Using temperature-dependent embryonic growth models to predict time of hatch of American lobster, Homarus americanus, in nature

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Canadian Journal of Fisheries and Aquatic Sciences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>cjfas-2015-0355.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>05-Feb-2016</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Miller, Erin; University of New Brunswick, Biology Haarr, Marthe; University of New Brunswick, Biology Rochette, Rémy; University of New Brunswick,</td>
</tr>
<tr>
<td>Keyword:</td>
<td>TEMPERATURE EFFECTS &lt; General, MARINE FISHERIES &lt; General, LOBSTERS &lt; Organisms, EMBRYOLOGY &lt; General, MODELS &lt; General</td>
</tr>
</tbody>
</table>

https://mc06.manuscriptcentral.com/cjfas-pubs
Using temperature-dependent embryonic growth models to predict time of hatch

of American lobster, Homarus americanus, in nature

Erin Miller, Marthe Larsen Haarr, and Rémy Rochette

Dept. of Biology, University of New Brunswick Saint John,
P.O. Box 5050, Saint John, NB, E2L 4L5

Corresponding Author – Erin Hope Miller
Email – Erin_Hope.Miller@unb.ca

Second Author – Marthe Larsen Haarr
Email – marthe.haarr@unb.ca

Third Author – Rémy Rochette
Email – rochette@unb.ca
Abstract

Hatch time of American lobster, *Homarus americanus*, varies between years and regions, which affects temperature experienced by the developing larvae and hence the time and distance these drift before settling. Hatch time can be assessed by working with fishermen and inspecting the brood of gravid females caught in their traps. However, this would require frequent sampling as the hatch period is protracted (≈7-12 weeks), and would require dedicated sampling in many regions where hatching occurs outside of the fishing season. To address these limitations, we tested the accuracy with which hatch time can be predicted by taking egg samples during the fishing season and estimating embryo development using embryonic eye size (Perkins Eye Index) and lab-derived temperature-dependent development functions. Using a linear development function and observed variability in Perkins Eye Index at hatch, we successfully predicted 100% of the observed 50-day hatch period, and 96% of predicted hatch dates fell within this period. Our results suggest that samples can be obtained in collaboration with fishermen to predict the timing and progression of hatch of American lobster.

Keywords: Temperature effects, marine fisheries, lobsters, embryology, models
Introduction

The American lobster, *Homarus americanus* (Milne Edwards 1837), is of considerable economic importance to coastal communities in Atlantic Canada and the northeastern United States (DFO 2014, NOAA 2014a). The fishery is managed through 45 separate Lobster Fishing Areas (LFAs) in Canada and 7 management areas in the United States (DFO 2014, NOAA 2014b). Henceforth, the term Fishing Area (FA) will be used to refer to both Canadian and American lobster management jurisdictions, the boundaries of which are based primarily upon political and socioeconomic considerations and generally do not reflect biological units or stocks (Miller 1995). As management practices are not tailored to discrete biological stocks of lobsters and recruits of one FA may be largely supplied by lobsters managed in a different jurisdiction (Miller 1997, Xue et al. 2008, Chassé and Miller 2010), it is important to understand connectivity among FAs, which is a function of dispersal by juveniles and adults on the seafloor and pelagic larvae in the water column (Ennis 1995).

The larval phase has considerable dispersal potential and is generally assumed to be the most important contributor to connectivity throughout the species’ range (Ennis 1986, Harding and Trites 1988). After a period of embryonic development that lasts up to 12 months in Canadian waters (Templeman 1937), newly released larvae drift near the surface of the water column for up to 8 weeks (MacKenzie 1988), undergoing three molts prior to reaching the post-larval stage, which eventually becomes competent to settle on the seafloor (Ennis 1995). The length of time these larvae drift with currents is primarily a function of temperature, with warmer waters significantly accelerating development (MacKenzie 1988). It is estimated that larvae may disperse up to several
Since the late 1980’s biophysical models have been developed to predict dispersal of American lobster larvae, including for the Gulf of Maine (Harding and Trites 1988, Incze and Wahle 2006) and the Gulf of St Lawrence (Chassé and Miller 2010). Using physical data of oceanographic conditions such as water temperature, ocean currents, and wind-driven surface currents, these models predict the movement of larvae from their point of origin to their settlement location. The accuracy of these predictions is, however, dependent upon the accuracy of biological inputs such as the location and quantity of larvae released as well as rates of larval development and survival (Annis et al. 2007, Hudon and Fradette 1988). Another potentially important input into these models is the time of hatching, as this will affect the currents and temperature experienced by the larvae, which in turn will affect the direction, duration and extent of their dispersal.

Timing-of-hatch can be determined through direct observation of females in the process of hatching, as these can be easily discerned by reduced clutch volume and the appearance of gluey “cement” on pleopods. However, it is logistically difficult to obtain such information across a large geographic area. Collaborating with lobster fishermen could in principle enable such direct observations of hatch. However, this would require dedicated and expensive sampling in areas where much or all of the hatch period occurs out of fishing season, such as in Atlantic Canada, where fishing is generally closed during later summer months to protect reproductive females and to avoid competition with peak U.S. landings and late summer molting (Miller 1995).
Previous efforts have been made to determine the timing-of-hatch of American lobster in nature (Templeman 1940; Fogarty 1983 and references therein; Harding and Trites 1988; Miller et al. 2006). The majority of these studies used larval tows to infer the timing and progression of hatch, based on the presence of stage I larvae in the water, although Templeman made direct observations of clutches of ovigerous females held in lobster pounds and in situ. The results of these studies, and of a recent analysis of a long time-series of egg-bearing female data from the southern Gulf of Saint Lawrence (M. Haarr, In preparation), indicate that hatching occurs from late spring to late summer months, that the duration of hatch is variable (e.g., ≈9-12 weeks in Scarratt 1964, 7 weeks in this study) and that the timing and progression of hatch vary considerably between years and regions. For example, the onset of hatch over the past 25 years has varied among regions of the southern Gulf of Saint Lawrence by as many as 8 weeks in any given year, and by as many as 7-9 weeks in different years for a same region (M. Haarr, In preparation). Similarly, the progression of hatching in a given region also varies considerably from year to year, sometimes showing a marked peak period of hatching and in other years showing more uniform hatching over time. For example, using time series presented in Scarratt (1964) on the abundance of stage I larvae in the Northumberland Strait we estimate that the inter-week variance in amount of hatching ranged from 9 times less to almost 6 times more than the mean variance of the 13-year data set (1949 to 1961).

Importantly, all studies conducted to assess hatch time of American lobster have dependent on substantial sampling efforts and required extended time at sea. Alternatively, if time of hatch can be predicted using eggs from ovigerous females
sampled (a) at a single point in time and (b) during the fishing season, then hatch could potentially be determined across the species’ range with minimal sampling effort and cost if working in collaboration with lobster fishermen.

A method for predicting time of hatch based on water temperature and embryonic development was first developed by Perkins in 1972. Ovigerous females from southeast New England were held in the laboratory at either constant or fluctuating seasonal temperatures and the development of embryos was monitored by measuring their eye size (Perkins 1972). Perkins used embryo eye size to build a linear temperature-dependent function of embryonic development, and more recently a logarithmic variant of this was proposed (Gendron and Ouellet 2009). By using these development functions along with temperature data and eye size of embryos at hatch as an endpoint, time to hatch can be estimated. Perkins (1972) concluded that hatch in southeast New England occurred when the embryo’s eye reaches ≈560 µm in diameter, while Gendron and Ouellet (2009) report a mean eye size at hatch of 550 µm in the Magdalen Islands and Helluy and Beltz (1991) report an estimate of 570 ± 20 µm in Massachusetts. Whereas these studies suggest relatively limited geographic variation in mean eye size of lobster embryos at hatch, at least one study revealed considerable variation among and within females of a same location (Gendron and Ouellet 2009), suggesting that using a single mean eye-size-at-hatch value as an endpoint could reduce the accuracy of hatch predictions made for a particular study site.

The primary objective of this study is to test the accuracy with which *in situ* hatch time of ovigerous *H. americanus* can be predicted using embryonic eye size as a measure of development, published temperature-dependent embryonic growth...
functions and both fixed and variable embryo eye sizes as development endpoints. Our
objective was not to develop new development models, but rather to determine whether
existing models, which had been developed in the lab, could actually be used to predict
hatch in nature. We estimate hatch dates of wild-caught embryos using site-specific
temperature data and both Perkins’ (1972) linear and Gendron and Ouellet’s (2009)
logarithmic embryonic growth functions, and compare these predictions to observed
hatch dates for the area obtained by sampling with fishermen. Specifically, we aim to
test (1) whether the timing of hatch can be accurately predicted based on egg samples
obtained from ovigerous females within the fishing season, but prior to the start of
hatching, (2) which embryonic growth function performs better, and (3) whether
consideration of inter- and intra-female variability of embryonic eye-size-at-hatch
improves predictions over a generalized eye-size-at-hatch obtained from the literature.
By attempting to validate this approach in nature we wish to determine whether regional
hatch times in American lobster can be accurately predicted by taking egg samples at a
single point in time during the fishing season, which would enhance our ability to predict
spatial connectivity via dispersal of lobster larvae over large geographic areas.

Methods

Sampling Location and Frequency

All sampling took place off Cheticamp, Nova Scotia, Canada over a 15-week
period from May 3rd to August 16th 2012, in an area covering ≈7.5 km² and varying in
depth from 6 to 28 m. Sampling of ovigerous females was done in conjunction with
commercial fishing from May 3rd until June 23rd, and then with a vessel chartered out-of-
season until the end of the study, to capture the period over which female eggs hatched at the study site. Two boats participated in the in-season sampling, each doing 1-3 trips per week and hauling on average 240 traps per trip. The out-of-season sampling was done by 1 boat making 3-4 trips per week and sampling on average 60 traps per trip. Traps were always soaked for 24 hours before being surveyed, at which time the size (carapace length) and developmental status (see below) of ovigerous females were recorded. Carapace length (CL) was measured as the distance from the posterior edge of the eye socket to the posterior edge of the cephalothorax, on a line parallel to the centerline of the carapace.

Water temperature

Two HOBO Pro v2 Data Loggers (U22-00) were attached <1 m above the bottom using a stationary mooring at a depth of ~18 m inside Cheticamp Harbour, within the fishing ground, and programmed to record temperature at 30 minute intervals from June to August. We estimated daily temperature values by taking the average of the 48 recordings made by each logger during a given day. Unfortunately, the time period over which the loggers were deployed was not sufficient to predict the entire hatch period, and we thus had to estimate the missing temperature data (August 16th – September 17th). We projected the missing data by fitting a second order polynomial to the existing data (m = -0.0026x^2 + 0.4539x, b = +0.7504) (Fig. 1), which provided a very strong fit (R^2 = 0.96) and mimicked the decrease in water temperature that is known to occur in the region in late summer, a pattern that we confirmed using both historical SST data for our study site (DFO 2013a) and 2012 bottom temperature data (DFO, pers. comm.) for
Pleasant Bay, a fishing ground located ~10km from our study site. Note that we also used a second approach to estimate the missing temperature data, based on difference in bottom temperature between our study site and Pleasant Bay, and the two approaches yielded similar temperature estimates and led to identical conclusions, which is not surprising given that hatch time of samples collected within the fishing season (the focus of this study) was almost entirely estimated using actual (rather than estimated) temperature data from the study site. For example, using in-season samples obtained 18 days before the beginning of hatch, the percentage of predicted hatch dates that fell after August 15th, and hence required some reliance on estimates of temperature, was ≤6% for the different models.

Observed Timing of Hatch

Timing of hatch in the field was observed by monitoring the progression of egg development of ovigerous females. Each female’s clutch of eggs was categorized as one of four developmental stages, based primarily on the colour and appearance of the eggs in the clutch. Eggs in a stage I clutch have no visible “embryo eye”, are small, dark green to black in colour and are tightly packed together. Eggs in a stage II clutch have visible embryo eyes and they are dark–medium brown in colour. Eggs in a stage III clutch are light brown to orange in colour, comprise fully developed embryos, are loosely packed together and are close to hatching. A stage IV clutch comprises eggs that are hatching and adhesive glue can be seen on the female’s abdomen along with empty egg cases (MacKenzie et al. 2011). By observing the proportion of ovigerous females with eggs in each of these stages over the course of the spring-summer we
were able to monitor the temporal progression of embryonic development and obtain an estimate of the period of time over which hatch occurred (henceforth the “hatch period”) based on the presence of stage IV clutches.

Predicting Timing of Hatch

Timing of hatch in the field was predicted using small clusters of eggs removed from a total of 227 ovigerous females with stage II or stage III clutches, 100 of which were sampled prior to the hatch period (13\textsuperscript{th} and 23\textsuperscript{rd} of June; n=50 per trip) and 127 that were sampled during the hatch period (23 separate trips between July 4\textsuperscript{th} and August 16\textsuperscript{th}). Fine tweezers were used to sample 8 small clumps of eggs spread throughout the egg mass of each of these females. The eggs were placed in a glycerine-ethanol solution (35:65%) in order to preserve the embryos and their size at sampling.

The degree of embryonic development was measured by calculating the “Perkins Eye Index” (henceforth PEI), which is the mean of the greatest width and length of an embryo’s oval-shaped eye (Perkins 1972). We used a microscope (Leica S8AP0) at 80 X to measure the PEI on each of five eggs (preliminary tests with 4 females indicated CV between 0.04 and 0.08, mean = 0.06) haphazardly selected from within each female’s egg sample (eggs from 11 of the 227 females could not be processed due to poor preservation). This measure of embryonic development (PEI) was then used in conjunction with bottom temperature and Perkins’ (1972) and Gendron and Ouellet’s (2009) embryonic development formulas to predict a future hatch date for each individual embryo processed. Embryonic development (i.e., increase in PEI) was
estimated on a daily basis and was added to the previous day’s PEI, beginning with the initial PEI measurement and progressing until reaching PEI-at-hatch. This exercise was carried out using two different embryonic growth formulas and different values of PEI-at-hatch. In terms of growth formulas, we used Perkins’ (1972) linear function:

\[
y = -8.3151 + 2.6019(x)
\]

where \( y \) is the embryonic growth rate expressed as change in PEI in microns per week (we divided by 7 to obtain a daily rate), and \( x \) is water temperature, as well as Gendron and Ouellet’s (2009) logarithmic function:

\[
PEIR = 0.00002 \times t^{2.25008}
\]

where \( PEIR \) is the change in PEI in mm per day, and \( t \) is water temperature.

We used two approaches for assigning values of PEI-at-hatch: (1) a fixed value of 560 µm for all embryos, which is the mean of the three values reported in the literature (Gendron and Ouellet 2009 (550 µm), Helluy and Beltz 1991 (570 µm), Perkins 1972 (560 µm)), and (2) a frequency distribution of PEI-at-hatch values observed during our study, which was obtained by measuring the PEI of newly hatched prezoea that were found among egg samples taken from females in the process of hatching larvae (60 prezoea from 7 females). The prezoea likely serves as an appropriate indicator of PEI-at-hatch as it is a brief phase, thought to last only 24 hours,
taken by a newly hatched lobster prior to its molt to larval stage I (Ennis 1975). As the number of prezoea available to estimate PEI-at-hatch varied among females \((n = 4 – 16)\), the proportion of each female’s prezoea that fell within each 1µm PEI interval (460µm – 611µm) was calculated in order to avoid creating a frequency distribution skewed by one or two females. A log-normal function was then fitted \((R^2 = 0.56, p = 0.0007)\) to this adjusted distribution of prezoea PEI and used to generate an estimate of PEI-at-hatch for each embryo measured. This log-normal function was used to generate PEI-at-hatch for all embryos with no consideration of maternal effects (e.g., female size) as the majority of variability in prezoea PEI was found within clutches (64%) and there was no significant relation between maternal size and PEI-at-hatch \((R^2 = 0.42, p > 0.10)\).

In total, therefore, four sets of predicted hatch dates were generated for five embryos from each of 216 females through combinations of the linear and logarithmic development functions and the two estimates of PEI-at-hatch.

Predicted versus Observed Timing of Hatch

To determine if, and how well, this approach can be used to predict hatch of lobster in nature we addressed four questions: (1) do the predictions made by the four models reflect the observed hatch period for Cheticamp?; (2) do the predictions made by the four models reflect the observed progression of hatch in the hatch period?; (3) does either of the two temperature-dependent embryonic development functions perform better than the other?; (4) is the quality of the hatch predictions affected by the estimate of PEI-at-hatch used?
As the primary objective of this study is to determine whether the timing of larval release can be predicted by sampling ovigerous females during the fishing season, all main analyses are based on the two samples of females taken in-season, and prior to the observed start of hatch (before July 1st). To assess the performance of different models at predicting the hatch period (question 1) we compared the (a) number of predicted hatches that fell within the hatch period, and (b) portion of the hatch period that was covered by predicted hatch values. To assess the performance of different models at predicting the progression of hatch (question 2) we compared predicted and observed cumulative percentage of hatch on each of the 23 sampling dates (1-4 day intervals) during the 50-day hatch period. To compare our four models’ ability to predict the progression of hatch we used Akaike Information Criterion (AIC), where each model’s residual sum of squares (RSS) was the sum of the squared differences between observed and predicted cumulative hatch values for each of the 23 sampling dates. We also included in this comparison a “null model”, which predicted a constant proportion of hatch occurring on each of the 23 sampling dates (i.e., 4.3%).

Results

Water temperature at our study site was 6.9°C when we took our first sample of eggs on June 13th, then it increased to a maximum of 20.3°C on August 8th, before decreasing slightly to 19.0°C on September 17th, which is the latest day any of our samples and models predicted hatching at our study site (Fig. 1).

Observed Timing of Hatch
Ovigerous females with hatching (stage IV) clutches were observed on each sampling date between July 4th and August 16th 2012 (Fig. 2). Hatching likely started sometime between July 4th and June 23rd (the previous sample), given the abundance of stage III females (close to hatching) in the June 23rd sample. Hatching ended on or shortly after August 16th, our last date of sampling, because egg-bearing females sampled on this day were either in the process of hatching (11% stage IVs) or had newly extruded clutches that would not hatch until the following year (89% stage Is) (Fig. 2). Due to the uncertainty around the exact day when hatch began (between June 23rd and July 4th) and ended (on or shortly after August 16th), the first and last observed hatch dates plus 3 days was used to signify the beginning (July 1st) and end (August 19th) of hatch and create a 50-day hatch period. Whereas the addition of 3 days to the first and last observed hatch dates was somewhat arbitrary, it likely resulted in a more accurate description of the true hatch period. More importantly, all general conclusions reached in this study were unchanged when we ran the analyses on a “non-modified” hatch period or one that was further expanded (see examples in Discussion).

Predicted Timing of Hatch: 8-day versus 18-day samples

Using samples obtained 8 days prior to the beginning of hatch, hatch was forecast 1–63 and 1-35 days in the future with the linear and logarithmic models, respectively. Using samples obtained 18 days prior to hatch, these numbers increase to 11-75 and 9-46 days (Fig. 3). Predictions made with samples obtained 8 and 18 days before the beginning of hatch were remarkably similar (Fig. 4) and we only present
hereafter results pertaining to eggs sampled 18 days prior to the beginning of hatch, for simplicity.

**Predicted versus Observed Timing of Hatch: The Hatch Period**

The vast majority (91-100% for different models) of predicted hatch dates of individual embryos sampled 18 days prior to the beginning of hatch fell within the observed hatch period, both for the linear (96 % using both the fixed and variable PEI-at-hatch values, respectively) and the logarithmic (91 % or 100 %) development functions. However, the linear development functions did a better job at predicting the entirety of the hatch period than the logarithmic functions (Fig. 5). For example, the proportion of days of the 50-day observed hatch period that was forecast by the linear temperature-dependent functions was very high (84 or 100% using the fixed and variable PEI-at-hatch values, respectively) and markedly greater than that forecast by the logarithmic temperature-dependent function (54 or 58%) (Fig. 5). In particular, and in contrast to the linear temperature-dependent functions, the logarithmic temperature-dependent functions failed to capture later hatches, predicting no hatching during the last 22 (fixed PEI-at-hatch) or 21 (variable PEI-at-hatch) days of the 50-day hatch period.

Samples obtained out of season, after hatch had begun, predicted increasingly later hatch dates on average (Fig. 5), likely because they comprised increasingly fewer of the earlier hatching females as these would already have hatched their eggs and been removed from the population. More surprisingly, however, predictions made from
samples obtained after mid-July began predicting hatches extending beyond the observed hatch period, and also beyond hatch dates predicted by in-season samples. The predictive ability of the models was also affected by the PEI-at-hatch values used as hatching endpoints, although this effect was less pronounced than that of the temperature development functions (Fig. 5). Using PEI-at-hatch values observed during this study produced a greater range of predicted hatch dates (17 and 11-day increase for the linear and logarithmic temperature-dependent functions, respectively) compared to using the mean PEI-at-hatch from the literature, and it increased the portion of the observed hatch period that was predicted by 16% and 4% for the linear and logarithmic development functions, respectively.

Overall, the hatch period was best predicted by the model that used the linear temperature-dependent development function and the site-specific frequency distribution of PEI-at-hatch. Using this model and samples obtained 18 days prior to the beginning of hatch, 96% of predicted hatch dates of individual embryos fell within the hatch period and 100% of the 50-day period of hatch was forecast.

Predicted versus Observed Timing of Hatch: Progression during the Hatch Period

In addition to predicting the timing and range of the hatch period with a high level of accuracy, the models also tracked the cumulative progression of hatch within the hatch period with relative success (Fig. 6). The observed hatch rate was fairly constant over the hatch period, only showing a modest peak around days ≈15-20 of the 50-day hatch period (Fig. 6).
Of the 5 models compared, the linear development function with variable PEI-at-hatch and the null model based on “uniform hatch” best predicted cumulative hatch throughout the 50-day hatch period. These two models performed similarly (Table 1), yielding nearly identical AICc values and weights (Anderson 2008). Using the null model, the average deviation between predicted and observed cumulative frequency of hatch across the 23 timesteps was 8.6%, and the single greatest deviation was at 18 days into the hatch period, when the model predicted 39% of hatching to be completed when in actuality 61% of hatch had been observed (22% deviation). Using the linear model with variable PEI-at-hatch the average deviation between predicted and observed cumulative frequency of hatch was 8.1%, and the single greatest deviation was at 13 days into the hatch period, when the model predicted 26% of hatching to be completed when in actuality 49% had been observed (23% deviation). The other three models produced average deviations ranging from 14.2-24.1% and maximum deviations between 31.7-51.4%.

Discussion

Our study indicates that we can predict with accuracy hatch of American lobster up to 67 days in the future using egg samples collected up to 18 days prior to the beginning of hatch, temperature-dependent development functions developed in the lab and development endpoints based on prezoea eye size. The strength and usefulness of a best model in this context is a function of two complimentary and necessary properties: (1) its predictions must fall within the observed hatch period, and (2) the observed hatch period must be predicted in its entirely. If all predictions fell within a
large hatch period but only captured a small proportion of this hatch period, then our
model would be weak, because predictions of hatch time and dispersal would only
cover a small portion of reality. Conversely, if our predictions covered all days of the
observed hatch period because they ranged widely and often extended beyond the true
hatch period, then our model would again be weak. A useful model in this context is one
that has both of these properties.

Of the two different temperature-dependent embryonic growth functions
proposed in the literature, Perkins’ (1972) linear function outperformed the logarithmic
function (Gendron and Ouellet 2009) in terms of forecasting hatch, based on the two
criteria outlined above. More specifically, using 250 eggs sampled 18 days prior to the
beginning of hatch, the linear temperature-dependent function successfully predicted
100% of the 50 days comprising the observed hatch period, and only 1% (variable PEI-
at-hatch) of predicted hatch dates were more than five days before (max 6 days) or after
(max 8 days) this period. In contrast, the logarithmic temperature-dependent function
appeared to overestimate the rate of embryonic development, as with both the variable
and fixed PEI-at-hatch values the logarithmic models predicted hatching to end 21 or 22
days earlier than the end of the 50-day hatch period.

In addition to the error surrounding our predicted hatch dates, there was also
some uncertainty surrounding the true hatch period, caused in part by the fact that we
did not sample every day. However, this uncertainty is relatively small and it seemed to
have negligible impact on the accuracy of predictions. For example, if we had not semi-
arbitrarily extended the tails of the hatch period by 3 days (44 days instead of 50 days)
relative to the dates when females with hatching eggs were actually sampled, the
proportion of predicted hatch dates that would have fallen within the hatch period using
the linear model with variable PEI-at-hatch, which is the model with the greatest range
of predicted hatch dates (i.e., provides the most stringent comparison), would only have
dropped from 96% to 92%. Similarly, if we had extended the hatch period by 10 days
(instead of three) on both ends (64 days total instead of 50 days), we would still
successfully have predicted 100% of the hatch period and 100% of predictions would
have fallen in this period. In fact, the uncertainty surrounding the real hatch period likely
means that our results may to some degree understate the true accuracy of the
approach. Furthermore, that this uncertainty had such a small impact on the accuracy of
model predictions also means that our conclusion that the linear development function
with variable PEI at hatch best modeled the hatch period is unlikely to be compromised
by this error. The small adjustment we made to the “observed hatch period” almost
certainly decreased the error surrounding this estimate, but most importantly it did not
affect the main inferences of our study.

Another potential source of error surrounding our observed hatch data relates to
“catchability” of female lobsters with stage IV clutches, or more specifically whether the
propensity of these individuals to enter our baited traps changed over the course of the
study. The catch-per-unit-effort (CPUE) of adult lobsters has been shown to correlate
with their abundance on the bottom, although the match is not perfect due to density-
and environmentally-mediated changes in lobster behavior and trap saturation (e.g.,
Watson and Jury 2013). Whereas we have no means of directly assessing such
potential errors, we made efforts to eliminate them and don’t believe they challenge our
main inferences. In particular, our traps were the only traps in the water during the
hatching period and we maintained a relatively constant sampling effort in our ≈7.5 km² study area during this period (3-4 boat trips/week, 60 traps/trip and a 24-hour soak time). Also, water temperature during the hatch period varied between 13-20°C, a range of temperatures over which movement rates and catchability of American lobsters do not appear to vary (McLeese and Wilder 1958). Although not enabling a rigorous test of the catchability bias hypothesis, our catch data of egg-bearing females do not raise any concerns over the presence of such a bias as they clearly show the stage II-III clutches gradually becoming stage IV clutches, and then these stage IV clutches being gradually replaced by females with new clutches (stage I) that will hatch the following year. A catchability bias is greatly limited here by the fact that we are using catch data to assess changes in the abundance of a same type of lobster (females with stage IV clutches) over a relatively small area and time period and under fairly constant sampling effort and environmental conditions.

In addition to accurately predicting the overall hatch period, our four models also did a reasonable job predicting the progression of hatch within this period, and here again the best linear model (with variable PEI-at-hatch) outperformed the best logarithmic model (with fixed PEI-at-hatch). For example, the average and maximum deviations between observed and predicted cumulative hatch over the 23 sampling dates were 8.1% and 23.1 % for the former versus 14.2% and 31.7% for the latter, and these differences arose mainly because the log model predicted hatch to be completed ≈3 weeks earlier than was observed. The average (8.1%) and maximum (23.1%) deviations between observed and predicted cumulative hatch for our best model (linear with variable PEI at hatch) were comparable to deviations obtained with the null model.
of uniform hatch (8.6% and 22.2%, respectively), as were their respective AICc scores and weights. We do not believe these results reflect failure of our best model, but rather the fact that hatch did indeed progress relatively uniformly throughout the hatch period during our study, given the small and similar deviations between observed and predicted progression of hatch for our best model and the null model. The progression of hatch in American lobster has been shown to vary markedly in different years and locations (Fogarty 1983, Scarratt 1964), sometimes showing a marked peak period of hatch and in other years showing more uniform hatching over time. We believe it is likely that the temperature-dependent development functions will track to some extent deviations from uniform hatch where and when that occurs, and are currently undertaking further validations of these outcomes.

Considering Variability in PEI-at-Hatch Improves Model Predictions

The addition of a development endpoint based on variability of PEI-at-hatch of prezoea observed within our samples increased the range of predictions made by both temperature-dependent functions, relative to using a fixed mean value derived from the literature, and it resulted in a better fit with the observed hatch period. For example, when PEI-at-hatch was based on the size-frequency distribution of values found for prezoea obtained in our samples, the proportion of the hatch period predicted increased from 84% to 100%, and from 54% to 58%, for the linear and the logarithmic development functions, respectively, and the mean deviation between observed and predicted cumulative hatch frequency decreased from 18.1% to 8.1% for the linear development function (see below for logarithmic function).
This positive effect of including PEI-at-hatch in our predictions is not surprising, given that it varied considerably among prezoea (460-611µm, n = 60). Interestingly, 64% of this variability was between prezoea of a same clutch, which is consistent with the observation that a same female can hatch its embryos over a period of 2-4 weeks (Ennis 1975). Within the clutch of a single female, the range of PEI-at-hatch was as high as 492-611µm (n=16 prezoea), which is almost the entire range of PEI-at-hatch values observed among the 60 prezoea we sampled from seven females. Between these seven females, mean PEI-at-hatch varied by as much as 49µm. Somewhat surprisingly, however, for the logarithmic model the mean deviation between observed and predicted cumulative hatch values increased (not decreased) slightly from 14.2 % to 24.1 % with inclusion of this variable endpoint. This small change may not be meaningful, considering the errors associated with our different estimates, but it does support our conclusion that the later stages of lobster embryo development may be better described by the linear than the logarithmic function, given that the inclusion of observed variability of PEI-at-hatch undoubtedly better approximates reality than using a mean value.

Although variability of PEI-at-hatch within or among females has not been systematically quantified, it is evident from at least two studies (Helluy and Beltz 1991; Fig. 1.1 in Gendron and Oulette 2009). Those findings, along with results of our study, suggest that improved forecasting can likely be achieved by further characterization of the variability in PEI-at-hatch. Interestingly, the mean PEI-at-hatch determined in this study (520µm) was substantially smaller than that previously reported in the literature (550µm -570µm), suggesting that future studies are also needed to more rigorously contrast PEI-at-hatch among regions.
Temporal Trends in Predicted Hatch Dates

There was a strong temporal trend in the hatch dates predicted, with samples obtained after the beginning of hatch predicting increasingly later aggregated hatch dates. This pattern is easily explained. As sampling progresses past the beginning of hatch, early hatchers are lost, leading to later samples predicting later aggregated hatch dates than the samples taken prior to the start of hatch, when all embryos to be hatched in the coming season are still present.

There was, however, another temporal pattern in our data that is not as readily explained, which is that females sampled mid-July onwards (well into the hatch period) generated some predicted hatch dates beyond the observed hatch period, and also beyond hatch dates predicted by in-season samples. One possible explanation for this pattern is that later samples may have comprised females that migrated to the sampling site from deeper waters after the fishing season, and hence were not present in pre-hatch samples. For example, in the Gulf of Maine larger ovigerous females migrate to deeper offshore waters during the gestation period and then move inshore to release their larvae (Cowan et al. 2007). If lobsters from our study area in the southern Gulf of St Lawrence behave similarly, then such behavior may lead to a staggered regional hatch period. However, if such offshore-inshore movements are displayed by female lobsters in our study area they do not appear to be related to female size, as the carapace length of sampled females did not change over the course of the study ($N = 227, m = 0.077, R^2 = 0.009, P = 0.16$) and the mean carapace length of females with embryos predicted to hatch within and outside the hatch period was similar ($t_{214} = 1.97$,
p < 0.32). This does not, of course, preclude a change in the population of egg-bearing females over the course of the sampling period unrelated to body size.

**Application and Recommendations**

In this study the first egg samples were collected during the fishing season 18 days prior to the start of hatch, which proved adequate to predict the entire hatch period, up to 67 days in the future. Importantly, the fishing season is sufficiently close to the beginning of hatch throughout much (but see below) of the species' range for such relatively short-term forecasting to be sufficient.

The method outlined in this paper may be most immediately applicable to the development of bio-physical models of larva dispersal used to elucidate connectivity amongst management areas. For example, larval release simulations made with a new dispersal model for lobster larvae encompassing the species' geographic range (Brickman and Drozdowski 2012; Quinn 2014) indicate that hatches occurring 2 weeks later than observed during our study would have resulted in drift distances increasing by ~40 km (B.K. Quinn, University of New Brunswick, pers. comm.). These findings indicate that accurate estimates of hatch time will likely markedly improve connectivity estimates made by bio-physical models of larva dispersal.

Although our findings unequivocally demonstrate the usefulness of the approach to predict hatch time of lobster larvae in nature, and includes recommendations of particular development functions and endpoints to use, more work should be done to refine the method and better understand its limitations and context specificity. First, it must be realized that our comparisons of development models are specific to the time
period over which forecasting was done, which is short (≈60 days) relative to the complete embryo development period (≈300 days). In other words, the models that were successful at predicting development and hatch in this study may not do a good job at predicting development from spawn to hatch. Whereas such long-term forecasting was not the objective of this study, it would nevertheless be important to assess the ability of the method to accommodate areas where there exists a larger disconnect between the end of fishing season (and potential sampling) and the end of hatch (for example, ≈90 days in LFA 33 and LFA 34 in Canada (DFO 2004, DFO 2013b)).

Second, over the time period investigated, the linear development function with variable PEI-at-hatch was the best of the four models tested to predict the hatch period, but none of the 4 study models outperformed the “null model” of uniform hatch to predict the progression of hatch during the hatch period (the best “biological model” and the null model appeared equally adequate). More work is needed to assess the generality of this finding, and we expect that a biological model will in most natural scenarios better predict the progression of hatch than the null model, despite the results found in this study, where observed hatch rates did seem relatively uniform over the hatch period.

Third, it will similarly be important to repeat these tests in different regions, to ensure the development functions perform well in different temperature conditions. Finally, it would be worth doing additional tests in nature where egg-bearing females would be confined and have their eggs monitored on a regular basis, which would enable a more direct correlation between predicted and observed hatch of individual females instead of the “aggregated relation” quantified in this study.
Acknowledgments

Berried female data and egg samples were provided by Raja Wetuschat, Julie Murdoch, Michel Comeau, Leonard Leblanc and the Gulf Nova Scotia Fishermen’s Coalition. This project was funded by grants to R. Rochette from the Lobster Node of the NSERC Canadian Fisheries Research Network (CFRN) and the NBIF Research Innovation Fund, as well as a NSERC Discovery grant. E. Miller and M. Haarr were also funded by the CFRN.

References


Predicting hatch time of American lobsters in nature


Predicting hatch time of American lobsters in nature


(Homarus americanus): Quantitative staging and characterization of an embryonic molt 

Hudon, C., and Fradette, P. 1988. Planktonic Growth of Larval Lobster (Homarus 
americanus) off îles de la Madeleine (Quebec), Gulf of St. Lawrence. Can. J. Fish. 

Incze, L., and Naime, C.E. 2000. Modelling the transport of lobster (Homarus 
americanus) larvae and postlarvae in the Gulf of Maine. Fish. Oceanogr. **9**: 99-113. doi: 
10.1046/j.1365-2419.2000.00125.x.

Incze, L.S., Wahle, R.A., Wolff, N., Wilson, C., Steneck, R., Annis, E., Lawton, P., Xue, 
H., and Chen, Y. 2006. Early Life History and a Modeling Framework for Lobster 

between stage durations, mortality, and growth in laboratory-reared Homarus 
10.1016/0022-0981(88)90248-1.


Table 1. Comparison by Akaike’s information criterion (corrected for small sample size) of accuracy of predictions of cumulative hatch made by five models based on egg samples obtained 18 days prior to the beginning of hatch.

<table>
<thead>
<tr>
<th>Model</th>
<th>RSS</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>wi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>0.271995</td>
<td>-42.32483</td>
<td>0.248596</td>
<td>0.467843</td>
</tr>
<tr>
<td>Linear Fixed</td>
<td>1.590994</td>
<td>-24.68137</td>
<td>17.89206</td>
<td>6.9E-05</td>
</tr>
<tr>
<td>Logarithmic Fixed</td>
<td>0.791062</td>
<td>-31.6609</td>
<td>10.91252</td>
<td>0.002262</td>
</tr>
<tr>
<td>Linear Variable</td>
<td>0.26531</td>
<td>-42.57342</td>
<td>0</td>
<td>0.529764</td>
</tr>
<tr>
<td>Logarithmic Variable</td>
<td>1.624387</td>
<td>-24.47388</td>
<td>18.09954</td>
<td>6.22E-05</td>
</tr>
</tbody>
</table>

*aThe five models compared are a null model of constant hatch throughout the hatch period and four different temperature-dependent models of embryo development and hatch: linear formula of embryo development with fixed PEI-at-hatch; logarithmic formula of embryo development with fixed PEI-at-hatch; linear formula of embryo development with variable PEI-at-hatch; and logarithmic formula of embryo development with fixed PEI-at-hatch.

*bRSS is residual sum of squares

*cAICc is Akaike’s information criterion (corrected for small sample size)

*dΔAICc is the difference between the AICc score of each model and the score of the best model

*ewi is Akaike weight.
Figure 1: Observed (black circles) and estimated (white circles) temperature in the lobster fishing grounds in Cheticamp, Nova Scotia, between June 1st and September 30th 2012. Observed temperatures were based on the average daily temperature recorded by 3 temperature loggers. Temperature data extending beyond the sampling period (August 16th – September 30th) was estimated by fitting a second order polynomial to the existing data \( m = -0.0026x^2 + 0.4539x, b = +0.7504 \) (\( R^2 = 0.96 \)). The dashed line marks the latest date at which a lobster embryo was predicted to hatch based on samples collected during the fishing season.
Figure 2: The observed hatch window indicated by the proportion of ovigerous females sampled from early-May to mid-August 2012 in Cheticamp, NS, with eggs at different stages of development. Each female’s clutch of eggs was categorized as one of four developmental stages, based primarily on the colour and appearance of the eggs in the clutch. Eggs in a stage I clutch have no visible “embryo eye”, are small, dark green to black in colour and are tightly packed together. Eggs in a stage II clutch have visible embryo eyes and they are dark–medium brown in colour. Eggs in a stage III clutch are light brown to orange in colour, comprised of fully developed embryos, are loosely packed together and are close to hatching. A stage IV clutch has begun hatching and adhesive glue can be seen on the female’s abdomen along with empty egg cases.
Figure 3: Number of days after sampling that hatch was predicted to occur using samples collected 8 (white box) and 18 (shaded box) days prior to hatch in 2012 in Cheticamp, Nova Scotia, based on our four different temperature-dependent models of embryo development and hatch: linear function of embryo development with fixed PEI-at-hatch (LINF); logarithmic function of embryo development with fixed PEI-at-hatch (LOGF); linear function of embryo development with variable PEI-at-hatch (LINV); and logarithmic function of embryo development with fixed PEI-at-hatch (LOGV). Boxes show 25th, 50th, and 75th percentile, whiskers represent 10th and 90th percentiles and black circles represent the 5th and 95th percentiles.
Sampled 8 days prior to the beginning of hatch

Sampled 18 days prior to the beginning of hatch

Figure 4: Relation between cumulative timing of hatch predicted using eggs samples obtained 18 and 8
days prior to the beginning of hatch in 2012 in Cheticamp, Nova Scotia, using four models: linear formula
with fixed PEI-at-hatch (A); logarithmic formula with fixed PEI-at-hatch (B); linear formula with variable
PEI-at-hatch (C); and logarithmic formula with fixed PEI-at-hatch (D). Each point represents one of 23
sampling days during the 50-day hatch period.
Figure 5: Relation between predicted (circles) and observed (gray area) time of hatch in 2012 in Cheticamp, Nova Scotia. Hatch time was predicted by measuring eye size of embryos in eggs of ovigerous females sampled prior to hatch (gray circles) and after hatching had begun (black circles), and projecting embryo development using different combinations (four panels) of two temperature-dependent development functions: linear function with fixed PEI-at-hatch (A); logarithmic function with fixed PEI-at-hatch (B); linear function with variable PEI-at-hatch (C); logarithmic function with variable PEI-at-hatch (D) (see Methods). The observed hatch period is based on the presence of ovigerous females with recently hatched eggs (see Fig. 2) in samples obtained by sampling out-of-season with fishermen.
Figure 6: Observed (X), null (black circles) and predicted (4 other symbols) cumulative timing of hatch in 2012 in Cheticamp, Nova Scotia. The observed progression of hatch is based on the presence of ovigerous females with recently hatched eggs (see Fig. 2) (see Methods), whereas predictions are based on egg samples obtained 18 days prior to the beginning of hatch and four different temperature-dependent models of embryo development and hatch: linear function of embryo development with fixed PEI-at-hatch (LINF); logarithmic function of embryo development with fixed PEI-at-hatch (LOGF); linear function of embryo development with variable PEI-at-hatch (LINV); and logarithmic function of embryo development with fixed PEI-at-hatch (LOGV). Each point represents one of 23 sampling days during the observed 50-day hatch period.