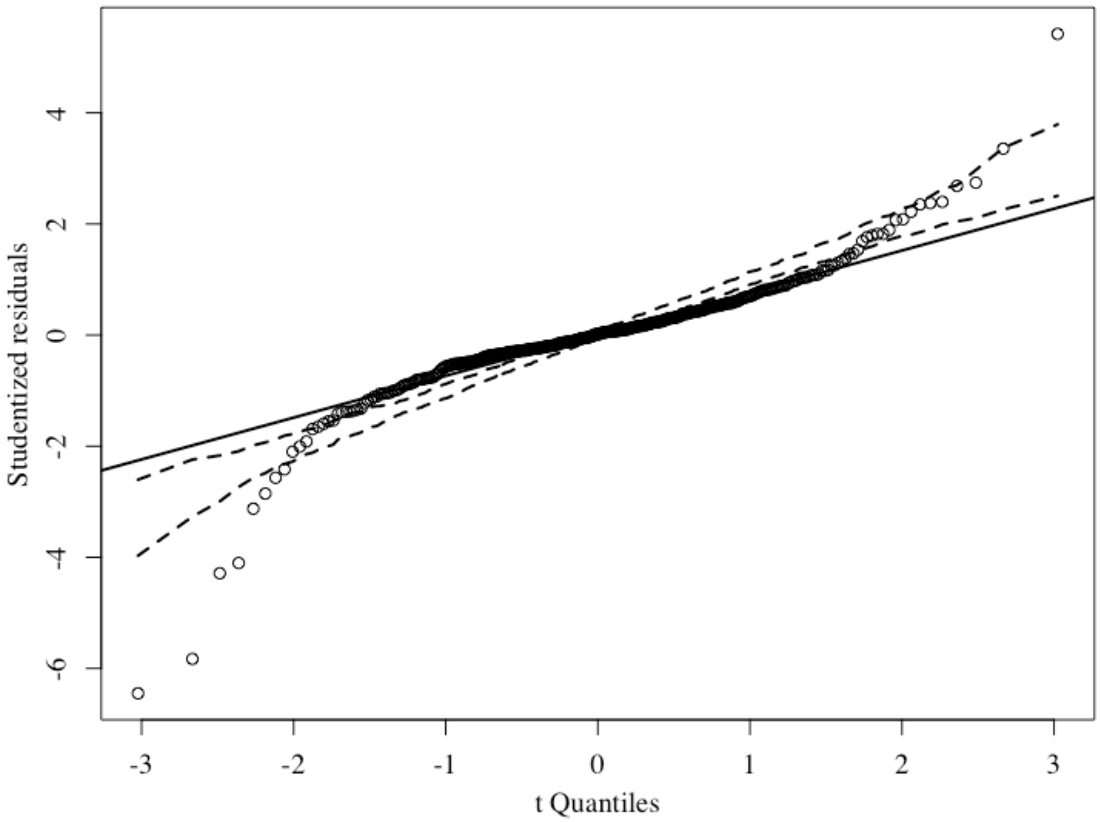
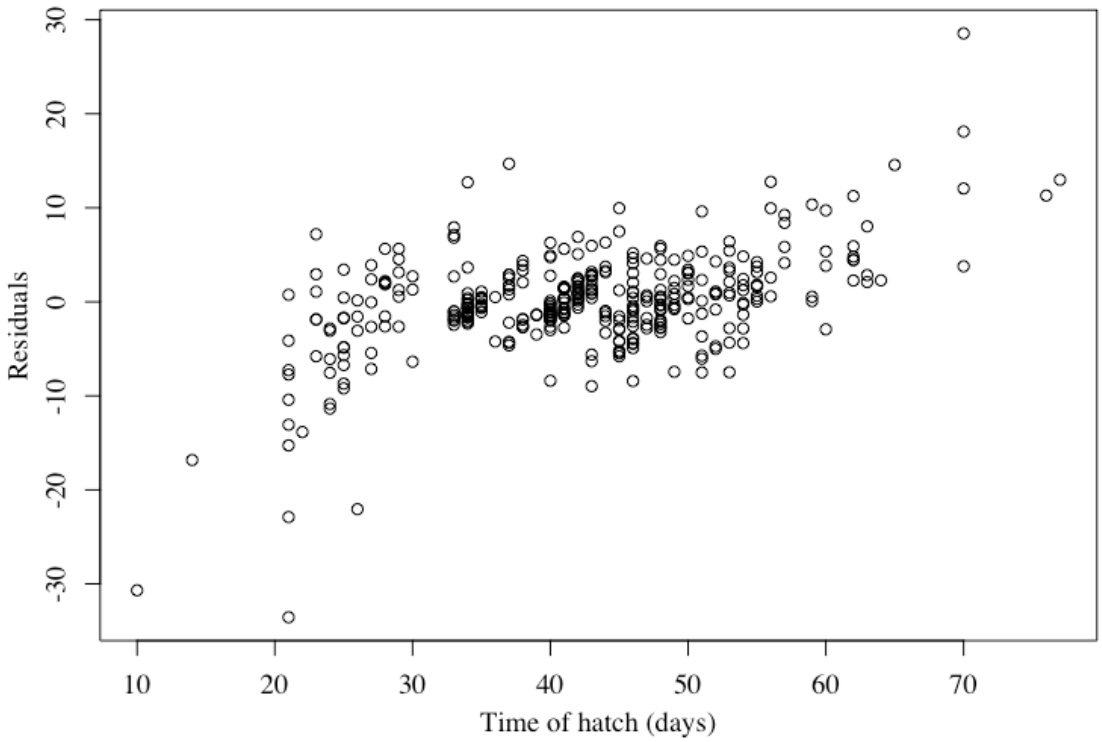
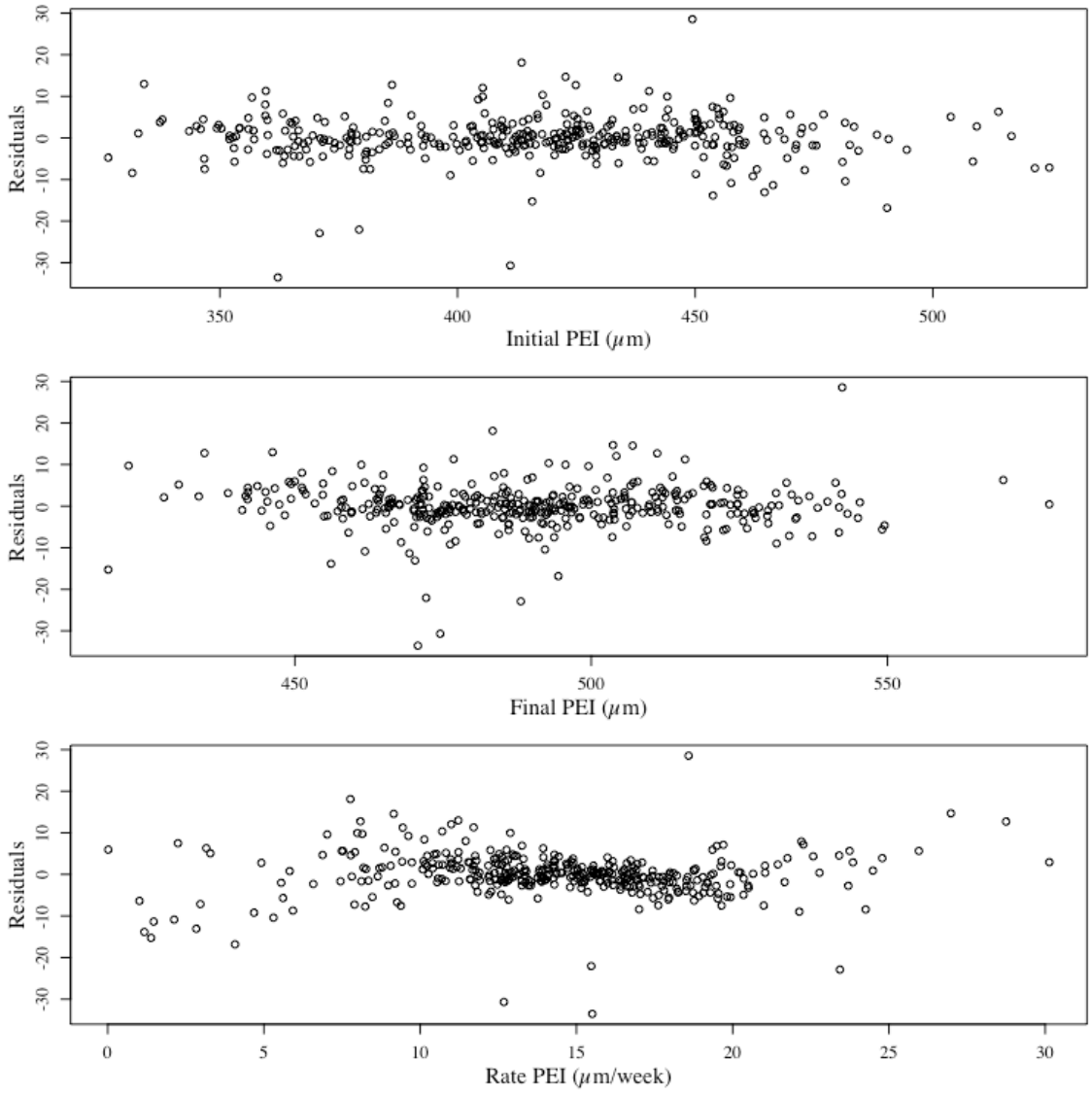
Appendix



Appendix 1. Normal Quantile-quantile plot for the global linear model Time of Hatch = Initial PEI + Final PEI + Development Rate. Dashed lines represent 95% confidence interval around normal line.



Appendix 2. Residuals versus fitted plot for the global linear model Time of Hatch = Initial PEI + Final PEI + Rate PEI. Each predictor is a continuous variable.



Appendix 3. Residuals versus predictors plots for the global linear model Time of Hatch = Initial PEI + Final PEI + Rate PEI. Each predictor is a continuous, and was determined to be significant for predicting time of hatch through AIC model comparisons.