Validating a method of predicting timing of hatch of American lobster, *Homarus americanus*, in nature

by

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ABSTRACT

Models have been developed in the laboratory to predict hatch of American lobsters based on temperature and embryonic eye-size. These models have not, however, been validated in different locations in nature. I compared hatch predictions using two models that differed only in the assumed functional relationship between temperature and embryo development rate to observed hatch in two locations in Atlantic Canada. The best model predicted 91-100% of the range of observed hatch dates and 90-95% predictions fell within the observed hatch periods, although it was not the same model for each location. Although I was able to predict the mean hatch date at the population level, on average only 31% of predictions fell within the observed hatch periods of individual lobsters. Models appear useful to predict hatch, however future research is necessary to determine what influences model success in different locations.

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Introduction

The American lobster (*Homarus americanus*) fishery is the most economically important fishery in Canada. In 2017, lobster landings were valued at $1.2 billion CAD domestically and $2.1 billion CAD internationally (DFO 2019a, 2019b). Over the past decade lobster landings have increased steadily, with a record-breaking total of 97,542 metric tons reported in 2017 (DFO 2019c). In Canada, this fishing industry is divided into 45 Lobster Fishing Areas (hereafter "LFAs") across the five Atlantic Provinces (DFO 2015). The boundaries of each LFA are determined based upon geographic and socio-political considerations (Williamson 1992), rather than with respect to the biological stock structure of lobsters (Miller 1995). Management decisions such as the timing of the fishing season, gear restrictions, minimum legal size, the presence or absence of a maximum legal size, and number of license holders, vary among LFAs (Phillips et al. 1980, Miller 1995). These management decisions are tailored to the local industry within each LFA, and do not take into account potential connectivity of the lobster resource among LFAs, despite the fact that the annual provision of new recruits in one LFA may be largely supplied by lobsters a different LFA (Miller 1997, Xue et al. 2008, Chassé and Miller 2010, Quinn et al. 2017).

Without adequate understanding of stock connectivity, it is difficult to predict how changes in management strategies within one LFA may affect lobster abundances in that particular LFA, as well as in surrounding fishing

areas. Both the larval and adult phases of lobster are thought to contribute to connectivity among LFAs via dispersal of pelagic larvae in the water column and walking by settled animals on the seafloor, respectively (Ennis 1995, Morse et al. 2018). The larval phase in particular is thought to have considerable dispersal potential, with movement largely mediated by oceanographic conditions, such as wind and currents (Ennis 1986, Harding and Trites 1988). After a period of embryonic development lasting up to 12 months (Templeman 1940), newly hatched larvae drift near the surface of the water column for 2–8 weeks or more depending on temperature (MacKenzie 1988, Annis et al. 2007). Pelagic larvae undergo three moults prior to reaching the postlarva stage, which eventually becomes competent to settle on the seafloor (Ennis 1995). The length of time that larvae drift with currents is primarily a function of water temperature, with development time being inversely correlated with temperature (Templeman 1936, MacKenzie 1988). It is estimated that larvae can disperse up to several hundred kilometers from their location of hatch depending on environmental conditions (Incze and Naimie 2000, Xue et al. 2008, Quinn et al. 2017).

Biophysical models have been developed to predict dispersal of American lobster larvae for the Gulf of Maine (Harding and Trites 1988, Incze et al. 2006), the southern Gulf of St. Lawrence (Chassé and Miller 2010), and, most recently, the northern Gulf of St. Lawrence, the Scotian Shelf, and western Newfoundland (Quinn et al. 2017). Using physical oceanographic data such as water temperature, salinity, bathymetry, ocean circulation, and wind-

driven surface currents, these models predict the movement of larvae from their point of origin to their potential settlement location. The usefulness of these predictions hinges not only on accurate oceanographic inputs, but also upon biological information such as the location and quantity of larvae released, rates of larval development and survival, and how and when all of these parameters interact (Annis et al. 2007, Hudon et al. 1986). Another important biological input for such models is the timing of hatching, as this will affect the currents and water temperature experienced by larvae, which in turn will affect the direction, duration, and extent of their dispersal.

Several studies indicate that hatch time in American lobster is an important determinant of the dispersal of their larvae, and that this in turn affects settlement success. Larval release simulations made with a new dispersal model encompassing most of the lobster's range (Quinn et al. 2017) provided evidence that timing-of-hatch of lobsters is optimized to reduce drift time and, to a lesser extent, drift distance of larvae (Haarr 2018, *PhD thesis*). In particular, Haarr demonstrated that larval release (i.e. hatching) dates resulting in minimum predicted drift time fell within the estimated hatch period of lobsters in 19/22 hatching locations across the species' range in Canadian waters. Reduced drift time may increase settlement success. A recent study that simulated larval release in the Magdalen Islands, Quebec, found that larvae released later in the season, when temperatures were warmer, spent significantly less time in the water column, and dispersed over shorter distances, compared to larvae that were released earlier, and found that

settlement success correlated negatively with drift time (Gendron et al. 2018). Other studies, however, have suggested that late hatching larvae are less likely to successfully recruit to the bottom compared to those entering the water column earlier (Gendron and Ouelett 2009, Chassé and Miller 2010), indicating that optimal timing of hatch likely varies among release locations, and potentially from year to year.

Timing of lobster larval release is related to temperature, as their embryonic development is strongly temperature dependent (Templeman 1940). Perkins (1972) used the relationship between temperature and a proxy for development to create a linear temperature-dependent function of lobster embryonic development. Specifically, Perkins used the developing embryo eye size as a proxy for developmental status (hereafter the Perkins Eye Index, or PEI), and proposed that by pairing this development index with known eye-size-at-hatch, an embryo's timing-of-hatch could be predicted based on the temperatures it experiences. Perkins' method of using embryonic eye size as a proxy for development has since been widely used in studies investigating not only *H. americanus* (e.g. Helluy and Beltz 1991, Gendron and Ouelett 2009) and the related European lobster *H. gammarus* (e.g. Branford 1978, Charmantier and Mounet-Guillaume 1992), but also a number of other commercially harvested decapod crustaceans, such as the rock lobster *Jasus edwardsii* (Tong et al. 2000), snow crab *Chionoecetes opilio* (Moriyasu and Lanteigne 1998), and blue king crab *Paralithodes platypus* (Stevens 2006).

Since Perkins' (1972) study, a second function has been developed by Gendron and Ouellet (2009) to describe the relationship between temperature and development of American lobster embryos, as ascertained by eye size as a proxy for developmental status. Perkin's development function predicts a constant linear increase in rate of development related to increasing temperature:

$$y = -8.3151 + 2.6019(x)$$

Gendron and Ouellet's development function predicts an exponential response, where development rate is predicted to increase progressively faster with every degree increment in temperature:

$$PEIR = 0.000002 * t^{2.25008}$$

These two functions predict similar rates of development between 0 and ≈8°C, but Gendron and Ouellet's function predicts faster development than Perkin's function at temperatures above 8°C, and the difference between these functions' predictions increases markedly with additional increments in temperature (Appendix A). If development rate in PEI can be predicted based on temperature, and PEI at hatch can be estimated, then PEI at the sampling time in conjunction with water temperature can be used to estimate how many days until the predicted hatch PEI is reached. However, both functions were developed in laboratory settings and were not validated using samples from the field, where embryos of egg-bearing female lobsters may experience more variable environmental conditions.

Historically, *in situ* timing-of-hatch has been determined by sampling female lobsters whose eggs were in the process of hatching in the field (Campbell 1986), holding ovigerous lobsters in pounds (Templeman 1940), or by monitoring the presence of stage I larvae in the water column (Scarratt 1964, Annis et al. 2007). In Atlantic Canada, however, most LFAs prohibit lobster fishing activities during the late summer months, when the majority of hatch occurs, in order to protect reproductive females, avoid summer moulting, and avoid competition with peak US fisheries landings (Miller 1995). This regulation therefore eliminates the opportunity for scientists to collaborate with fishermen to collect this particular data, as fishermen are not on the water during the timeframe at which it can be collected. Organizing out-of-season sampling for the duration of the ~3 month hatching period (Templeman 1940) is possible, but can be both expensive and logistically challenging, especially if the goal is to assess hatch over large geographic areas. To address this problem, in a previous study (Miller et al. 2016) we conducted a field-based experiment that evaluated a method of predicting timing-of-hatch using (i) embryos collected from egg-bearing females sampled during the fishing season, (ii) embryonic temperature-dependent development functions, and (iii) temperature data. Using PEI and both Perkins' (1972) and Gendron and Ouellet's (2009) temperature-dependent development functions, we predicted timing-of-hatch in nature based on samples collected from Cheticamp, Nova Scotia, Canada, in 2012 (Miller et al. 2016). The objectives of that study were both to (i) validate the use of temperature-dependent development functions to predict timing of

hatch *in situ*, and (ii) assess this technique as a potential substitute for observation-based acquisition of timing-of-hatch data. The results of this study suggested that this method is an effective means of forecasting timing of hatch in nature, as the best model, which was based on Perkin's development function, did a good job at predicting the observed hatch period. More specifically, we were able to predict a range of hatch dates that encompassed 100% of the 50 day hatch period observed at the site, and of 500 hatch predictions, 480 fell within the 50 day hatching period (Miller et al. 2016). The results of this study also showed that we can do a fair job at predicting cumulative hatch within the hatching period, with the best model having a mean absolute deviation of 8% (<1%–23%) over 23 time-steps of the 50-day hatch period (Miller et al. 2016).

A second finding of our recent study in Cheticamp was that newly hatched lobsters (prezoae) collected from our sampling site had a markedly greater range of PEI-at-hatch values (460–611µm, $\bar{x}$=520 µm, n = 60) than reported in Perkins' (1972) study (~560 µm in diameter) and more recent studies (Helluy and Beltz 1991, Gendron and Ouellet 2009) (~550-570 µm in diameter). Incorporating this variability in PEI-at-hatch into our models increased the number of days of the hatch period that were successfully predicted by 24% compared to when a fixed PEI-at-hatch endpoint (PEI = 560 µm) was used (Miller et al. 2016). This finding suggests that the modeling of larval release timing in nature can be improved by the addition of more accurate PEI-at-hatch data.

This new approach of estimating timing-of-hatch *in situ* requires further field validation, in different locations and years. Although we were able to successfully predict timing-of-hatch for lobsters in Cheticamp, NS, in 2012, it is unknown how well these findings transfer to other sampling contexts, including whether Perkin's development function will always generate more accurate predictions than Gendron and Ouelett's development function. Cheticamp is a relatively warm-water region, and we do not know how well this method will hold across the species' thermal range, including areas where summer temperatures are much cooler. We also do not know how well this approach holds up to repeated tests in the same sampling region from year to year. Finally, embryos were collected no more than 2.5 weeks prior to the beginning of hatch, which would not be possible in LFAs where there is a greater disconnect between the timing of hatch and the closure of the fishing season (e.g., LFAs 33 and 34; DFO 2004, 2013), making it important to assess how close to hatch sampling must be conducted to generate sufficiently accurate hatch predictions.

Before marine biophysical models (e.g., Quinn et al. 2017) can be used to inform management and sustainable exploitation of lobsters, we need to know how accurate their predictions are. Whereas a model is often the end point of an ecological study, this should not be the case, and more attention needs be given to the transferability of such models over space and time (Pilkey and Pilkey-Jarvis 2007, Stow et al. 2009). One approach to validating a model's predictive ability is "cross-validation", where a model that has been derived

from only a subset of available data (a training set) is challenged to predict the behaviour of the remaining data (a test set). Whereas this approach provides some test of a model's predictive ability, the training and test sets of data are not independent of one another and validating a model in this way alone risks favourably biasing the assessment of the model's usefulness to that particular sampling scenario. A second and preferable method of model validation is to use the model to predict the behaviour of data that exist outside of the initial context under which the model was developed, such as different geographic areas or in different years.

Confirming whether this approach to modeling larval hatch is transferable across spatial domains and years is essential before it can be routinely used to feed large-scale models of larval dispersal. Thus, the objective of my thesis is to assess the spatial and temporal transferability of the models and approach used by Miller et al. (2016) in 2012 in Cheticamp, NS. In this study, I (i) repeated the same experiment at 2 additional locations in eastern Canada, which have different thermal regimes when lobster embryos are developing, and (ii) examined the impact of the timing of sampling by comparing the accuracy of hatch predictions made using embryos sampled early (beginning of the fishing season) versus those sampled late (near the closure of the fishing season). If the predictive ability of this method can be consistently demonstrated in different contexts (e.g., different locations, thermal regimes, and years), then we can be more confident in using it to feed biophysical models of larval dispersal.

Methods

This study comprised two main components, both conducted in the field in 2015. The first component involved sampling free roaming ovigerous females from two study locations, and the second involved monitoring caged females from one of these locations. In both cases, I predicted the timing of embryo hatch using the two temperature-dependent development functions described above and compared the predictions to observed hatch dates.

The first component of this study was conducted within ~10 km^2 on each of two fishing grounds, one in Bonaventure, Quebec, and the second in Dingwall, Nova Scotia (Fig. 1). Initial sampling of lobster embryos and prezoea at each location was done in conjunction with the local spring-summer lobster fishery, beginning shortly after the opening of the fishing season in May and continuing as late as possible prior to the closure of the season in July. Sampling of ovigerous females to determine timing-of-hatch began in conjunction with the fishery and continued after the fishing season, using chartered vessels, until hatching females were no longer observed in samples from the fishing ground. All ovigerous female lobsters were released where they were captured after data collection.

The second component of this study was conducted in Dingwall Harbour, less than 100 m from shore and 2 km from the Dingwall fishing ground. Twenty-four ovigerous females were captured early in the fishing season (May), caged (see below), and held until hatching was completed.

Embryos were collected from each female on each of seven occasions, separated by seven to eleven days, and the predicted dates of hatch of these embryos was compared to observed hatch dates of both caged and free-roaming females. The caged ovigerous lobsters were released into the Dingwall fishing grounds after the completion of the experiment.

Sea sampling

All lobster sampling occurred during the spring-summer of 2015. For each ovigerous female sampled, 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, and clutch condition was recorded as one of four developmental stages based primarily on the colour and appearance of the eggs in the clutch (MacKenzie et al. 2011). Eggs in a stage I clutch lack visible embryo eyes, are small, dark green to black in colour and are tightly packed on the female's abdomen. Eggs in a stage II clutch have visible embryo eyes and are dark-medium brown in colour. Eggs in a stage III clutch are light brown to orange in colour, comprise fully developed embryos, and are loosely packed. A stage IV clutch comprises eggs that are hatching and adhesive glue on the female's abdomen along with empty egg cases (MacKenzie et al. 2011). Using fine tweezers, ~50 embryos were haphazardly sampled from the clutches of up to 50 stage II or III females on each embryo sampling date. Females with stage II and III clutches were selected because these clutches would be hatching in the coming several

months, whereas females with stage I clutches would not hatch their eggs until the following year. Recently hatched embryos (henceforth prezoea) were collected opportunistically from stage IV females in order to determine the PEI-at-hatch for each sampling location.

In Bonaventure, 28 sampling trips were done by one vessel over a 17-week period, 16 during (May 7th–July 8th) and 12 after (July 14th–August 24th) the fishery. From May 7th to 28th one sampling trip was done every week, with a mean of 221 traps (90–235) being hauled per trip after a 24 h soak period. Up to a maximum of 150 ovigerous lobsters (42–150, $\bar{x}$=89) were used to assess the size (CL) and clutch stage of ovigerous lobsters in the fishing grounds each sampling day. From June 1st to July 8th sampling frequency was increased to every 3rd day, to increase the precision of the estimate for the beginning and ending of the hatch period, based on the presence of females with a stage IV clutch (see *Observing timing-of-hatch in the field*). I sampled embryos to use to make hatch predictions during four in-season trips, beginning on May 7th and concluding July 8th, and opportunistically sampled prezoea on July 8th. In total ~10,000 embryos from 200 females, and 133 prezoea from 22 females, were preserved in 20 ml vials containing a solution of 35:65 glycerol and ethanol (70%). Of the four embryo sampling dates in Bonaventure, three were determined to have occurred prior to the beginning of hatch. In order to observe the hatch period in Bonaventure, starting July 9th (i.e., after the closure of the fishery), and until August 24th, the number of traps was reduced to 18, the soak

period increased to 72 h, sampling continued to occur every 3rd day, and the clutch stage of every ovigerous lobster was recorded. Sampling was concluded after the hatch period had been determined to have ended based on the absence of more than one (0-1) stage IV females for three consecutive sampling days.

In Dingwall, 23 sampling trips were done alongside local fishers over a 20-week period, 8 trips during (May 21st–July 9th) and 15 after (July 18th–October 5th) the fishery. From May 21st to July 9th sampling trips were conducted every 1–14 days ($\bar{x}$=6.8), with a mean of 275 traps (4–424, $\bar{x}$=98.4) being hauled per trip after a 24 h soak period. Every ovigerous lobster captured on each trip was measured and assessed for clutch stage. Three in-season sampling trips between May 21st and July 2nd were used to sample embryos. In total ~7500 embryos from 150 females, and 115 prezoea from 21 females, were preserved in 20 ml vials containing a solution of 35:65 glycerol and ethanol (70%). Of the three embryo sampling dates in Dingwall, two were found to have occurred prior to the beginning of hatch. Starting July 18th (i.e., after the closure of the fishery), and until October 5th, sampling continued using a chartered vessel and a reduced number of traps (n= 6), but with the same soak period (24 h). Post-season sampling frequency was typically twice a week (occurring every 2–7 days, $\bar{x}$=3.5) until the end of August, to increase the precision of the estimate for the beginning and ending of the hatch period based on the presence of females with stage IV clutches. High intensity sampling was concluded on August 29th based on logistical considerations, although there

were two additional sampling days in September and in October to confirm the absence of stage IV females.

Water temperature

Temperature recording data loggers (HOBO Pro V U22-00) were deployed on the lobster fishing grounds in Bonaventure (n=3) and Dingwall (n=2) (Fig. 1), with each logger attached to a stationary mooring <1 m above the bottom and recording temperature every 30 minutes. In Bonaventure, loggers were deployed on May 7th at depths varying from 5.9 m–11.3 m; Logger 1 was moored at 5.9 m and was lost, Logger 2 was moored at 8.3 m and was retrieved on December 14th, and Logger 3 was moored at 11.3 m and was lost due to a boat cutting the buoy line. In Dingwall, loggers were deployed on May 21st at 14 and 16.5 m depth; one logger was not recovered due to a lost buoy, and the identity (i.e., depth) of the recovered logger is uncertain due to a miscommunication among technicians. The daily mean temperature on each fishing ground was determined by taking the mean of the 48 daily recordings, and in the case of Bonaventure averaging the values obtained for the two recovered loggers.

Observing timing-of-hatch in the field

Timing-of-hatch in the field was observed on the basis of the occurrence of ovigerous females with stage IV clutches (i.e., with hatching embryos) in the catches. The start of the "observed hatch period" was estimated to be the trip when the first female with a stage IV clutch was captured, and its end to be the

last trip that a female with a stage IV clutch was encountered. The "maximum possible hatch period" was taken as the sampling day immediately preceding the first day when a female with a stage IV clutch was captured, plus one day, to the day following the last sampling day when a female with a stage IV clutch was captured, minus one day.

The progression of hatch during the hatch period was estimated based on the percentage of ovigerous females that were observed to have stage IV clutches on each sampling date. To avoid potential bias resulting from sampling days with low catch values, sampling dates with less than 10 ovigerous females were pooled together with the next date, or dates, until n of at least 10 was reached. This resulted in three pooled sampling dates in Bonaventure (August 12th – August 21st) and three pooled sampling dates for the Dingwall fishing ground (August 24th – September 19th). Although this approach does not account for the temporal shift in fishing effort that occurred between the in-season and out-of-season sampling, it produces similar estimates of hatch per sampling date (r=0.67 and r=0.98 for Bonaventure and Dingwall, respectively) and cumulative hatch (r=0.98 and r=0.99 for Bonaventure and Dingwall, respectively) when compared to estimates based on catch per unit effort of stage IV females, reduces potential error caused by changes in berried female catchability over time, and both methods identified the same best models.

Embryo measurements and predicting timing-of-hatch

A microscope (Leica MZ12s) at 40x and a digital camera (Nikon CoolPix 4500) were used to capture an image of the polar view (Appendix B) of the left and right eye of each of 10 embryos haphazardly selected from each female's egg sample. The degree of development of each embryo was estimated with Image J software (Schneider et al. 2012) based on the average size of its left and right Perkins Eye Index (PEI), which is the mean of the greatest width and length of an embryo's oval-shaped eye (Perkins 1972).

$$PEI = (embryo\ eye\ height + embryo\ eye\ width)/2$$

Embryonic PEI was then used in conjunction with mean daily bottom temperature and both Perkins' (1972) linear

$$y = -8.3151 + 2.6019(x)$$

and Gendron and Ouellet's (2009) logarithmic

$$PEIR = 0.000002 * t^{2.25008}$$

functions of embryonic development to estimate daily embryonic development for each embryo. In Perkin's linear function *y* is embryonic development rate expressed as change in PEI in microns per week, and *x* is water temperature. In Gendron and Ouellet's logarithmic function *PEIR* is the change in PEI in mm per day, and *x* is water temperature. For values estimated with these two functions to be expressed in the same units (microns/day), I divided the output of the linear function by 7 and multiplied the logarithmic

function's output by 1000. Daily estimates of development were added to the previous day's PEI, beginning with the initial PEI measurement and progressing until reaching the individual's assigned PEI-at-hatch (randomly selected from the PEI-at-hatch values of prezoea collected from the same sampling location), resulting in both a linear and a logarithmic estimate of timing-of-hatch for each embryo. I excluded as statistical outliers values exceeding 1.5 times the interquartile range above the third quartile or below the first quartile in PEI-at-hatch values (3.8% of prezoea from Bonaventure and 0% from Dingwall). If the PEI-at-hatch assigned to any embryo used to make a hatch prediction was less than the PEI of that embryo, the timing-of-hatch of the embryo was back-calculated to determine when the embryo would have had that eye-size, based on the temperature data for the site, and this date was used as timing-of-hatch for the embryo. This only occurred in 16 of the 3984 (0.04%) egg samples used to predict hatch in this study. In total 1482 embryos collected on 3 sampling dates from Bonaventure ($\bar{x}$=485 each date), and 982 embryos collected on 2 sampling dates in Dingwall ($\bar{x}$=491) were used to make hatch predictions for free roaming females.

Caged females

Twenty-four ovigerous lobsters (CL: 82.4–100.4 mm; $\bar{x}$=87.2 mm) with stage II clutches were collected from the Dingwall fishing ground between May 21st–28th and placed inside a custom-built cage that was submerged in Dingwall Harbour (Fig. 1) at a depth of 6.4 m. The cage was made of lobster trap wiring

and corrugated plastic (91.5 cm x 43 cm x 61cm), with individual compartments (9 cm x 10 cm x 53.3 cm) for up to 24 lobsters (Appendix C). The compartments were designed to allow water to flow into the cage. A temperature logger (HOBO Pro V U22-00) was zip tied to the cage and the mean of the logger's 48 daily recordings (once every 30 minutes) was used as an index of daily water temperature experienced by the lobsters.

The cage was hauled once per week to assess each lobster for clutch stage and take a small sample (~20–50) of embryos from each clutch, which was preserved in a 35:65 glycerol: ethanol (70%) solution. Two of the 24 females were not used to assess development and hatching; embryos on one female did not hatch during the study period and one female died prior to the beginning of hatch. In total, 1520 embryos collected from 22 females (60–73 per female, $\bar{x}$=69.1) and across 7 sampling dates (200–220 per date, $\bar{x}$=217.1) were processed. Once hatching had begun, 95 prezoea from 15 females (1–10 per female, $\bar{x}$=6.3) over 5 sampling dates (10–33 per date, $\bar{x}$=19) were collected. A fifth clutch stage ("Clean") was used to denote that the lobster had completely finished hatching, and no embryos remained on the abdomen. Due to inclement weather, there was an interval of 11 days between June 30th and August 11th where sampling did not occur. As with sea-sampling, observed hatch period of each female was expressed both as the days that the female was observed to have a stage IV clutch (i.e., the "observed hatch period"), and more liberally as the day(s) where hatching was observed +/- the number days where

the female was not observed (i.e., the "maximum hatch period"). The lobsters were fed bait fish ~once per week, coinciding with cage haul-out days.

Statistical analysis

Water temperature

To explore whether water temperature contributed to differences in model performance at the two main study sites, as well as between free roaming and caged females at Dingwall, I used t-tests to compare the temperature embryos experienced at these "site pairs" over each of three time intervals: (i) female sampling period, (ii) before hatching, and (iii) during hatching. Mean temperatures per calendar date were used as replicates, and in all site-pair comparisons the number of days of temperature data used was made to be the same for the two sites. For temperature over the female sampling period, the same calendar dates were used for the two sites of a particular comparison; the first date used was the first sampling date of the site where sampling started the latest, and the last date used was the last sampling date of the site where sampling ended first. For comparisons of temperature before and during hatching, the selection of days to be compared was not based on calendar date, but on the timing of hatching (observations of females with stage IV clutches); comparison of temperature before hatching was based on the number of days (when temperature data were available) preceding hatching at the site from which this number was smallest, and comparison of temperature during hatching was based on the number of days of hatching at the site where the

hatch period was shortest, and beginning on the day when hatching was first observed at each site. Temperature over these three time-intervals was also compared between the pilot study in Cheticamp, NS, in 2012, and each of my two main study sites.

In addition to the above comparisons of mean temperatures values, I also compared the number of days embryos experienced temperatures at or above 9°C, as the two development functions predict similar development rates below this temperature but increasingly different rates above it (logarithmic > linear) (Appendix A). The number of days that developing embryos experienced water temperatures above 9°C could have affected which function better predicts hatch at the different study sites.

Predicted vs observed hatch

Predicted timing-of-hatch of individual embryos was compared to observed timing-of-hatch of ovigerous females (i.e., with stage IV clutches) at each study location in order to contrast the predictive accuracy of six models for Bonaventure and four models for Dingwall, which were based on (i) a particular date when embryo samples were obtained (three dates at Bonaventure and two at Dingwall), (ii) embryo development estimates based on the linear or logarithmic development function, and (iii) a randomly assigned site-specific PEI-at-hatch for each embryo. This model assessment exercise was based on 3 key metrics: (i) the difference between the observed and predicted mean date of hatching:

$$Mean\ hatch = \frac{Summed\ observations\ of\ hatch\ dates\ (day\ of\ year)}{Total\ observations\ of\ hatching}$$

(ii) overlap in the observed and predicted hatch periods:

$$Hatch\ period\ overlap$$
$$= (\%\ of\ range\ of\ observed\ dates\ predicted)$$
$$- (\%\ of\ dates\ predicted\ that\ fell\ outside\ the\ observed\ range)$$

and (iii) the difference between the observed and predicted progression of hatch over time. Metric (iii) was investigated in two ways, with both approaches being based on the same number of time-steps for observed and predicted values of hatch during the hatch period (11 in Bonaventure, 16 in Dingwall, 6 in Dingwall Harbour (i.e., caged females)), but differing with respect to the inferred fit between observed and predicted values. First, I evaluated the correlation between observed proportion of ovigerous females with stage IV clutches and the number of embryos predicted to hatch in each time-step, with the best model considered to be the one that had the highest positive *r* value. Second, I made a similar comparison, but this time based on the cumulative predicted and observed percentage of hatch at each time-step. For this analysis, residual sum of squares (RSS) were used to determine which model deviated the least from what was observed.

$$RSS = \sum(observed\ cumulative\ hatch - \ predicted\ cumulative\ hatch)^2$$

Although the choice of best model could have been based on RSS alone, given models compared always involved the same sample size (number of time-steps) and number of factors, I computed AICc values (Akaike's information criterion corrected for small sample size) to estimate the relative explanatory power of each model (i.e., AICc weights) (Anderson 2008).

As the objective of this study is to determine under what contexts development models can be used to predict hatch based on samples collected within the fishing season, and prior to the beginning of hatch, I did not assess predictions made using samples collected after the beginning of hatch, meaning samples collected on July 2nd in Dingwall, on July 8th in Bonaventure, and after July 10th for the caged females in Dingwall Harbour.

Statistical analysis of caged female data

Comparison of observed and predicted timing-of-hatch for the caged females followed the same general approach as for free roaming females, with a few distinctions. Most importantly, comparisons between observed and predicted values were made at the level of individual females, in addition to the sampled population of females. Predictions for a single female used all embryos from that female collected across all sampling dates due to low number of embryos processed for each date per female (n=8–11, $\bar{x}$=9.6). Predictions for

the whole population of caged females were based on all embryos from all females pooled, irrespective of sampling date. In order to assess the accuracy of hatch predictions based on using embryos collected at different times, I additionally pooled embryos by sampling date and compared the cumulative trajectory of hatch predictions made by embryos sampled on each date with pooled observed cumulative hatch.

Given that the same females were repeatedly sampled in the Dingwall caging experiment, I was also able to compare observed and predicted development of embryos (i.e., in addition to hatch), based on the two embryo development functions. I used the mean PEI value of all embryos collected on week 1 to predict the mean PEI of the same embryos on weeks 2 through 7, using each development function, and then compared these predictions with the observed mean PEI values of new embryos from the same clutches. This was used to estimate RSS by comparing each observed value to the mean predicted value for each female on each date. Finally, I used variance component analysis (VCA) to evaluate the contribution of intra-brood variability, inter-brood variability, and sampling date to variability in embryo PEI.

Inter-site differences in PEI-at-hatch were evaluated by comparing the average PEI-at-hatch for each female using a non-parametric Kruskal-Wallis test.

Results

Bonaventure

Observed timing-of-hatch

The majority of clutches at the Bonaventure study site were stage II in May samples, at the beginning of sampling, and the incidence of stage III and IV clutches increased through June, July, and August (Fig. 2). From mid-July to the end of sampling in August the incidence of newly spawned stage I clutches (to be hatched the following year) increased (Fig. 2). The hatching period in Bonaventure was estimated to be between 48 and 54 days. Ovigerous females with hatching (stage IV) clutches were observed on 92% of sampling trips, beginning on July 5th and ending on August 21st, 2015, for a minimum 48-day hatching period (Fig. 2). Based on the intervals of time between observed hatch (July 5th–August 21st) and the sampling dates before and after hatching was observed, the maximum estimated hatch period for Bonaventure was 54 days (July 1st–August 23rd). The mean observed date of hatch was July 17th. The proportion of eggs hatched between sampling events stayed relatively constant, only showing a modest peak around days ≈1–4 (i.e., calculation time-steps 1–2) (Fig. 3).

Predicted timing-of-hatch

Predictions made using the logarithmic function and samples collected 59 days prior to hatch had a mean hatch date closest to the mean observed hatch

date (predicted: July 20th; observed: July 17th), and the mean hatch dates predicted using the logarithmic function were closer to observed mean hatch than predictions made using the linear function (Table 1). The mean hatch dates based on hatch predictions for each of the three sampling dates for Bonaventure were July 20th, July 22nd, and July 24th for the logarithmic function and August 8th, August 10th, and August 14th, for the linear function (Table 1).

Using samples obtained 20 days prior to the observed beginning of hatch, hatch in Bonaventure was forecast 16–152 and 11–80 days in the future with the linear and logarithmic functions, respectively. Using samples obtained 41 days prior to observed hatch, these numbers change to 0–147 and 0–94 days, and using samples obtained 59 days prior to observed hatch they change to 35–174 and 31–113 days. Predictions made with samples obtained 20, 41 and 59 days prior to the beginning of observed hatch were extremely similar. For example, the correlation of the cumulative hatch predicted by samples collected 20 and 59 days prior to observed hatching was r=0.99 for the linear function and r=0.98for the logarithmic function, while that of the raw hatch predictions for the two sampling dates were r=0.92 and 0.78 when the linear and logarithmic development functions were used, respectively (Fig. 4).

For Bonaventure, all model-sample date combinations predicted the entirety (100%) of the observed hatch period, and most predicted hatch dates of individual embryos fell within the observed 48-day hatch period: 78%, 68%, and 71% for the linear function, and 90%, 91%, and 95% for the logarithmic function, based on eggs sampled 59, 41, and 20 days prior to hatch, respectively

(Fig. 5) (Table 1). Consequently, the “hatch period overlap”, which was defined as the percentage of the range of observed dates that was predicted minus the percentage of dates predicted that fell outside this observed range, was less for the linear (78%, 68%, and 71%) than the logarithmic (90%, 91%, and 95%) function. This was also the case when the maximum 54-day hatch period was considered, in which case the hatch period overlap increased slightly for both the linear (84%, 71%, and 77% for 59, 41, and 20 days prior to hatch respectively) and logarithmic (95%, 98%, and 98%) function.

At the Bonaventure study site, models using the logarithmic function of embryo development always outperformed models using the linear function at predicting the progression of hatch during the observed hatch period. For each of the three sampling dates, the logarithmic function outperformed (lower AICc) the linear function, and overall the three models involving the logarithmic function carried 99% of the AICc weights, compared to <1% for those involving the linear function (Fig 3) (Table 1). The best model was the logarithmic development function paired with embryos collected 59-days prior to observed hatch, which had 70% of AICc weight (Fig. 3) (Table 1). The other two models involving logarithmic development functions, based on samples obtained 41 and 20 days before observed hatch, had higher AICc scores and lower AICc weights (Table 1).

The best model (logarithmic function paired with embryos collected 59 days prior to observed hatch) had a mean deviation of 7% between predicted and observed cumulative frequency of hatch over the 11 sampling time-steps,

with the single greatest deviation being 15% at 13 days into the hatch period, when the model predicted 33% of hatching to be completed while 48% had been observed (Fig. 3). In contrast, the linear models predicted a much slower progression of hatch and greater deviations from observed values (Fig. 3). For example, using the best linear model, which was also based on embryos collected 59 days prior to observed hatch, the mean deviation between predicted and observed cumulative frequency of hatch was 36% (compared to 7% for logarithmic model) (Table 1), with the single greatest deviation being 56% (versus 15%), observed at 23 days into the hatch period, when the model predicted only 20% of hatching to be completed when 76% had been observed (Fig. 3).

None of the models were able to accurately predict the proportion of hatching females at individual time-steps within the hatch period, but those based on linear functions of embryo development seemed particularly bad at doing so (Fig. 6). Whereas these two variables were not related when using the three logarithmic models (r=-0.11 to 0.12; all $P > 0.55$), they were significantly negatively related with the three linear models (r=-0.70 to -0.62; all $P < 0.01$), and hence predicted greater hatch on dates when the proportion of hatching females was lesser (Table 1).

Dingwall

Observed timing-of-hatch

The sampling of ovigerous females in the Dingwall fishing ground reflected expected seasonal clutch development, with the majority of clutches being stage II in May samples, at the beginning of sampling, and the incidence of stage III and IV clutches increasing in turn through the months of June, July and August (Fig. 2). From late-August to the end of sampling in October the incidence of newly spawned stage I clutches (to be hatched the following year) increased. The hatching period in Dingwall was estimated to be between 94 and 114 days. Ovigerous females with hatching (stage IV) clutches were observed at this site from June 18th to September 19th, for an estimated 94-day "observed" hatching period (Fig. 2). Based on the intervals of time between observed hatch (June 18th –September 19th) and the sampling dates before and after hatching was observed, the "maximum estimated hatch period" for Dingwall was 114 days (June 13th–October 4th). Hatching was observed on the majority (89%) of sampling dates during the observed hatch period; however, only one stage IV female was observed on June 18th (representing less than 1% of the observed catch of ovigerous females on this date), and then hatching was not observed again until July 9th. The observed mean date of hatch was August 3rd (Table 1). The observed incidence of females with hatching clutches was close to constant over the hatch period, although there was a small peak from days ≈40–43 (i.e., calculation time-steps 8–9) (Fig. 3).

Predicted timing-of-hatch

For the Dingwall site, predictions made using the linear function and samples collected 6 days prior to hatch had a mean hatch date closest to the mean observed hatch date (predicted: August 2nd; observed August 3th), and the mean hatch dates predicted using the linear function were closer to observed mean hatch than predictions made using the logarithmic function (Table 1). The mean hatch dates based on hatch predictions for each of the two sampling dates for Dingwall were July 19th and July 25th for the logarithmic function and August 2nd and August 12th for the linear function (Table 1).

Using samples obtained 6 days prior to the beginning of observed hatch, hatch in Dingwall was forecast 14–126 and 12–74 in the future with the linear and logarithmic functions, respectively. Using samples obtained 28 days prior to observed hatch, these numbers change to 36–135 and 33–92 days. Predictions made with samples obtained 6 and 28 days before the beginning of observed hatch were similar, with a correlation cumulative hatch predicted by the two sampling dates of r=0.93 for the linear function and r=0.94 for the logarithmic functions. Correlations of the raw hatch predictions of the two sampling dates of r=0.64 and 0.66 for the linear and logarithmic functions, respectively (Fig. 4). However, the predictions made using the first samples suggested fewer embryos hatching earlier during the hatch period compared to the predictions made using samples collected later (Fig. 4).

For Dingwall, most predicted hatch dates of individual embryos fell within the observed 94-day hatch period: 99% and 95% for the linear function,

and 100% for the logarithmic function, based on eggs sampled 28 and 6 days prior to hatch, respectively (Fig. 5) (Table 1). However, the linear function predicted the more of the observed hatch period (90% and 91%) than the logarithmic function (64% and 67%), and therefore the hatch period overlap was greater for the linear function (89% and 86%) than the logarithmic function (64% and 67%) (Fig. 5) (Table 1). This was also the case when the maximum 114-day hatch period was considered, and in this case the hatch period overlap changes slightly for both the linear (87% for both 28 and 6 days prior to hatch respectively) and decreases for the logarithmic function (52% and 54%).

The best model at predicting hatching over time in Dingwall was the linear development function paired with embryos collected 6 days prior to hatch, which had 99% of AICc weight (Table 1). This model's mean deviation between predicted and observed cumulative frequency of hatch across 16 sampling time-steps was only 4%, and the greatest deviation was 14% (55 days into the 94-day hatch period) when the model predicted 83% of hatching to be completed when only 69% had been observed (Fig. 3) (Table 1). In contrast, the best logarithmic model's mean deviation was 16% and the maximum deviation was 41% (46 days into the hatch period) when the model predicted 89% of hatching to be completed when only 50% had been observed (Fig. 3) (Table 1). Predictions made by the logarithmic and linear functions paired with embryos collected 28 days prior to hatch were similar in their amount of deviation from the observed, although they predicted different patterns of hatching, with the logarithmic function consistently predicting hatching to occur more quickly

than what was observed, and the linear model predicting hatching to occur more slowly than was observed (Fig. 3) (Table 1).

When predicted hatch was compared to the proportion of hatching females on each sampling date, the performance of the different models was similar to what was observed in the AICc model selection of predicted vs observed cumulative hatching. The best model (linear function with samples 6 days prior to hatch) had predictions that correlated positively and most strongly with the observed the proportion of stage IV females over time (r=0.53, P=<0.01) (Fig. 7) (Table 1). The second-best models (linear and logarithmic with samples 28 days prior to hatch) made predictions that also correlated positively and significantly with hatch (linear: r=0.42, P=0.05; logarithmic: r=0.44, P <0.05), whereas the lowest ranking model (logarithmic with samples 6 days prior to hatch) did not (r =0.01, p=0.95) (Fig. 7).

Caged females in Dingwall Harbour

Over the course of the study period one ovigerous female perished due to the barriers of the cage failing to protect it from conspecifics, and a second female had undeveloped eggs and had not started hatching when the study was ended, although went on to hatch later. The 22 remaining ovigerous females progressed from stage II clutches (intermediate development) through to clean clutches (completely hatched) (Fig. 8).

Development of embryos

The amount of embryo development (based on increment increase of PEI per clutch per time-step) over the course of the experiment varied markedly (from 12.3–210.8 µm) among the clutches of embryos (22 females, 60–73 total embryos/female, 9–11 embryos per female/time-step). The two development functions were similarly successful at predicting the development of these embryos, with mean PEI values modeled with the logarithmic and linear functions only deviating from the mean observed values at different time-steps by 0–6% and 0–7%, respectively. Neither function consistently outperformed the other at predicting PEI at different time-steps (Fig. 9); however, by the final time-step the RSS was slightly less when the linear function was used (Fig. 9) (Table 2). Variance component analysis indicated that 53% of variability in embryonic PEI was attributable to sampling date, 40% to intra-brood variability, and only 7% to variability among females.

Observed timing-of-hatch

The hatching period for the caged females in Dingwall Harbour was estimated to be between 36 and 48 days. Ovigerous females with hatching (stage IV) clutches were observed on each sampling date between July 10th and August 14th for an observed hatch period of 36 days and a maximum estimated hatch period of 48 days (July 3rd–August 19th). Each of the 22 females' clutches were classed as clean on August 20th, meaning that hatching was completely finished by this time (Fig. 8). The observed mean hatch date was July 27th

(Table 1). The proportion of eggs hatched between sampling events stayed relatively constant (Fig. 10).

Predicted versus observed timing-of-hatch

Predictions made using the logarithmic function and pooled-female samples collected 0, 7, 21, and 28 days prior to hatch all had a mean hatch date closest to the mean observed hatch date (predicted: July 25th observed July 27th) and the mean hatch dates predicted using the logarithmic function were closer to observed mean hatch than predictions made using the linear function (Table 1). The range of mean hatch dates based on these two sets of predictions were July 21st–July 25th for the logarithmic function and August 4th–August 12th for the linear function (Table 1).

When all embryos of all females are pooled (60–73 [$\bar{x}$=69.1] embryos for each of the 22 females), the linear and the logarithmic function predicted 100% and 92% of the 36-day observed hatch period, respectively. However, only 67% of hatch predictions made by the linear function fell within the observed hatch period, compared to 98% of the logarithmic function predictions. Consequently, the logarithmic function had a greater hatch period overlap with a value of 90% compared to 67% for the linear function (Fig. 11) (Table 1). When the maximum 48-day hatch period was used, the linear function's hatch period overlap increased to 85%, while the logarithmic function's hatch period overlap was reduced to 82%.

Success at predicting the hatch period of individual females caged in Dingwall Harbour was low compared to the ability to predict the hatch period of the population of caged females (Fig. 11), as well as compared to the population of females roaming at-large in the Dingwall fishing ground (Fig. 5). The caged female's hatch periods ranged from an observed 1–28 days to a maximum of 7–40 days for different females, with a mean of 6.8 (observed hatch period) and 20.8 (maximum hatch period). These observed and maximum estimates of the hatch period were significantly shorter than those predicted using the linear ($\bar{x}$=48.0 [31-78]; versus observed: 6.8: t=-13.2, df=21, P<0.0001; 20.8: t=-7.6, df=21, P<0.0001) and logarithmic ($\bar{x}$=28.6 [21-41]; versus observed; 6.8: t=-11.2, df=21, P=<0.0001;versus maximum: 20.8: t=-3.3, df=21, P=0.003) functions of embryo development (Fig. 11).

Of the 22 females that hatched during the experiment, three hatched all their embryos between two sampling dates, and hence did not have an "observed" hatch period. These females could therefore not be used to estimate the percentage of hatch predictions that fell within the "observed" hatch period (i.e., n=19 females for this endpoint). For two of these females I was nevertheless able to conclude that 100% of the observed hatch period was covered by the hatch predictions, because these predictions spanned the two dates between which hatch occurred (i.e., n=21 females for this endpoint).

Importantly, prediction success decreased markedly when considering individual females, although the logarithmic function still performed somewhat better than the linear function. Using the linear function, hatch predictions only

fell within the observed hatch period of individual females on average 12% of the time (0–67%, n=19), while predicting on average 96% of a female's observed hatch period (38–100%, n=21). The logarithmic function performed somewhat better, predicting hatch dates that fell within the observed hatch period on average 31% of the time (0–90%, n=19), and predicting on average 87% (0–100%, n=21) of a female's observed hatch period. The mean hatch period overlap was only 8% for the linear model and 18% for the logarithmic model. When the maximum hatch period was considered, predictions fell within it on average 42% (1–90%, n=22) of the time for the linear function and 59% (10–100%, n=22) of the time for the logarithmic function, while the percentage of the range of hatch dates predicted fell slightly to an average of 89% (43–100%, n=22) for the linear function and 83% (45–100%, n=22) for the logarithmic function. The mean hatch period overlap when the maximum hatch period was considered was 31% and 42% for the linear and logarithmic functions, respectively.

Overall, models using the logarithmic development function predicted cumulative hatch with more accuracy than those that used the linear function, with a combined AICc weight of 97% compared to 3% for the linear models (Table 1). Of the 16 function-date combinations compared, the logarithmic model applied to all embryo samples pooled best predicted cumulative hatch throughout the 6-step observed hatch period (AICc weight = 0.17) (Fig 10) (Table 1). This model's mean prediction error was 7%, and the single greatest deviation was 14% at 9 days into the hatch period, when the model predicted

17% of hatching to be completed when ~32% had been observed (Fig. 10). This function-pooled date combination was found to be statistically similar based on AICc delta all other logarithmic models (AICc Δ=0.008–1.63) (Table 1). The linear models did not perform as well, and consistently predicted hatching to occur more slowly than was observed. The mean prediction error for the best linear model was 25%, and the single greatest deviation was 48% at 20 days into the hatch period, when the model predicted 30% of hatching to be completed when 78% had been observed (Fig. 10). The linear predictions were almost all similarly accurate, including the model that used all sampling dates combined (AICc Δ=1.34–2.41, $\bar{x}$= Δ 1.78 from the best linear model) (Table 1).

The pooled predictions made using the logarithmic function showed a strong, significant correlation with the proportion of hatching females at a given time-step (r=0.82, P=0.001) (Fig. 12). Conversely, the pooled predictions made using the linear function produced a negative, insignificant correlation with observed hatching over time (r=-0.22, P=0.50).

Water temperature

Water temperature at the study site in Bonaventure, QC, was 0.9°C when sampling started on May 7th, then it increased relatively gradually to a maximum of 21.0°C on August 26th (Fig. 13). The temperature on the first day of observed hatch was 8.0°C on July 5th, and was 15.2°C on August 21st, which was the last day of observed hatch at the study site (Fig. 13). Water temperature at the study site in the Dingwall fishing ground was 3.6°C when sampling

started on May 21st, and then increased to a maximum of 19.6°C on August 24th (Fig. 13). The temperature on the first day of observed hatch was 8.1°C on June 18th, and was 17.6°C on September 19th, which is the last day of observed hatch at the study site (Fig. 13). Water temperature was 3.8°C when I caged ovigerous females in Dingwall Harbour on May 21st (Fig. 14). It then increased to a maximum of 19.8°C on August 24th. Water temperature was 14.1°C on August 14th, which was the last day hatching was observed at this site.

Spring and summer temperatures differed between Bonaventure and Dingwall fishing grounds, although the amplitude and direction of these differences varied depending on the time periods compared (see Methods) (Fig. 13). When comparisons were based on temperatures recorded on all calendar dates when sampling for ovigerous females occurred at both study sites (May 21st–August 24th), the daily temperatures in Bonaventure and Dingwall were nearly identical, averaging 10.8°C ($t=-0.06$, $df=190$ $P=0.95$). However, mean daily temperature was significantly warmer in Bonaventure than in Dingwall when comparisons were based not on calendar days but on temperatures recorded prior to the onset of observed hatch at either site (9.7°C versus 4.9°C; $t= -9.91$, $df=54$, $P<0.0001$) or during the observed hatching periods at both sites (12.8°C versus 11.8°C; $t= 2.00$, $df=94$, $P=0.04$) (Fig. 13)

Spring-summer temperatures also differed between this study and the recent study in Cheticamp (Miller et al. 2016). Mean temperature during the ovigerous female sampling period (June 1st –August 15th) was significantly greater in Cheticamp (13.1°C) than in Dingwall (11.0°C; $t=2.59$, $df=150$,

P=0.01) and Bonaventure (11.2°C; t=2.54, df=150, P=0.01), as was mean temperature during the observed hatch period (Cheticamp [17.5°C] versus Dingwall [12.0°C]: t=10.5, df=98, P<0.0001; Cheticamp versus Bonaventure [12.8°C]; t=9.96, df=94, P<0.0001). Mean temperature prior to observed hatch was also greater in Cheticamp (6.8°C) than in Dingwall (5.0°C; t=-2.59, df=54, P=0.01), but was significantly cooler than Bonaventure (9.6°C; t=3.91, df=58, P<0.001). Embryos from the three fishing grounds also experienced a different number of days > 9°C in the 28 days preceding observed hatch (number of days preceding hatching at the site from which this number of days with temperature data was smallest), with 19 days in Bonaventure, 10 in Cheticamp, and 0 in Dingwall.

The onset of the observed hatch period in Dingwall was based on the observation of a single hatching female in on June 18th, after which hatching was not observed again until July 9th. When temperature was compared among sites assuming July 9th as the day of hatch onset in Dingwall, instead of June 18th, only two differences in temperature between sites pairs changed; mean daily temperature during the observed hatch period became greater in Dingwall (14.9°C) than Bonaventure (12.8°C; t=3.76, df=94, P<0.001), and pre-hatch temperature became greater in Dingwall (8.9°C) than Cheticamp (6.7°C; t=-3.00, df=58, P=0.003). Also, using July 9th as the day of hatch onset in Dingwall, the number of days >9°C becomes 19 days in Bonaventure, 10 in Cheticamp, and 18 in Dingwall.

Water temperature in the Dingwall fishing ground vs Dingwall Harbor

Water temperature during the ovigerous female sampling period (overlapping calendar days) was very similar in Dingwall Harbour (10.5°C) and Dingwall fishing ground (10.7°C; T-test, df=182, t=0.44, P=0.66), but temperature in the days prior to hatch (t=-10.8, df=54, P<0.0001) and during hatch the hatch period (t=5.1, df=70, P<0.0001) were both greater in Dingwall Harbour (9.6°C and 14.0°C, respectively) than in Dingwall fishing ground (4.9°C and 10.9°C, respectively) (Fig. 14). The ovigerous females held in Dingwall Harbour experienced 18 days of temperature at or above 9°C prior to the beginning of hatch, while females in the fishing ground experienced 0.

Eye-size at hatch

As there was an unbalanced number of samples (n=1–43) representing each female from each sampling location, the mean PEI-at-hatch values for each female (excluding females with only one PEI-at-hatch value as a mean could not be calculated) were used for inter-site PEI-at-hatch comparisons.

Eye-size at hatch varied significantly among sampling locations (Fig. 15; Chi square = 9.76, P <0.05, df = 3). Pairwise comparisons using a Mann-Whitney-Wilcoxon test showed that prezoea from Cheticamp having smaller PEIs at hatch than prezoea Dingwall Harbour (P <0.01); all other site pairs (including Cheticamp; Miller et al. 2016) did not differ significantly from one another.

Discussion

I found that hatch models based on temperature-dependent functions of embryo development and estimates of embryo development status at hatch can be used, in conjunction with temperature data and embryos sampled up to ≈8 weeks prior to hatch, to predict the timing and duration of hatch of American lobster (*Homarus americanus*) in nature with useful accuracy. For example, the best hatch model found in each of my two study locations predicted the mean hatch date within 1–3 days of what was observed at each location. Sampling up to several weeks prior to hatch had no consistent effect on the accuracy of these predictions, which indicates the possibility of collaborating with lobster fishermen from any Lobster Fishing Area in Canada during the spring fishing season to obtain samples needed to make these predictions. One major limitation, however, is that the two development functions considered in my study performed differently at my two study locations, and I was unable to determine the cause of these differences. Temperature and geographic origin of the lobsters may have played a role in these differences in function performance, and future studies should be conducted to explain this outcome to allow general application of the approach.

The caged-female portion of this thesis demonstrated that these models' ability to predict timing-of-hatch at the "population level" does not result from accurately predicting timing of hatch of individual females, but rather reflects the ability to correctly predict the average behaviour of embryos within the population. For example, embryo hatch predictions had a hatch period overlap

of 91% observed for the population of caged females, this value decreases to 18% when considering hatch predictions and observations for embryos of individual females.

Which model works best?

My assessment of the usefulness of different hatch models was based on 3 key endpoints: (i) the ability of the models to accurately predict the mean date of hatching observed in nature, (ii) overlap in the observed and predicted hatch periods, and (iii) how well the predicted progression of hatch matches the observed temporal distribution of hatching females in nature. Using samples from Dingwall, NS, the best model predicted a mean hatch date of August 2nd, compared to an observed mean hatch date of August 3rd *in situ*. This model predicted 91% of the days of the 94-day observed hatch period, 95% of its 480 predictions fell within this timeframe, and it had a hatch period overlap of 86%. This model also did a good job of predicting the cumulative percentage of hatch over 16 sampling time-steps, with a mean absolute deviation from what was observed of only 4% (maximum 14%) across time-steps, and its predictions correlated significantly with the observed proportion of stage IV females at different time-steps within the hatch period. Similarly, the best model for samples from Bonaventure, QC, predicted a mean hatch date of July 20th, compared to the observed mean hatch date of July 17th. This model predicted 100% of the 48-day observed hatch period and 90% of its 500 predictions fell within this timeframe, and therefor had a hatch period overlap of 90%. This

model's predictions had a mean absolute deviation from the observed cumulative hatch of 7% (maximum 15%), but raw hatch predictions did not correlate with proportion of hatching females during the hatch period. It is important to stress, however, that some of these models' prediction errors might not actually reflect limitations of the model, but rather could be due to errors in the "observed" hatch period, which was estimated on the basis of the capture of ovigerous females with mature clutches in lobsters traps set at 1–15 ($\bar{x}$=5.4) day intervals.

The best model for each study location did a good job of predicting timing of hatch, however the best model in Dingwall and Bonaventure were based on different temperature-dependent functions of embryo development. In Bonaventure, the logarithmic function outperformed the linear function at predicting the mean hatch date (observed: July 17th; best linear model: August 8th; best logarithmic model: July 20th), the hatch period (best linear model: 100% of range , 78% predictions within range, 78% hatch period overlap; best logarithmic model: 100% of range, 90% predictions within range, 90% hatch period overlap) and the progression of hatch (best linear model mean deviation from observed hatch: 36%; best logarithmic model mean deviation from observed hatch: 7%). The logarithmic predictions represented a more condensed range of hatch dates than predictions made by the linear model, will always predict wider hatch periods than the logarithmic function, and this more condensed range of hatch dates aligned more closely with observed hatching in Bonaventure; the linear predictions overestimated the duration of hatching by

~9–11 weeks, and 19% of these predictions were later than the latest hatch observed in nature, compared to only 2% for the logarithmic function.

In contrast, in Dingwall the linear function of embryo development outperformed the logarithmic function at predicting the mean hatch date (observed: August 3rd; best linear model: August 2nd; best logarithmic model: July 25th), the hatch period (best linear model: 91% of range, 95% predictions within range, 86% hatch period overlap; best logarithmic model: 64% of range, 100% predictions within range, 64% hatch period overlap) and the progression of hatch during the hatch period (best linear model mean deviation from observed hatch: 4%; best logarithmic model mean deviation from observed hatch: 15%). Although 100% of the logarithmic model's predicted hatch dates fell within the observed hatch period for this location, these predictions failed to describe the entire duration of the hatch period, with only 64–67% of the observed range of hatch dates predicted for samples collected on different sampling dates, compared to 90–91% for the linear function. In particular, the relatively truncated logarithmic predictions failed to capture the ending of the hatch period, predicting hatching to end ~3.5 weeks earlier than what was observed.

To investigate whether the quality of the hatch predictions may have been related to the uncertainty surrounding my estimate of the true hatch period, due to the fact that sampling of ovigerous females was not continuous (3–15 days between samples in vicinity of the hatch period), I considered a modified definition of hatching duration, which was the period over which hatching was actually observed plus the days prior to and after hatching when

no samples were taken (hereafter maximum hatch period). For Bonaventure, this led to the addition of 4 days prior to the observed beginning of hatch and 2 days after the observed end of hatch, and for Dingwall it led to the addition of 5 and 15 days on either side of the observed hatch period. Whereas this change did affect performance metrics to some extent, it did not affect the identity of the development function that made the most accurate predictions. The results for Bonaventure were minimally affected by the hatch period considered. For example, both development functions predicted 100% of the 48 (observed) to 54-day (maximum) hatch period, and the proportion of hatch predictions that fell within the maximum hatch period only increased from 90 to 95% for the best model (90 to 95% hatch period overlap). Importantly, whether I considered the "observed" or the "maximum" hatch period, the logarithmic function best predicted hatch in Bonaventure. The impact of the uncertainty concerning the hatch period was similar in Dingwall, with the percentage of the range of the hatch period described by the best model falling from 91% for the observed hatch period to 88% for the maximum hatch period, with the percentage of hatch predictions occurring within this period increasing slightly from 95 to 99% (86% to 87% hatch period overlap). Notably, these changes were predominantly driven by the addition of 15 days onto the end of observed hatching in Dingwall, which was the result of a single hatching female (< 1 % of all hatching) observed in the middle of September. But again, whether I considered the "observed" or the "maximum" hatch period, the linear function

predicted hatch with greater accuracy then the logarithmic function in Dingwall.

Why did different models perform best in my two study locations?

The difference in the predictive ability of the two functions at the two study locations suggests that their performance may be context-dependent, and that no one function is overall better. Such context dependency could arise if the relative performance of the two development functions was influenced by the temperature experienced by the embryos (i.e., perhaps the two functions better capture development at different temperatures), and if embryos in my two study locations experienced different temperatures during their development. Although mean temperature was nearly identical at the two study sites over the study period (10.8°C), it was significantly higher in Bonaventure (where logarithmic function worked better) than in Dingwall (where the linear function worked better) during the weeks preceding hatch and during the hatch period. This introduces the possibility that temperature immediately preceding and during hatch may determine which development function best describes hatch at a study location. Results of our recent study in Cheticamp (Miller et al. 2016) are at least partly consistent with this hypothesis, as the period prior to the beginning of hatch was also cooler in Cheticamp than in Bonaventure, and hatch in Cheticamp was best described by the linear function, as in Dingwall. The only group of free roaming females that had hatch better described by the logarithmic function (Bonaventure) had a significantly warmer pre-hatch period

than the two locations where hatch was better described by the linear function (Dingwall and Cheticamp). A recent study by Goldstein and Watson (2015) provides support for this hypothesis. When the authors tracked the embryonic development and hatching of lobsters in the Southern Gulf of Maine they found that “inshore lobsters” hatched on average four weeks earlier than “offshore lobsters”, with the authors speculating that hatching in the inshore population occurred earlier due to the rapid spring/summer warming of the relatively shallow inshore waters (Goldstein and Watson III 2015). It may therefore be the case that the exponential development predictions (and hence earlier hatching predictions) made by the logarithmic function are capturing this phenomenon, as springtime temperature prior to the beginning of hatch was significantly warmer in Bonaventure, where the logarithmic function worked the best, than in Dingwall and Cheticamp, where the linear function worked the best.

Pre-hatch temperature is particularly likely to affect model performance if it is at or above 9°C; the two development functions make similar predictions below this threshold, but beyond 9°C the logarithmic function predicts development to exponentially accelerate with temperature, whereas the linear function continues to predict a constant increase in the rate of development, leading to increasing differences in development predictions made by the two functions with increasing temperature (Appendix A). Interestingly, embryos from Bonaventure, where the logarithmic function performed better, experienced a greater number of days above 9°C prior to hatch (19 days) than did embryos from the Dingwall fishing grounds (0) and Cheticamp (10), where

the linear function performed better. It is therefore possible that the logarithmic function better captures the true rate of embryo development at higher temperatures, although the conclusion that embryos in Dingwall spent 0 days at 9°C prior to the onset of hatch is questionable given the uncertainty concerning when hatch in the population started. More specifically, only one female was observed hatching on the first recorded day of hatch (June 18th), with the next hatching female not observed until ~3 weeks later (July 9th). By July 8th, Dingwall would have experienced almost as many days above 9°C (18) as Bonaventure (19), although mean pre-hatch temperatures were still somewhat cooler in Dingwall (7.1°C) than Bonaventure (8.2°C). If pre-hatch temperatures determine which model works best, then knowledge of spring temperatures prior to hatch *in situ* (relative to embryo development status) could be used to decide which function to use to predict the hatch period. Future manipulative studies involving the exposure of ovigerous females to manipulated pre-hatch temperatures may help determine if this is in fact the case.

It is possible that differences in model performance arose due to differences in the temperatures used to develop them. The logarithmic function was developed based on embryo responses to temperatures from 1–16°C (Sibert et al. 2004). The linear function, however, was developed based on embryo response based on temperatures ranging from 5°–25°C (Perkins 1972). In Bonaventure, where the logarithmic function outperformed the linear function, there were 33 recorded days prior to hatch with mean daily temperatures below 5°C, and therefore below the thermal threshold at which the linear function was

developed. However, this explanation for differences in model success is not likely, as the logarithmic function outperforms the linear function across all sampling dates in Bonaventure, including those that occurred after temperatures had increased. Further, the linear function-date combination that performed the best in Bonaventure was the linear function paired with samples collected earliest (59 days prior to hatch), and therefore had the most dates with mean temperatures below 5°C. Finally, differences in model performance based on temperature thresholds cannot explain the difference between the outcomes for the two Dingwall sites, which had an equal number of dates below 5°C but did not have the same best development function.

A different potential explanation for the context dependency of development function success is that there may exist genetically based differences in how embryos from different regions develop as a function of temperature. Results from my two studies (present and Miller et al. 2016) provide some support for this hypothesis, as hatch of free roaming females in the two study sites off the coast of Cape Breton (Dingwall and Cheticamp) was best predicted by the linear function, whereas hatch in the Gaspé peninsula (Bonaventure) was best predicted by the logarithmic function. A recent unpublished laboratory study indicated evidence of significant spatial variation in the rate of development of lobster embryos held at the same temperatures (Mawer 2018, *Honours thesis*), suggesting that the development rate of embryos may be adapted to geographic variation in environment conditions, including potentially temperature. It is important to note that the two

development functions tested during this study were derived using embryos from markedly different geographic regions, i.e., the Magdalen Islands for the logarithmic function (Sibert et al. 2004, Gendron and Ouellet 2009) and the New England region for the linear function (Perkins 1972). It is possible that this geographic variation in the source of study embryos is the reason why two different functions ended up being developed in the first place, and in turn that the success of these functions varies based on the locations where hatch was being predicted. Additionally, the predictive ability of the two functions depends not only on whether the correct functional relationship has been identified for a given sampling location but also the quality of parameter estimates. It is possible that the differences in a model's predictive ability among sampling locations is not because of different functional relationships of embryo development and temperature but rather because parameter estimates differ among locations.

Finally, it is also possible that the accuracy of hatch predictions made for different study locations/times with the linear and logarithmic models of embryonic development is a coincidence, and does not reflect true and repeatable differences in function performance related to temperature or geographic origin. If timing-of-hatch is overestimated (i.e., predict hatching later than it occurs) for a study location for any reason, then the logarithmic function, which will always predict faster development (particularly when temperatures exceed 9°C, Appendix A), will produce hatching estimates that better reflect observed hatch, compared to the linear function, which predicts

slower development and later hatching time at all temperatures. Conversely, if timing of hatch is underestimated for reasons unrelated to water temperature, the linear function will more accurately predict timing of hatch compared to the logarithmic function. Although timing of hatch appears to be predictable using development functions, embryos collected prior to the beginning of hatch, and temperature data, the conditions surrounding model success must be clarified with additional research before this technique can be implemented to forecast hatch *in situ*.

How does sampling date influence model predictions?

The timing of sampling within ≈8 weeks of the onset of hatch had little and inconsistent effect on the accuracy of hatch predictions. For both study locations, earliest (Dingwall: May 21st; Bonaventure: May 7th) and latest (Dingwall: June 12th; Bonaventure: June 15th) samples resulted in similar mean hatch dates (Dingwall: August 12th versus August 2nd; Bonaventure: July 20th versus July 24th), and more importantly the differences were in opposite direction at the two study locations. I similarly found only small differences in predictions of the progression of hatch between early and late samples, and again these differences were in opposite directions at the two study locations; in Dingwall the model that best predicted this endpoint was based on the sampling date closest to hatch, and in Bonaventure it was based on sampling date farthest from hatch. Therefore, from early-mid May, the amount of spring-summer embryo development remaining to be predicted did not appear to affect the

accuracy of hatch predictions for free roaming females. Similarly, there was no significant difference in hatch predictions made from repeated sampling of the same caged females over time. Using the logarithmic development function, hatch predictions of caged females based on different sampling dates were "essentially equivalent" (Delta AICc ≤ 2, Anderson 2008), and the best prediction overall came from pooling data from all sampling dates (all embryos from 7 dates combined). When using the linear function to predict hatch of caged females, two sampling dates produced hatch predictions that were worse than those made by the best date-function combination, but these were in the middle of the sampling period (i.e., not particularly early or late). Based on results of this project, and also those of Miller et al. (2016), it appears that there does not exist a direct effect of sampling date on the accuracy of model predictions, although given the sampling time frame of these studies it is not possible to determine how sampling earlier, for example during the autumn fishing season, would affect the accuracy of hatch predictions. Importantly, this result suggests that if samples are collected in the spring-summer months leading up to hatch, then this method of predicting timing-of-hatch may be applied across the lobster's range in Canada as every LFA has a spring fishery (DFO 2015).

How do predictions made for individual females differ from "population-level" predictions?

Success at predicting the hatch period of individual females held in Dingwall Harbour was low compared to success at predicting the hatch period of the population of these caged females. When hatch predictions of all caged females are pooled, 67% of the linear predictions and 98% of the logarithmic predictions fell within the observed hatch period, whereas when predictions were made for individual females they only fell within the observed hatch period on average 12% (0–67% for different females) of the time using the linear function and 31% (0–90%) of the time using the logarithmic function. When a less conservative estimate of the hatch period was used (i.e., maximum hatch), these values change to 85% of the linear predictions and 99% of the logarithmic predictions falling within the hatch period for the pooled population, and on average 42% and 59% for the individual females when the linear and logarithmic functions were used, respectively. These findings indicate that this approach to modeling hatch of lobster embryos best describes the "average behaviour" of embryos from multiple females, but that not all embryos are responding (in terms of development) to temperature in the same manner or hatching with an eye size that can be predicted based solely on the observed variability of this endpoint (e.g., development rate and development status at hatch may co-vary among embryos).

Both the linear and logarithmic functions predicted a significantly lengthier hatch period for the individual females (mean of 48.0 and 28.6 days

among females, respectively) than the observed (6.8 days) and least conservative (i.e., maximum) (20.8 days) estimate of mean observed hatch. One potential explanation for this result is that my approach to selecting PEI-at-hatch may have broadened the range of such values attributed to individual embryos in a manner that increased the range of hatch predictions beyond those experienced by embryos belonging to a same female. There are two aspects of my approach to modeling PEI-at-hatch that may have caused this. First, it is possible that PEI-at-hatch varies at the level of individual females (i.e., embryos from different broods/females hatching at a relatively larger or smaller PEI), and this variability would not have been accounted for in my study because I used a pooled PEI-at-hatch distribution to predict PEI-at-hatch of all embryos, given that our opportunistic sampling led to obtaining 0 prezoea from seven of the 22 females, and the range of prezoea obtained per female was only 1–11 ($\bar{x}$=6.3). A second, and potentially complementary explanation is that randomly drawing from a pooled PEI-at-hatch distribution may broaden the range of predicted hatch dates for a female if in reality the PEI-at-hatch of an individual embryo is dependent on its development rate and/or development status in spring/summer preceding hatch. For example, an embryo paired with a PEI-at-hatch that is smaller than what was true may cause a hatch prediction that is too early, and vice versa, therefore resulting in a hatch prediction that is too late. In a recent unpublished study, embryos that entered the spring warming period with a smaller PEI developed at a faster rate than those that entered the warming period with more development, and embryos that

developed more quickly in the spring tended to hatch with a larger PEI than those that developed more slowly (White 2018, *Honour's thesis*). These two processes would contribute to condensing the hatch period of individual females relative to my estimates. Although it has been previously demonstrated that the inclusion of variability of PEI-at-hatch at the population level improves the accuracy of hatch predictions at the population level (Miller et al 2016), uncertainty surrounding the mechanisms underlying variability in development and hatch of individual embryos, including why different embryos hatch at different stages of development (based on PEI), may be contributing to errors in hatch predictions.

Plasticity in patterns of embryonic development of lobsters has been noted in both studies from which the two development functions considered in this study were derived. In Perkin's (1972) study there was evidence that variability in the rate of development of embryos belonging to different females results in hatching synchronicity among females that had extruded their eggs at different times during the spawning season, and that brood-level rate of embryonic development depends not only on temperature, but also on the level of development attained by the brood at the time a temperature is experienced (Perkins 1972). More specifically, Perkins observed that embryos that had been extruded later in the summer spent less time in winter stasis than embryos that had been extruded earlier, allowing them to "catch-up", in terms of development, to earlier-spawned embryos (Perkins 1972). Although this observation was only based on the clutches of 5 females, and the exact ages of

all clutches was not known (not all broods had been extruded in the laboratory), the pattern appeared consistent, with embryos from the oldest known clutch spending 18 weeks in stasis between December and April, compared with those from the youngest clutch that did not stop developing at all during this period (Perkins 1972). Gendron and Ouellet noted the same phenomenon, where embryos from "early spawners" (defined as having clutches that were extruded in mid-July) and "late spawners" (defined as having clutches that were extruded in mid-late August) showed statistically different reactions to the winter cool-down period, with the least-developed embryos continuing to develop through the winter and more mature embryos showing no measurable change in PEI over the winter (Gendron and Ouelett 2009). These embryos were all competent to hatch during the summer months, when conditions are optimal for survival, drift, and settlement (Haarr 2018), which would not have been the case if all embryos followed the same developmental trajectory in response to temperature. Although plasticity in these two studies was observed in relation to winter dormancy ~7–2 month prior to hatch, it is not unreasonable to speculate that similar adaptation may have contributed to the functions' inability to track development of embryos belonging to different females in the weeks leading up to hatching. It is also possible that a similar phenomenon occurs at the scale of individual embryos within a clutch, where spring-summer development may be influenced by internal and/or external factors that modulate hatch time to match conditions that would be favorable to the emergent larvae. If this is true, then models that rely solely on temperature may

lack the sophistication necessary to predict embryonic behaviour at the individual or clutch level, as embryos are not all reacting to the environment in the same way. The functional significance of this plasticity is unknown, but possible explanations may be that it is an adaptation to optimize dispersal conditions (Haarr 2018, *PhD thesis*) or prey availability (Cushing 1990).

The considerable errors in my hatch predictions for individual females does not compromise the usefulness of this method for predicting timing-of-hatch of populations of females in nature, or to parameterize large-scale larval dispersal models. It does, however, provide evidence that this method may only work at a sufficiently low resolution, where the hatch time prediction for each embryo represents a sample of the behaviour of the population of embryos from a location, rather than that of that particular embryo. Further studies may be able to provide a more reliable model for predicting hatch time at the level of individual embryos and females by incorporating parameters that speak to the relationship between rate of development prior to hatch and the environment, such as how development status affects the rate of development at a given temperature during the spring warming period, and how this in turn may affect PEI-at-hatch.

Which model worked best for the caged females?

Neither the linear nor the logarithmic function consistently outperformed the other at predicting the mean development status of embryos from all caged females at different time-steps over the 7 weeks when these

broods were monitored. The two functions predicted nearly the same mean embryo PEI at time-steps 2 and 3, but the linear function better predicted PEI at time-steps 4 and 6, and the logarithmic function better predicted mean PEI at time-steps 5 and 7. Although the linear function had a somewhat lower total RSS compared to the logarithmic function when all 7 development time-steps are pooled, it did a worse job at predicting development at the final development time-step, as already indicated. More importantly, after the last sampling period, predictions made by the linear and logarithmic functions increasingly diverged as water temperature increased, and the linear function did a worse job at predicting timing of hatch than the logarithmic function. In particular, the linear function consistently under-predicted the rate of hatching over the hatch period, and predicted hatching to occur later than was observed, with a mean deviation from the observed cumulative percentage of hatch of 33%, compared to 7% for the logarithmic function. This outcome is consistent with my hypothesis that the logarithmic function will better predict hatching in water that is rapidly warming, as the weeks where the logarithmic function outperformed the linear function coincided with the greatest increases in temperature between sampling dates once temperatures had warmed to >9°C, by time-step 6. During the intervals between time-steps 4–5, and 6–7, when the logarithmic function better predicted amount of development between time-steps than the linear function, temperatures increased by 1.7 and 2.0°C, respectively, whereas the increase in temperature between time-steps 5–6, when the linear performed better, was only 0.9°C. Although the greatest temperature

increase between any two sampling intervals (i.e., 2.2°C between time-steps 3–4) occurred when the linear function performed the best, temperature during this period was below 9°C, which is the threshold at which the predictions made by the functions two substantially diverge.

That hatch of caged females in Dingwall Harbour was better predicted by the logarithmic than the linear function was somewhat surprising, given that the contrary was observed for females from the Dingwall fishing ground. One potential explanation for this difference is that the hatch period in Dingwall Harbour occurred during a warmer period than hatching in the fishing ground, which is consistent with the results of the other at-large study locations (see above), where cooler pre-hatch temperatures were associated with better performance of the linear (versus the logarithmic) function, and vice versa. It must be considered, however, that caging the females may also have altered development and timing of hatch in a way that went on to affect which model best predicted it. In particular, caging the females prohibited any movements they would have normally exhibited, potentially affecting clutch oxygenation. Although there is no data on the effect of clutch oxygenation on development of American lobster embryos, oxygenation has been linked to changes in development and hatching rate in embryos of blue king crab *P. Platypus* (Stevens et al. 2008), Norwegian lobster, *N. norvegicus* (Eriksson et al. 2006), and green crab *C. maenas* (Hartnoll and Paul 1982). Future "common-garden" style experiments could potentially determine if variations in temperature are the cause of differences in timing-of-hatch for females occupying the same

general area, for example by caging some females, and simultaneously tracking the movements of tagged, identifiable free-ranging females equipped with temperature loggers in the location where the cage is held and comparing their timing of hatch. If temperature does not explain observed differences then this would suggest that other variables, such as oxygen levels, are at play.

Hatching in marine decapods

Predicting hatch time of marine decapods relies on knowledge of environmental or endogenous factors, or a combination of these. The models used in this study used three inputs in order to predict hatching: one environmental (temperature) and two endogenous (development rate and PEI-at-hatch). Although these were sufficient to describe the average hatching behaviour of the populations of embryos we sampled, there are clearly additional factors modulating development and hatching of embryos belonging to different females that are not captured by these models. In some decapods, timing of hatch is entrained primarily by responses to environmental conditions (Forward 1987; Christy 2011), and in the simplest cases hatch is highly predictable and governed by a single environmental factor, and all embryos respond the same way to this factor, leading to synchrony of larval release. Decapod species that have highly synchronized hatching periods may be targeting particular environmental conditions that increase larval success, but exist within a relatively constrained timeframe, e.g., optimal dispersal potential through tidal conditions or peak prey abundance (Christy 2011). Notable

environmental hatching signals among littoral and supra-littoral crabs, for example, include lunar and diel cycles (e.g. Bermuda land crab, *Gecarcinus lateralis* (Klaassen 1975), striped-leg hermit crab, *Clibanarius vittatus* (Zeigler and Forward 2006)), and tidal cues (e.g. Atlantic fiddler crabs *Uca spp*. (Salmon et al. 1986)). For instance, both diel and lunar rhythmicity in larval release is observed in Bermuda land crabs *G. lateralis* (Klaassen 1975; Wolcott and Wolcott 1982), with crabs from certain populations predictably entering the water to release larvae for 4–5 days after the full moon during the reproductive season, between 2100 h–2400 h, regardless of tidal cycle (Wolcott and Wolcott 1982). The authors speculate that this mass migration to the sea was not related to improving dispersal conditions, as it was not tied to tidal rhythms, but instead was a predator satiation strategy, reducing the risk to any one individual crab larva as predators are confronted with more prey than they are able to consume (Wolcott and Wolcott 1982).

Sometimes hatch is not synchronized at the population level, but it is at the brood level. The spiny lobster *Panulirus guttatus*, for example, releases its entire brood of larvae in a single hatching event; however, not all females release larvae at the same time, with spawning and hatching occurring throughout the year (Ziegler and Forward 2007). These lobsters release their larvae into the ebbing tide at sunrise, and the phototactic larvae quickly swim to the surface where they are then transported on surface currents to nursery areas (Ziegler and Forward 2007). It is likely that for this species the function of synchrony of larval release is to take advantage of optimal larval transport

conditions (Zeigler and Forward 2007). Spiny lobsters *Jasus edwardsii* also have a relatively constrained hatching period with similar hatching behaviour, with ovigerous females with highly developed clutches migrating to areas with strong currents and initiating larval release at dawn (MacDiarmid 1985). The hatching process requires 1–5 days, and laboratory studies have determined that time to hatch across a range of constant temperatures can be accurately predicted within 2 days based on accumulated degree days, with variability of larval release dates determined by spawning date and the subsequent temperatures the embryos experience, and embryos belonging to different females developing in a very similarly and predictable manner when in the same conditions (Tong et al. 2000).

Sometimes, however, embryos from the same broods do not respond the same way to environmental factors, and hence hatch time becomes more protracted and more difficult to predict, even if the general mechanisms modulating development and hatch have been identified. Species with protracted hatch periods may be responding to environmental conditions that are less predictable, and therefore hedging their bets by releasing larvae over a timeframe that may encompass a variety of conditions, a proportion of which will be optimal for larval survival (Thatje et al. 2003; Christy 2011). Laboratory studies of king crabs (Lithodidae) have established that although embryonic development is temperature dependent, the oxygenation gradient within a tightly packed clutch likely causes considerable variability in development time within a brood. This results in hatching periods of ~four to seven weeks for a

single clutch at ambient temperature, despite embryos being extruded and fertilized on the same day, which makes timing of hatch difficult to predict at the level of the embryo (Thatje et al 2003; Stevens et al. 2008). The prolonged hatch period in king crabs is thought to be a bet-hedging strategy in response to the variability in timing of peak plankton blooms in the photic zone, combined with the crab's inability to perceive this bloom from their position on the benthos (Stevens 2006). It is hypothesized that if a crab is able to release its clutch slowly over the course of a period of several months, then it is more likely that some of its offspring will reach the surface at the time of peak prey abundance (Stevens 2006). American lobsters are another example of a species with a protracted hatch period, with hatching previously observed occurring as a nightly process over ~one to six weeks (Templeman 1937; Ennis 1975), which is nearly identical to the range of maximum estimated hatching periods of 7–40 days observed in my study for the individually-caged females. It is possible that American lobsters are engaging in a bet-hedging strategy similar to king crabs, with the function of extended release of individual broods into the water column being to increase the odds that a proportion of a female's larvae will encounter environmental conditions that allow rapid metamorphosis through the larval stages to settlement, such as warmer waters, more and/or better prey, and favourable currents.

Hatching and larval release of American lobsters are two separate, complimentary processes. Laboratory experiments have determined that embryos hatch successfully even if they are separated from the female (White

2018), and that females do not engage in larval release behaviours (i.e., pleopod pumping) in response to chemical cues from the hatching embryos (Ennis 1975). Instead, female pleopod pumping is initiated shortly after sunset in short bursts each night, with embryos hatching into prezoea throughout the 24 h intervals between these events (Davis 1964). In contrast, in some species with more synchronized hatching among embryos of the same brood, this is not the case, with pleopod pumping behaviour triggered by chemical cues released by ruptured eggs, creating a positive feedback loop that ensures relatively rapid larval release as more egg membranes are ruptured (e.g., Spiny lobster *P. argus*: 1 day [Ziegler and Forward 2007], Mud crab *Neopanope sayi*: 5 days [Swartz 1978, De Vries et al. 1991], Atlantic blue crab *Callinectes sapidus*: 1 day [Tankersley et al. 2002]). Experiments comparing the timing of hatch of embryos attached or detached from a female's brood support the conclusion that timing of hatch is mostly or entirely determined by embryos in American lobster, as there was no difference between the mean hatch date or amount of variability in hatch time of attached and detached embryos from the same female (Bo et al. in prep). This also suggests that normal levels of clutch-oxygen gradation within a clutch does not modulate timing of hatch, as is the case in king crabs. Future research should therefore focus on determining if the variability in hatch time observed among embryos is the result of genetic variability among embryos, differences in yolk allocation within the brood leading to differences in embryo metabolism and growth, or a combination of these.

Biophysical models of larval dispersal

This approach to modeling hatch is expected to improve larval dispersal and settlement predictions made by bio-physical models of larval dispersal. Currently, biophysical models of larval dispersal either rely on historical hatching estimates obtained using recorded observations of stage I larvae in the water column at different times and sampling locations (e.g. Incze and Naimie 2000; Quinn 2017), or back calculate hatch time based on the presence of settled lobsters observed *in situ*, larval development models, and temperature (e.g., Gendron et al. 2018). Relying on historical data does not account for inter-annual environmental variability, however, and may increase error if, for instance, temperatures are colder or warmer than the time-series upon which these estimates are based. Back calculating timing of hatch based on the presence of settlers requires logistically demanding sampling, as these necessary data cannot be collected alongside normal fishing activities, and the quality of hatch predictions derived from this approach will depend on the accuracy of larval development models tasked with estimating the duration of four distinct larval instars. In general terms, these approaches to estimating hatch of lobster larvae cannot readily be applied to the large spatial domain captured by larval dispersal models, or to capture inter-annual variability in hatch over this domain.

The approach used in this study, in contrast, integrates both real-time biological and environmental data, thereby reducing these errors, and can directly estimate hatch over large geographic areas. By using site and year

specific environmental data, it can account for the effect of inter-annual variability in spring/summer water temperature on hatch. It is likely that lobster embryo development rate, and hence the hatching period, will vary from year to year based on inter-annual variation in temperature. In addition to "normal" inter-annual variability, climate change projections suggest that sea temperatures will continue to rise (IPCC 2014), and it will be critical to not only use real-time data for hatching projections but also to monitor embryos in the field in order to track their response to unprecedented summer temperature conditions.

This approach of predicting timing of hatch also takes into consideration embryo development status at the time of sampling, which will vary annually based on temperature and timing of spawning in the year prior. It can also account for regional and inter-annual variability of PEI-at-hatch. Presently, the main limitation to application of this approach is the uncertainty concerning which development function to use for a given region and year. Future steps must therefore include further field validation of both development functions to ultimately understand which of these to use in what context. As we are able to predict the duration and mean date of hatch *in situ*, but can less reliably predict its progression over time, it is also clear that additional model parameters should be auditioned in order to improve hatch predictions based on this approach.

Summary

The results of this thesis improve our understanding of the usefulness, as well as the limitations, of estimating timing of hatch of American lobsters using temperature data and samples of embryos collected in nature. The immediate impact of my results is to demonstrate that we can forecast the timing and duration of the hatch period for lobsters across the species' range without the need for intensive and expensive out-of-season sampling of ovigerous females, requiring instead only minimal effort to sample eggs during the fishing season through collaboration with fishermen. These hatch predictions can then be used to feed biophysical larval dispersal models, which should in turn help improve our understanding of population connectivity in lobsters. However, some of the mechanisms underlying model success, and failures, remain unclear. In particular, this study suggests that different development functions might be needed to forecast the hatch of embryos from different regions and/or years, but I was unable to conclusively determine under what conditions each function should be used. This uncertainty represents the most important impediment to the use of this approach to predict hatch. Future research should assess the effect of pre-hatch temperature and geographic origin on embryonic development, and determine whether embryo development status in the spring, rate of development, and PEI-at-hatch are independent from one another, as my models assume. Once a process for model selection is in place, then this tool can be used to predict the timing and duration, and possibly

trajectory, of hatching *in situ* across the species' range with minimal sampling effort and cost.

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Table 1. Summary table of hatch prediction accuracy based on combinations of embryos collected from different sites and dates, temperature, and two temperature-dependent development functions.

Site	Sampling date	Function	Females	Embryos	Mean observed hatch	Mean predicted hatch	% Predictions within[a]	% Range predicted[b]	% Hatch period overlap[c]	Mean deviation[d]	*r* stage IV[e]	AICc weight[f]
Bonaventure	May 7th 2015	*Linear*	50	500	July 17th 2015	August 8th 2015	78	100	78	0.36	-0.70	<0.01
Bonaventure	May 7th 2015	*Logarithmic*	50	500	July 17th 2015	July 20th 2015	90	100	90	0.07	0.12	0.70
Bonaventure	May 25th 2015	*Linear*	49	458	July 17th 2015	August 10th 2015	68	100	68	0.42	-0.62	<0.01
Bonaventure	May 25th 2015	*Logarithmic*	49	458	July 17th 2015	July 22nd 2015	91	100	91	0.08	-0.08	0.26
Bonaventure	June 15th 2015	*Linear*	50	497	July 17th 2015	August 14th 2015	71	100	71	0.45	-0.64	<0.01
Bonaventure	June 15th 2015	*Logarithmic*	50	497	July 17th 2015	July 24th 2015	95	100	95	0.13	-0.11	0.03
Dingwall	May 21st 2015	*Linear*	50	502	August 3rd 2015	August 12th 2015	99	90	89	0.14	0.52	<0.01
Dingwall	May 21st 2015	*Logarithmic*	50	502	August 3rd 2015	July 25th 2015	100	64	64	0.16	0.55	<0.01
Dingwall	June 12th 2015	*Linear*	48	480	August 3rd 2015	August 2nd 2015	95	91	86	0.04	0.68	99.00
Dingwall	June 12th 2015	*Logarithmic*	48	480	August 3rd 2015	July 19th 2015	100	67	67	0.25	0.03	<0.01
Dingwall Harbour	May 28th 2015	*Linear*	22	220	July 27th 2015	August 7th 2015	84	92	76	0.30	-0.24	<0.01
Dingwall Harbour	May 28th 2015	*Logarithmic*	22	220	July 27th 2015	July 21st 2015	98	78	76	0.08	0.82	0.13

Site	Sampling date	Function	Females	Embryos	Mean observed hatch	Mean predicted hatch	% Predictions within[a]	% Range predicted[b]	% Hatch period overlap[c]	Mean deviation[d]	*r* stage IV[e]	AICc weight[f]
Dingwall Harbour	June 4th 2015	*Linear*	22	220	July 27th 2015	August 11th 2015	71	72	43	0.36	-0.26	<0.01
Dingwall Harbour	June 4th 2015	*Logarithmic*	22	220	July 27th 2015	July 24th 2015	100	81	84	0.10	0.81	0.07
Dingwall Harbour	June 11th 2015	*Linear*	22	220	July 27th 2015	August 12th 2015	56	94	50	0.41	-0.25	<0.01
Dingwall Harbour	June 11th 2015	*Logarithmic*	22	220	July 27th 2015	July 25th 2015	99	89	88	0.08	0.78	0.09
Dingwall Harbour	June 18th 2015	*Linear*	22	220	July 27th 2015	August 11th 2015	63	75	38	0.39	-0.30	<0.01
Dingwall Harbour	June 18th 2015	*Logarithmic*	22	220	July 27th 2015	July 25th 2015	100	78	78	0.09	0.78	0.08
Dingwall Harbour	June 25th 2015	*Linear*	22	220	July 27th 2015	August 9th 2015	67	100	67	0.34	-0.18	<0.01
Dingwall Harbour	June 25th 2015	*Logarithmic*	22	220	July 27th 2015	July 24th 2015	99	81	80	0.07	0.86	0.17
Dingwall Harbour	July 2nd 2015	*Linear*	22	220	July 27th 2015	August 9th 2015	64	100	64	0.34	-0.13	<0.01
Dingwall Harbour	July 2nd 2015	*Logarithmic*	22	220	July 27th 2015	July 25th 2015	98	89	87	0.09	0.67	0.09
Dingwall Harbour	July 10th 2015	*Linear*	20	200	July 27th 2015	August 4th 2015	66	100	66	0.25	-0.06	<0.01
Dingwall Harbour	July 10th 2015	*Logarithmic*	20	200	July 27th 2015	July 25th 2015	95	92	87	0.07	0.46	0.17
Dingwall Harbour	Pooled	*Linear*	22	1520	July 27th 2015	August 9th 2015	67	100	67	0.34	-0.22	<0.01
Dingwall Harbour	Pooled	*Logarithmic*	22	1520	July 27th 2015	July 24th 2015	98	92	90	0.07	0.82	0.17

[a]The percentage of hatch predictions that fell within the observed hatch period.

[b]The percentage of the range of the observed hatch period that was predicted.

[c] Mean absolute difference between predicted and observed cumulative progression of hatch across 16 (Dingwall), 11 (Bonaventure), and 6 (Dingwall Harbour) sampling time-steps.

[d] Overlapping hatch, which was defined as the percentage of range of observed dates predicted minus the percentage of dates predicted that fell outside the observed range

[e] Correlation between number of predicted hatching embryos and observed hatching female CPUE across 16 (Dingwall), 11 (Bonaventure), and 6 (Dingwall Harbour) time-steps.

[f]The relative explanatory weight of each model relative to other models used for a given sampling location according to Akaike's Information Criterion corrected for small sample sizes.

Table 2. Comparison by Akaike's information criterion (corrected for small sample size) of accuracy of predictions of cumulative progression of embryonic development made by linear and logarithmic models of temperature-dependent embryo development applied to all embryos from 6 different sampling dates prior to hatch in 2015 in Dingwall Harbour, Nova Scotia.

Model[a]	RSS[b]	AICc[c]	ΔAICc[d]	w_i[e]
Linear	0.00010	-26.63243	0	0.75434
Logarithmic	0.00024	-24.388624	2.24381	0.24566

[b]RSS is residual sum of squares.

[c]AICc is Akaike's information criterion corrected for small sample size.

[d]ΔAICc is the difference between the AICc score of each model and the score of the best model.

[e]w_i is Akaike weight, which is the relative explanatory weight of each model.

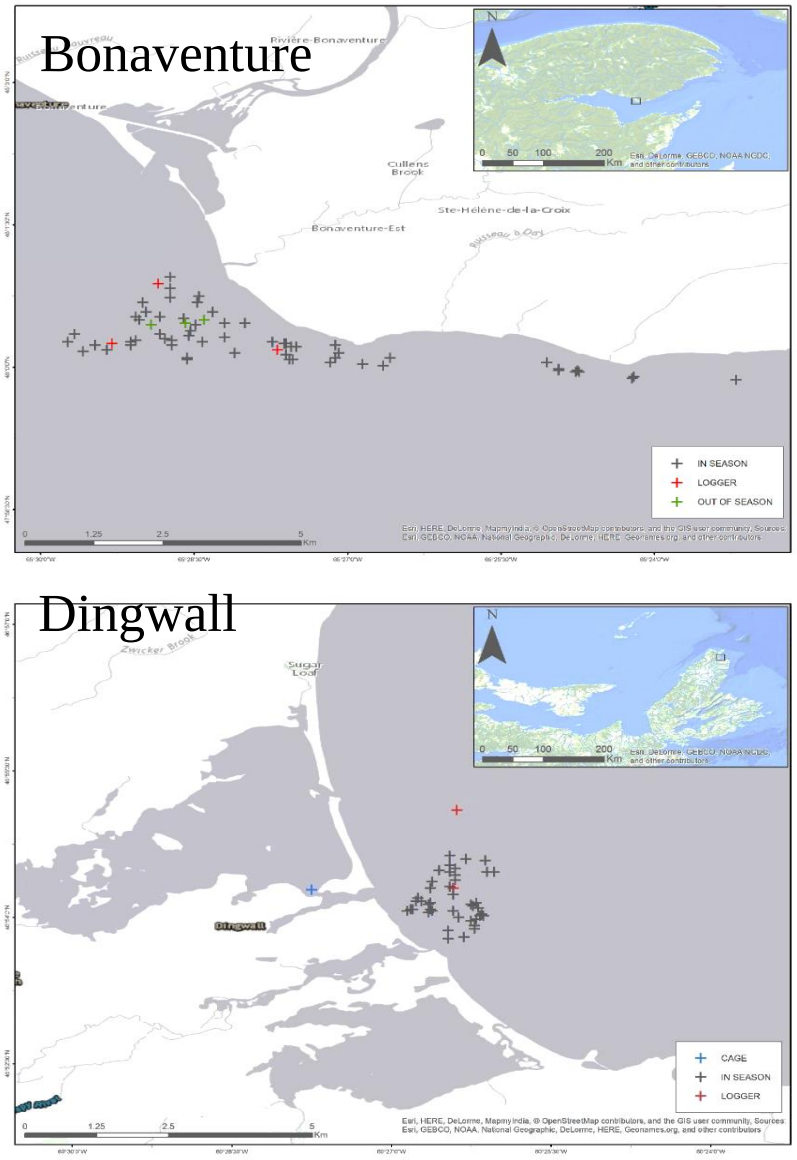

Figure 1. Location of sampling activities in Bonaventure, QC (upper panel), and Dingwall, NS (lower panel). Black crosses indicate locations within the lobster fishing grounds where samples were collected during the fishing season, and green crosses indicate locations where out-of-season sampling took place; whereas out-of-season data was collected in Dingwall, the locations of this sampling is not disclosed, at the request of the LFA 27 management board. Red crosses indicate where temperature loggers were moored in the fishing grounds. The blue cross marks where a cage containing 24 ovigerous female lobsters was held for the duration of the study in Dingwall Harbour.

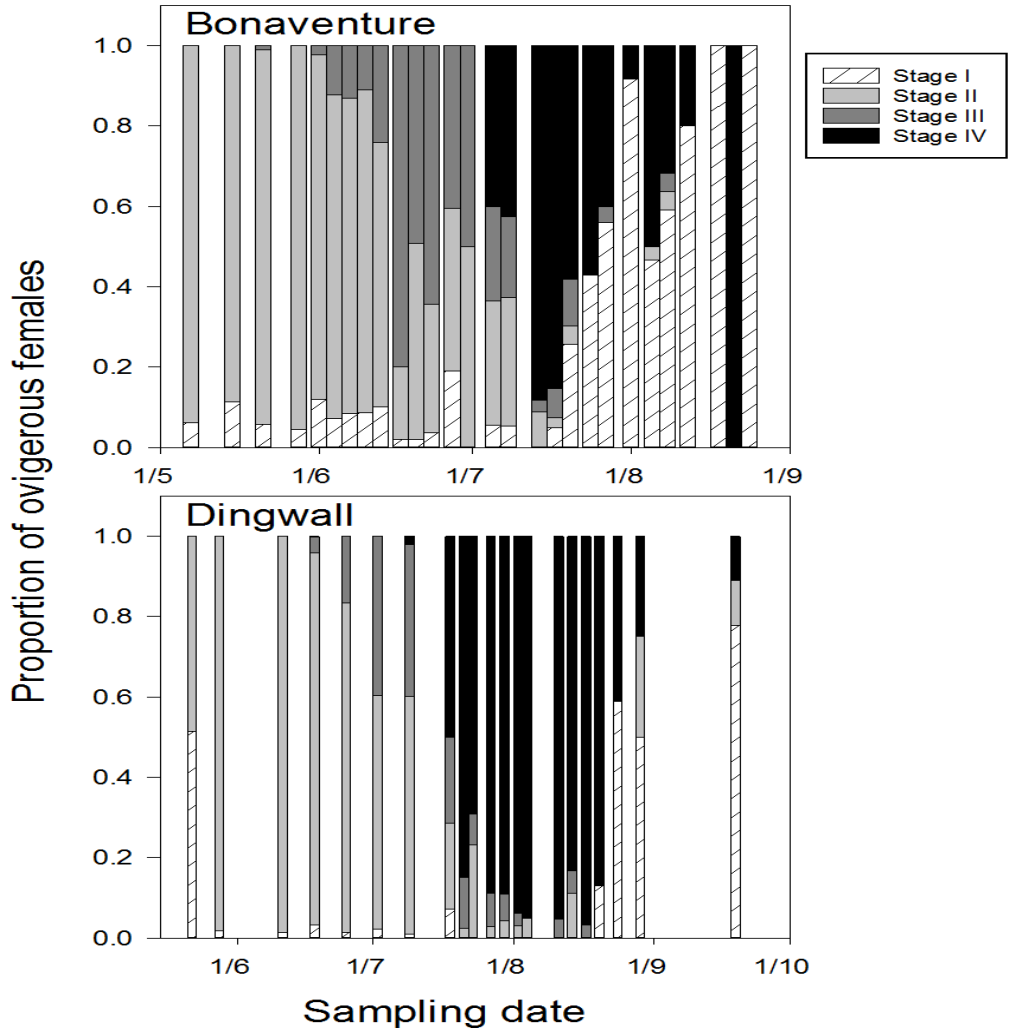

Figure 2. Observed hatch period of free-roaming female American lobster at our two study sites in 2015: Bonaventure, QC (top panel), and Dingwall, NS (bottom panel). The figures show the proportion of ovigerous females sampled with eggs at different stages of development. Each female's clutch of eggs was categorized as one of four developmental stages, based primarily on the colour and appearance of the eggs in the clutch (see Methods); stage I-III clutches have eggs at increasing stages of development, and a stage IV clutch has begun hatching. Hatch occurred from early July to late August in Bonaventure, and from June (one female on June 18th, not visible on figure) to mid-September in Dingwall.

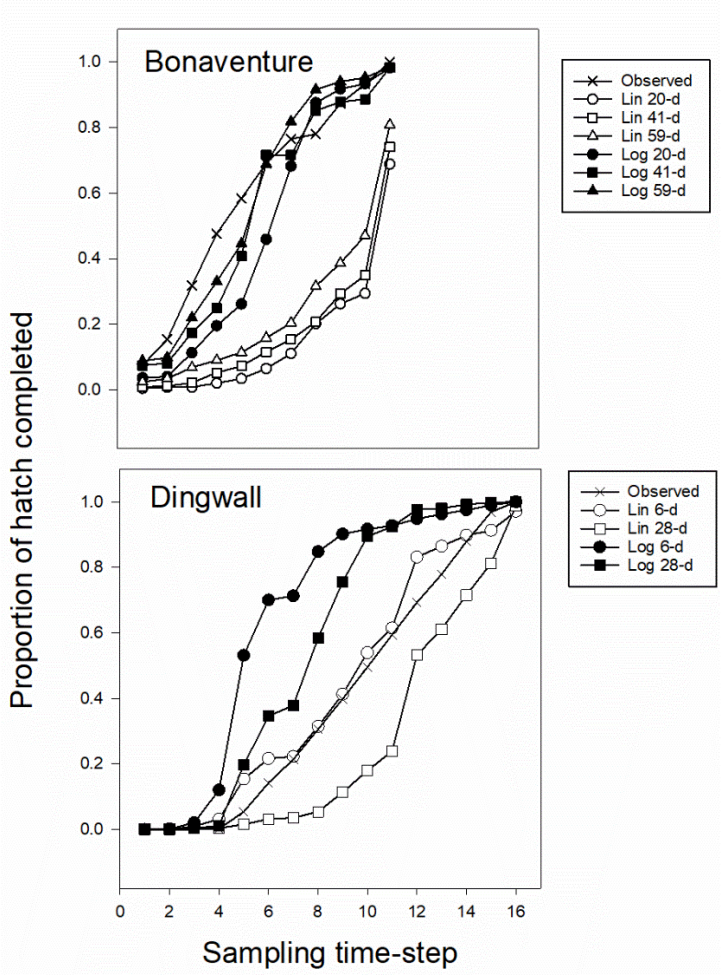

Figure 3. Observed (X) and predicted (other symbols) cumulative timing of hatch in 2015 in Bonaventure, QC (upper panel) and Dingwall, NS (lower panel). The observed progression of hatch is based on the presence of ovigerous females with recently hatched eggs (see Figure 2 and Methods), whereas predictions are based on egg samples obtained prior to the beginning of hatch (number of days shown in legend) and either a linear (white symbols) or a logarithmic (black symbols) temperature-dependent function of embryo development (see Methods). Each time-step represents one of 11 sampling time-steps during the 48-day observed hatch period in Bonaventure, Quebec, or one of 16 sampling time-steps during the 94-day observed hatch period in Dingwall, Nova Scotia.

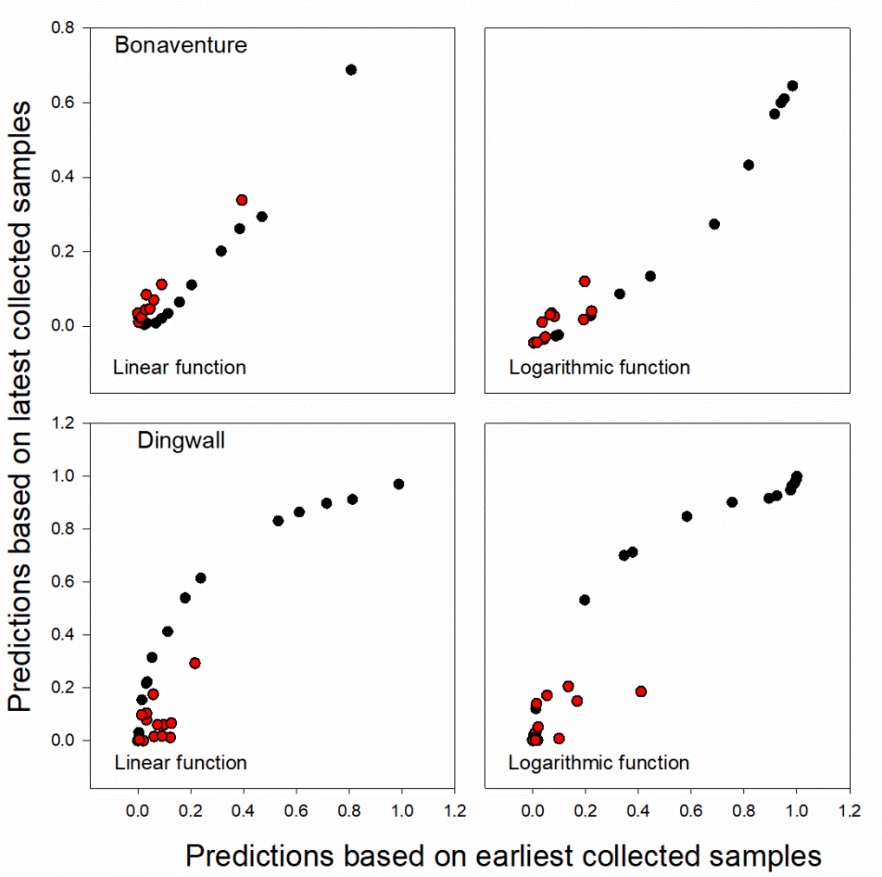

Figure 4. Relationship between predicted hatch using "early" and "late" embryo samples for each ovigerous female sampling date for our two study sites (Bonaventure, QC and Dingwall, NS) in 2015. Predictions are shown for cumulative hatch (black circles) as well as proportion of hatch predicted for each time-step (red circles) using two temperature-based development functions (linear and logarithmic). For Bonaventure, the two embryo sampling dates were 59 (early) and 20 (late) days prior to the beginning of hatch, and each point represents the prediction made for one of the 11 ovigerous female sampling time-steps during the 48-day observed hatch period. For Dingwall, the two sampling dates were 28 (early) and 6 (late) days prior to the beginning of hatch, and each point represents the prediction made for one of the 16 sampling time-steps during the 94-day observed hatch period.

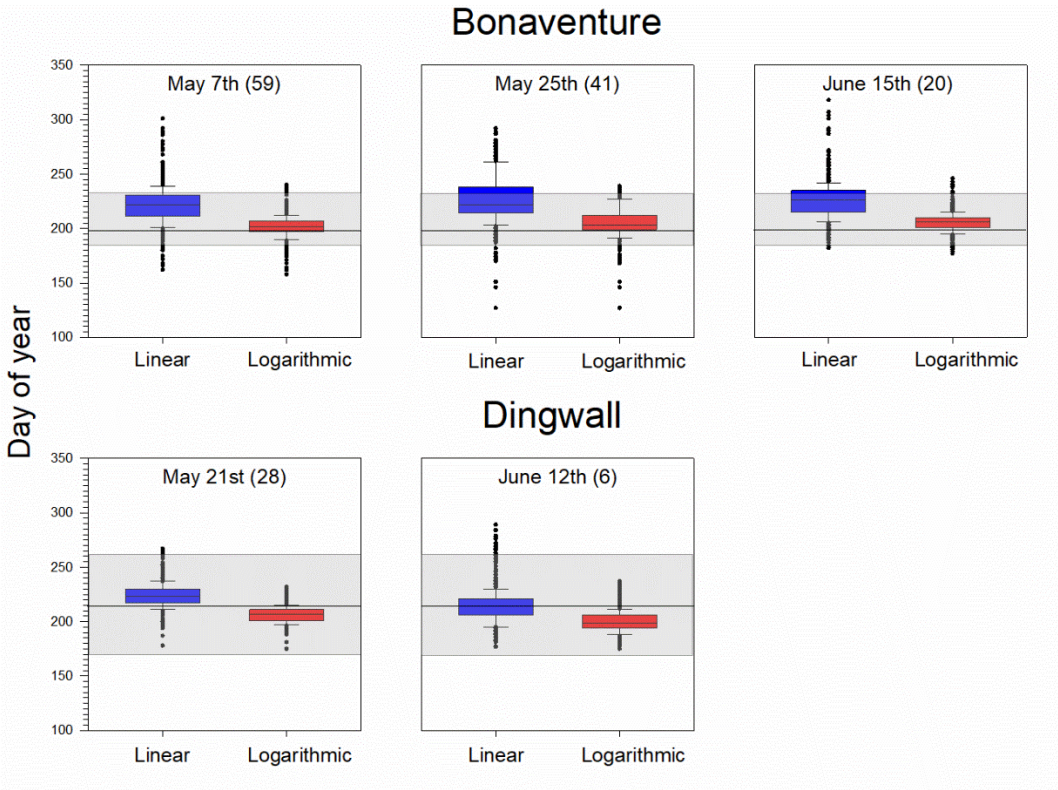

Figure 5. Predicted (boxplots) and observed (shaded area) time of hatch of lobster embryos in 2015 in Bonaventure, Quebec (above) and Dingwall, Nova Scotia (below). Hatch time was predicted by measuring eye size of embryos sampled from ovigerous females on different dates (shown on figure; numbers in parenthesis indicate days before hatching), and projecting embryo development using either a linear (blue boxplots) or a logarithmic (red boxplots) temperature-dependent function of embryo development (see Methods). The observed hatch period is shown as the shaded grey area and is based on the observation of ovigerous females with recently hatched eggs in the 11 (Bonaventure) and 16 (Dingwall) samples of ovigerous female obtained during sampling with fishermen. The black line signifies the average observed hatch date for the site. Boxes show 25th, 50th, and 75th percentiles, whiskers represent 10th and 90th percentiles and black circles represent data falling outside the 10th and 90th percentiles.

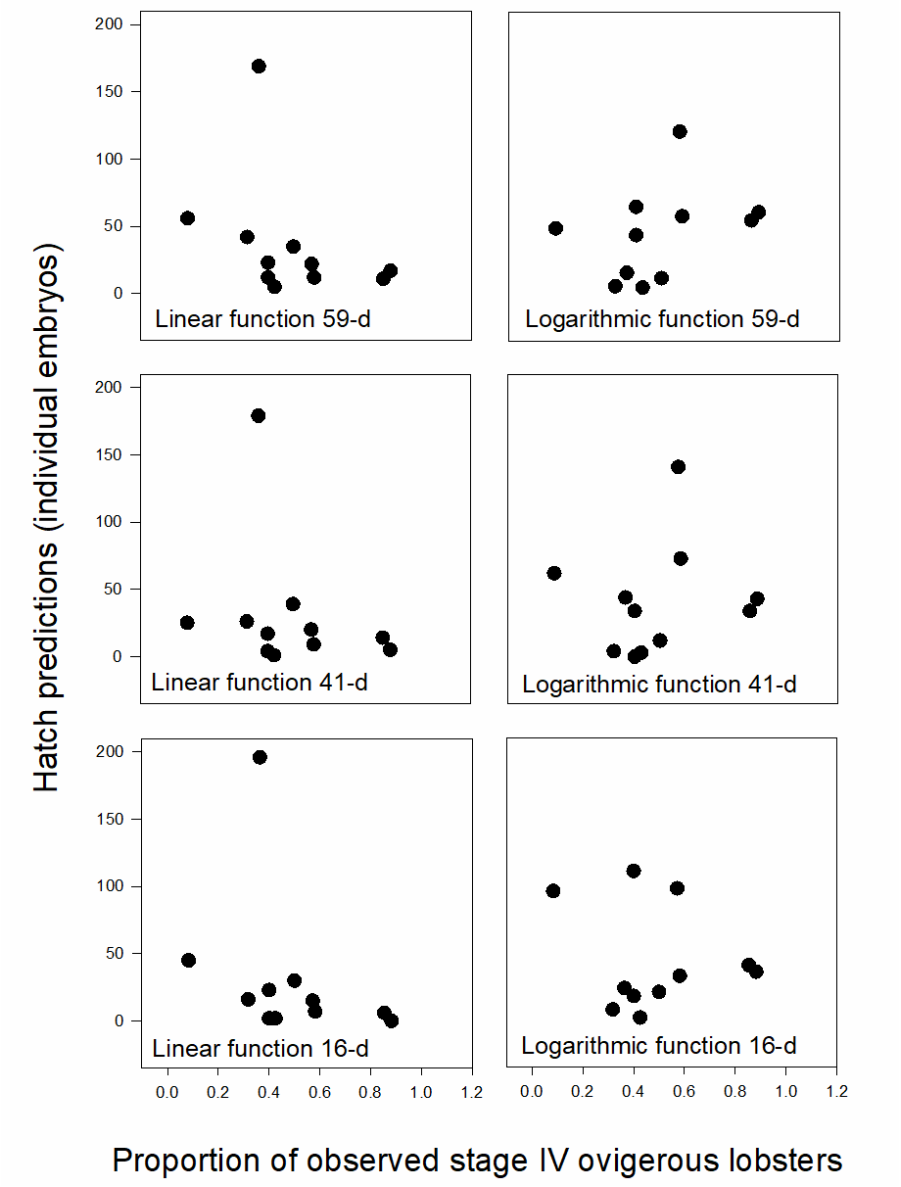

Figure 6. Relationship between the number of individual embryos that were predicted to hatch during each of 11 sampling time-steps in Bonaventure, QC, and observed proportion of stage IV ovigerous lobsters during each of these sampling days. Predictions were made using two temperature-dependent development functions (linear and logarithmic), and embryos (n=458–500) that were sampled 20, 41 and 59 days prior to the first observed hatch (female with stage IV clutch) at the site.

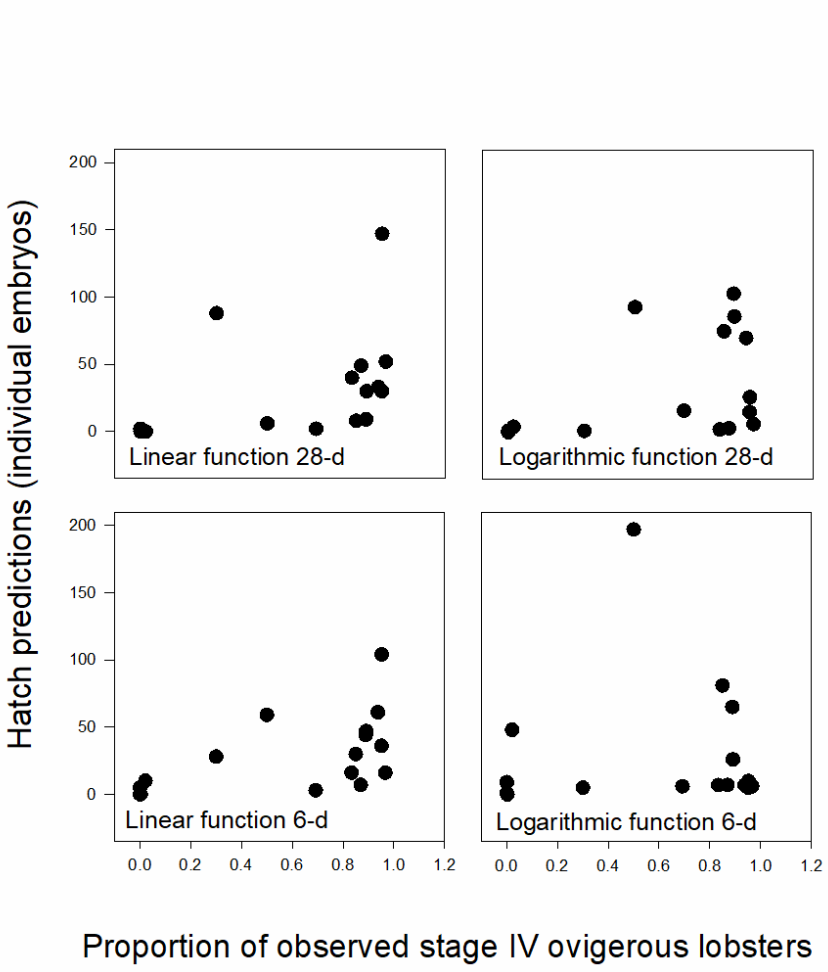

Figure 7. Relationship between the number of individual embryos that were predicted to hatch during each of 16 sampling time-steps in Dingwall, NS, and observed proportion of stage IV ovigerous lobsters during each of these sampling days. Predictions were made using two temperature-dependent development functions (linear and logarithmic), and embryos (n=480–502) that were sampled 6 and 28 days prior to the first observed hatch (female with stage IV clutch) at the site.

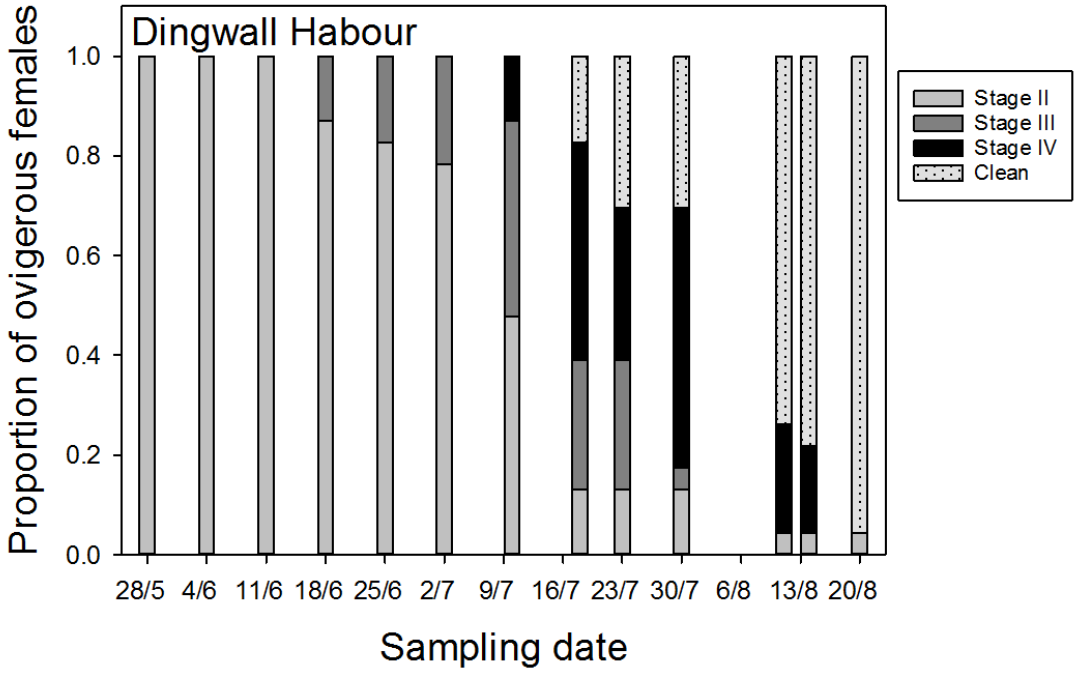

Figure 8. Observed hatch period of 22 ovigerous female American lobster caged in Dingwall Harbour, Dingwall, Nova Scotia, in 2015. The figure shows the proportion of females sampled from mid-May to late August that had eggs at different stages of development (see Methods). Clean denotes a female that had completely finished hatching, as evidenced by the absence of eggs on her abdomen. One female did not progress from stage II during the experimental period. The hatch period for the caged females was considered to be the period when females with stage IV clutches were observed, plus the number of days when no sampling occurred between samples when females with stage IV clutches were and were not observed, which in this case is 6 days. The hatch period of females caged in Dingwall Harbour is thus estimated to be between 36 and 36+12 days (see Methods).

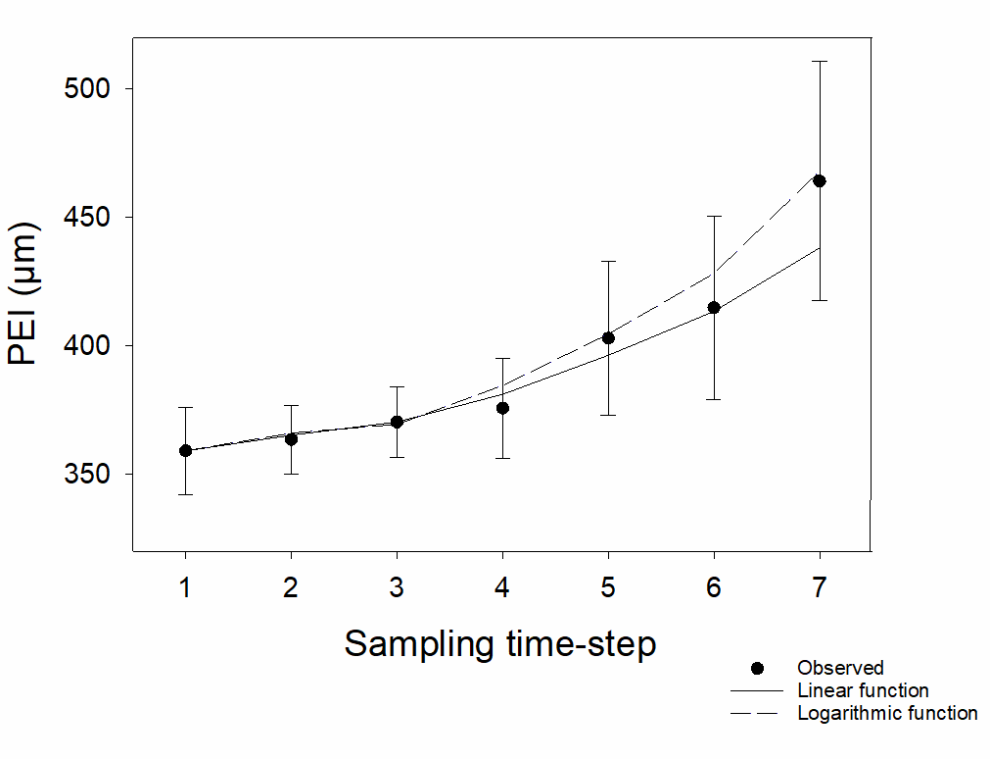

Figure 9. Mean (±SD) observed PEI of embryos from each of 22 caged females (see Results) sampled on 7 separate dates from May 28th to July 10th, 2015, in Dingwall Harbour, Dingwall, Nova Scotia (black circles). The figure also shows the mean predicted increase of PEI of the 220 embryos that were collected on the first sampling day (May 28th) over the remaining six sampling days, with predictions made using either a linear (solid line) or a logarithmic (dashed line) temperature-dependent function of embryonic development (see Methods).

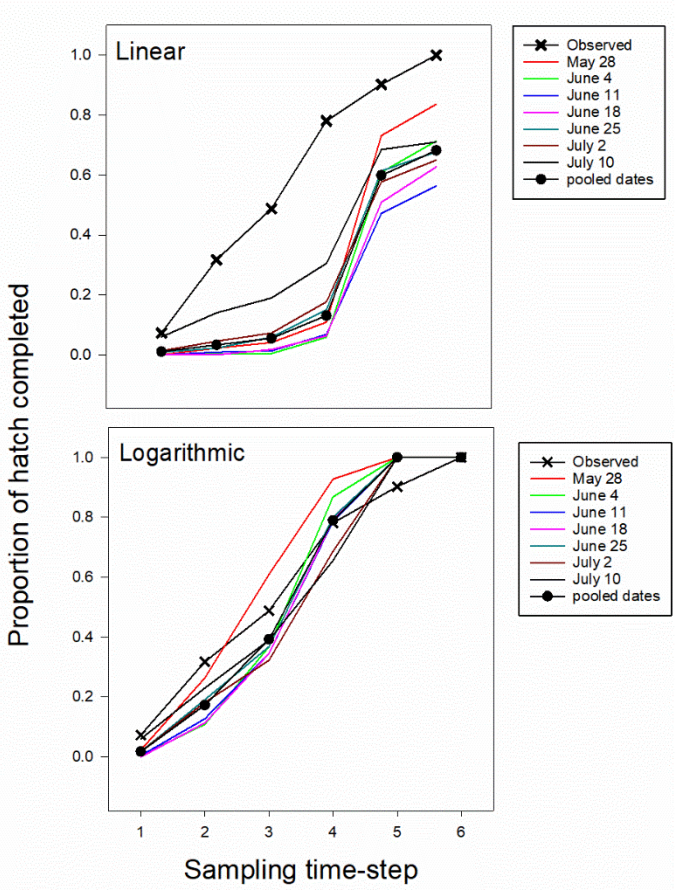

Figure 10. Observed (X) and predicted (other lines) cumulative timing of hatch in 2015 for the clutches of 22 caged females that went on to hatch in Dingwall Harbour, Dingwall, NS. Coloured solid lines represent hatch predictions made using samples collected on a particular sampling date. The line with black circles represents the hatch predictions made by pooling samples collected on all sampling dates. Observed cumulative hatch was obtained by tracking the proportion of females with hatching clutches over time (see Figure 8). Hatching was predicted by measuring eye size of embryos in eggs of ovigerous females sampled prior to hatch and collected up to and on day of the first observed hatching females (July 10th) and projecting embryo development using either a linear (upper panel) or a logarithmic (lower panel) temperature-dependent function of embryonic development (see Methods). Each time-step represents one of 6 sampling dates during the 36-day observed hatch period.

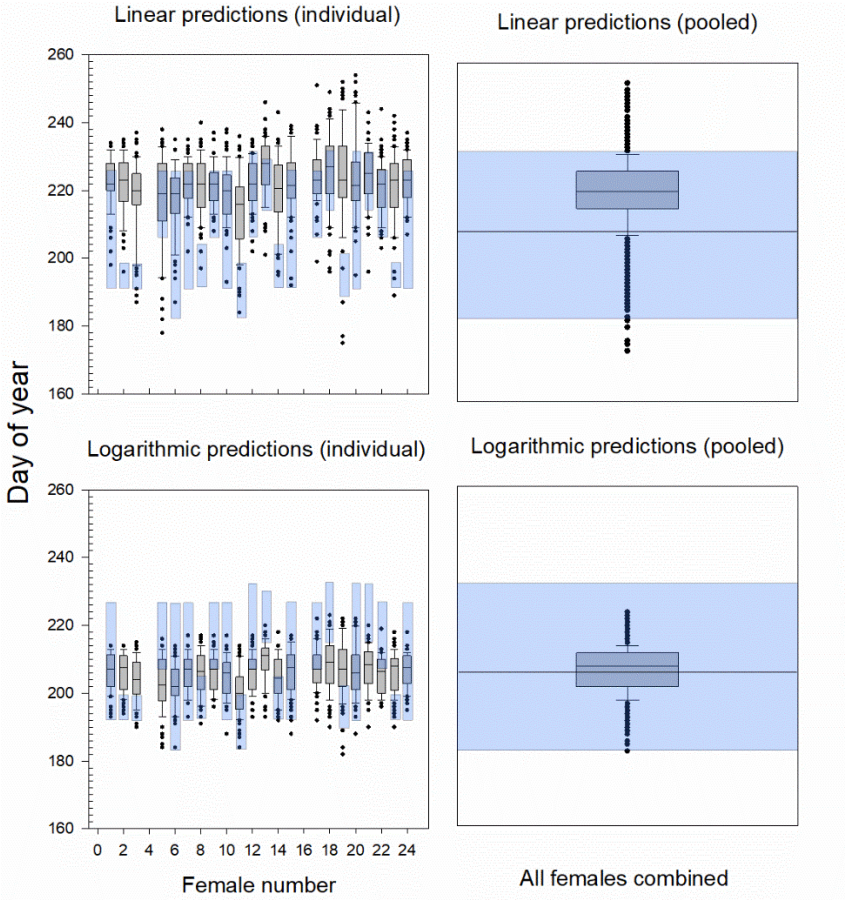

Figure 10. Predicted (boxplots) and estimated maximum (shaded area) time of hatch for 22 ovigerous female lobsters caged in 2015 in Dingwall Harbour, Dingwall, Nova Scotia. The black line signifies the average observed hatch date for the site. Hatch time was predicted by measuring eye size of embryos in eggs of ovigerous females sampled prior to hatch and up to the day the first hatching females were observed (July 10th), and projecting embryo development using either a linear or a logarithmic temperature-dependent function of embryonic development (see Methods). The maximum hatch period of each female is the first and last day hatching eggs were observed within its brood plus the number of days the female was not observed between the sampling dates prior to and after hatching embryos were observed (+/-6 d). Boxes show 25th, 50th, and 75th percentiles, whiskers represent 10th and 90th percentiles and black circles represent data falling outside the 10th and 90th percentiles

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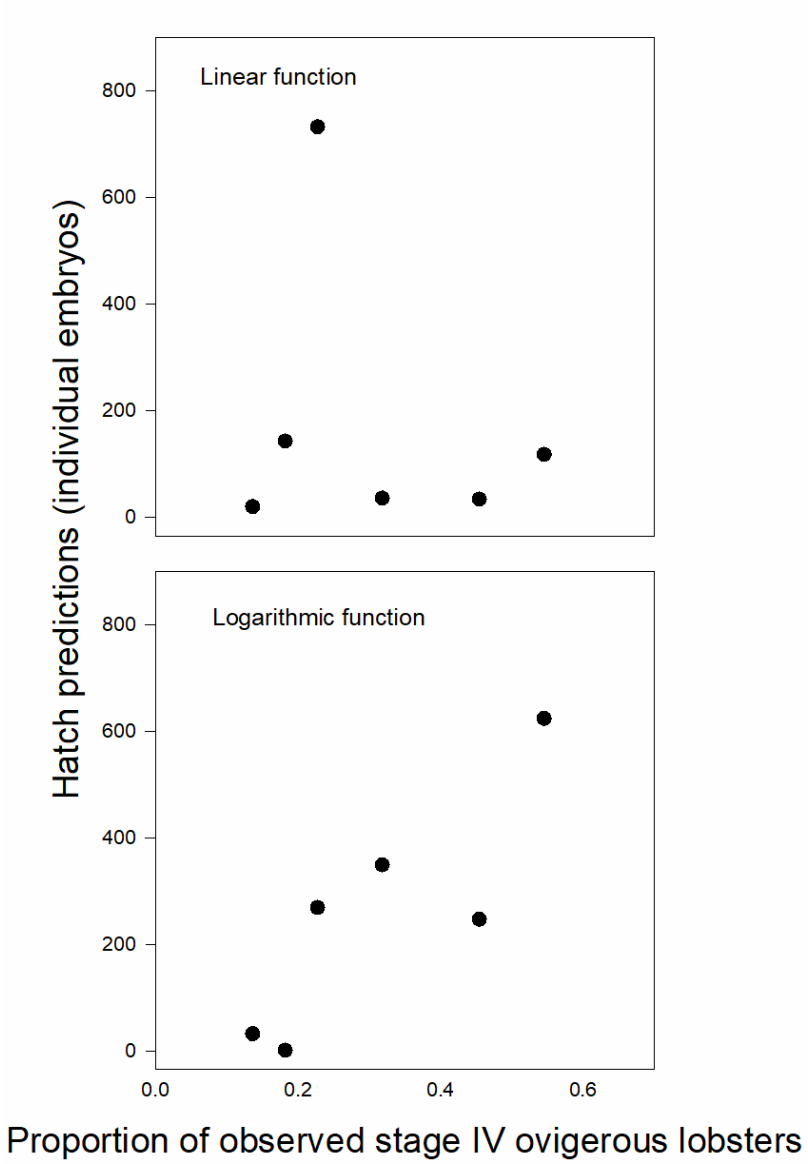


Figure 11. Relationship between the number of individual embryos that were predicted to hatch on each of 6 sampling days and observed proportion of caged ovigerous lobsters caged in Dingwall Harbour that have stage IV clutches and during each of these sampling days. Predictions were made using two temperature-dependent development functions (linear and logarithmic), and embryos (n=1520) collected from 22 females across 7 sampling time-steps preceding hatch (May 28th –July 10th, 2015).

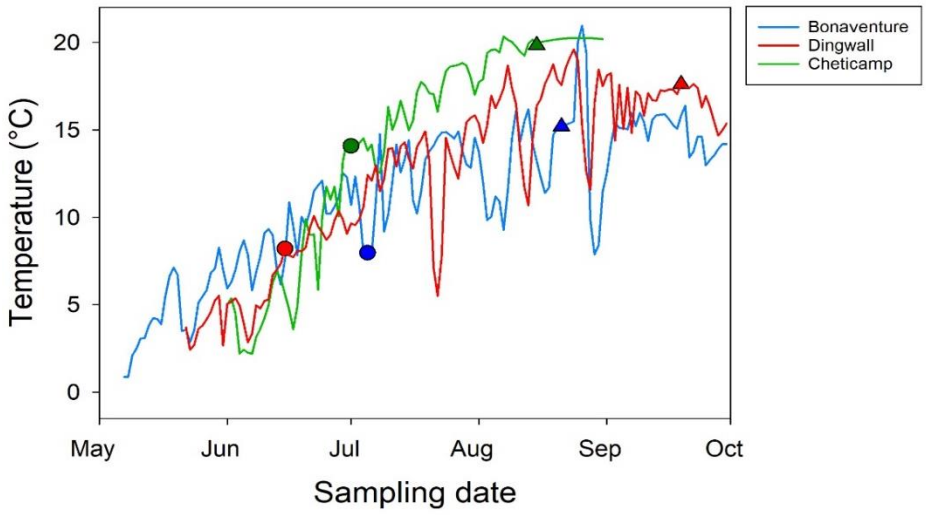


Figure 12. Temperatures from spring to fall recorded by a temperature logger placed on different lobster fishing grounds: 8.3 m depth in Bonaventure, QC, May–October 2015 (blue line); 14–16.5 m depth in Dingwall, NS, May-late October 2015 (red line); 18 m depth in Cheticamp, NS, June- early-October 2012 (green line). Temperature data from Bonaventure and Dingwall are from this study, and temperature data from Cheticamp are from Miller et al. 2016. Filled circles denote the first day of observed hatching, and the triangle symbols denote the final day of observed hatching.

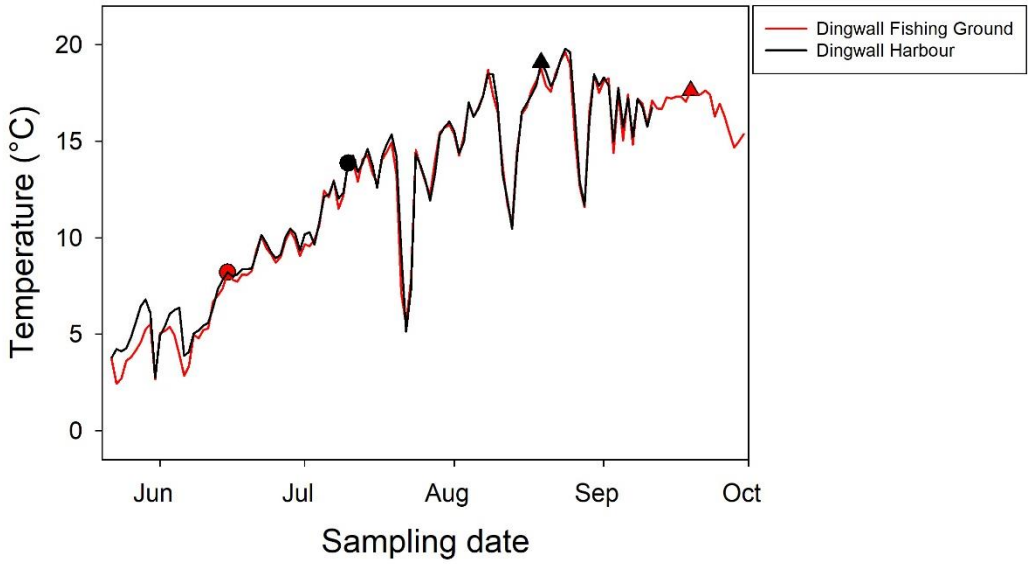


Figure 13. Temperatures recorded by a temperature logger placed on lobster fishing ground in Dingwall, NS, at 14 m depth from May–October 2015 (red line), and in Dingwall Harbour, NS, at 6.4 m depth, from May–September 2015 (black line). Filled circles and triangle symbols denote the first and last day of observed hatching, respectively.

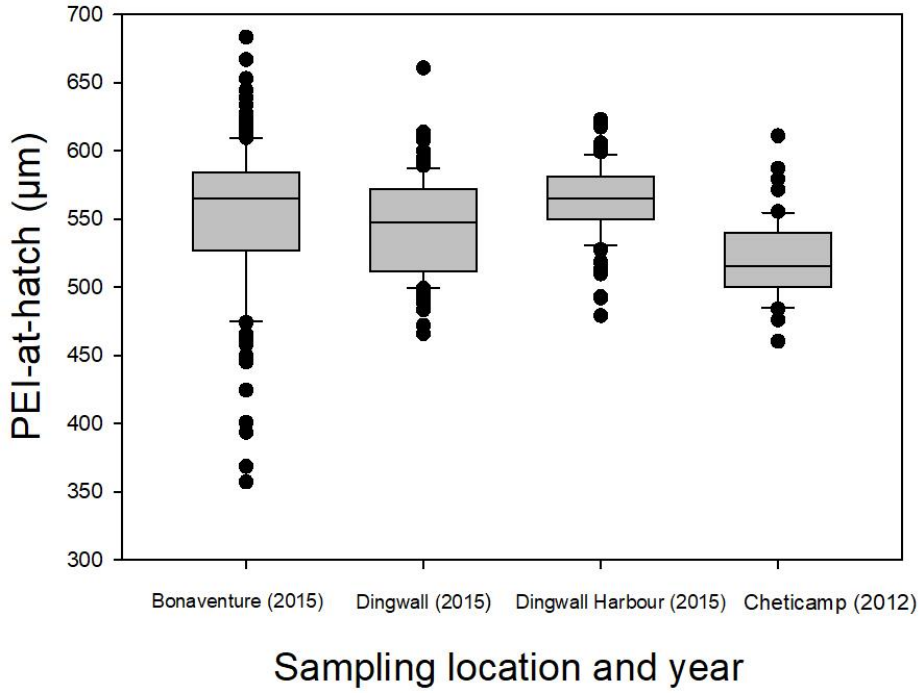
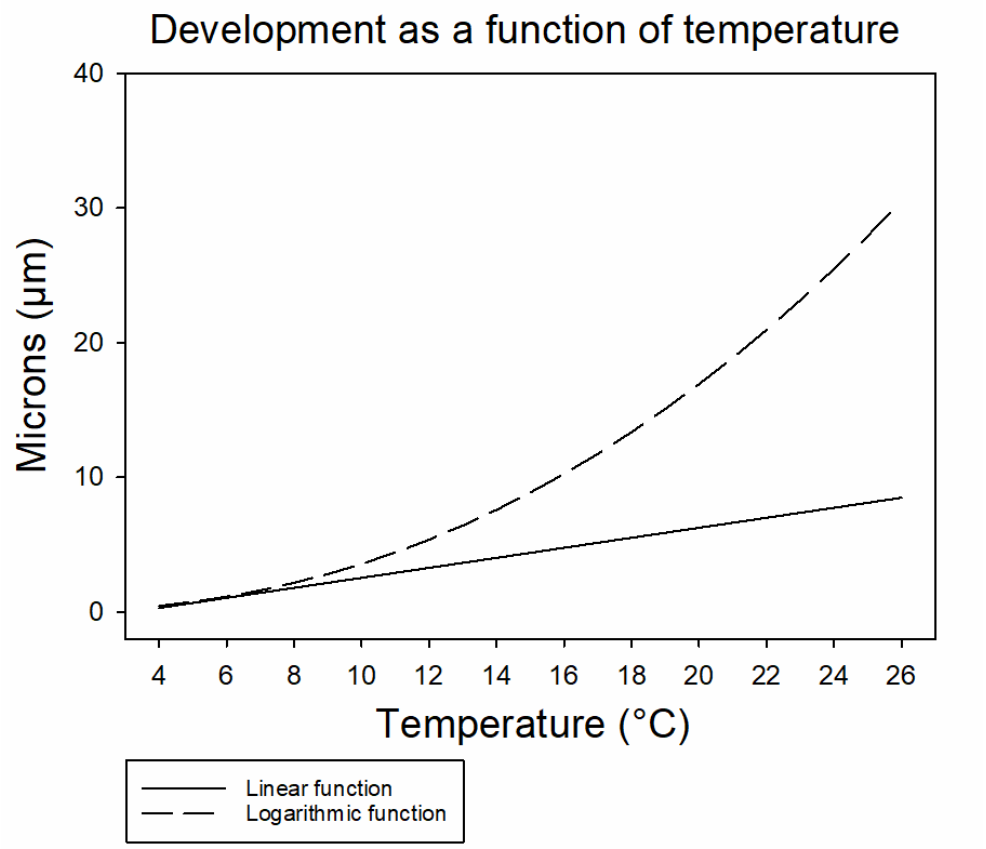
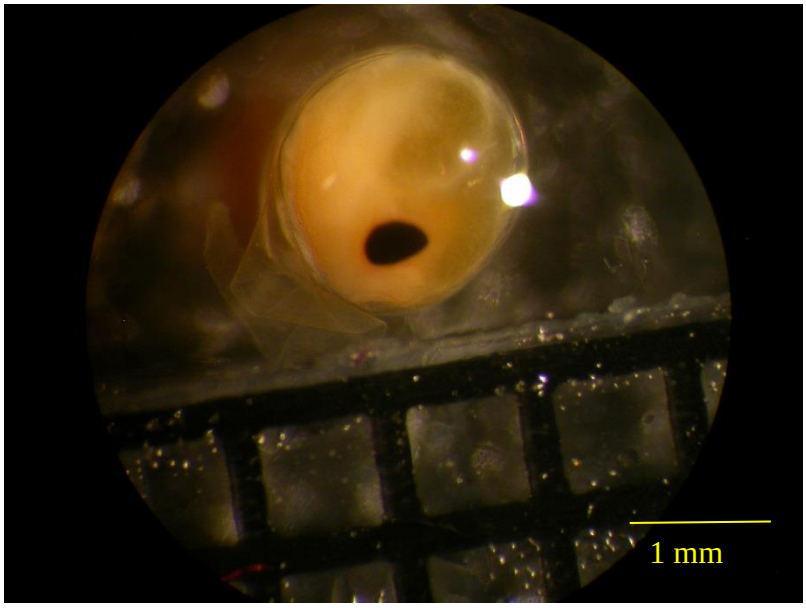


Figure 14. Frequency distribution of PEI-at-hatch for the three sampling locations used in this study in 2015, and one sampling location from a previous study in 2012 (Miller et al. 2016). Boxes show 25th, 50th, and 75th percentiles, whiskers represent 10th and 90th percentiles and black circles represent data falling outside the 10th and 90th percentiles.

Appendix A: Development as a function of temperature based on Perkin's (1972) linear model and Gendron and Ouelett's logarithmic model (2009).



Appendix B: Lobster embryo at 40X magnification with eye positioned toward camera.



Appendix C: Caged females held in Dingwall Harbour, Nova Scotia, May 21st, 2015.

Curriculum Vitae

Candidate's full name: Erin Hope Miller

Universities attended: University of New Brunswick Saint John. BSc. Marine Biology. First Class Honours. (2009-2014)

Publications:
Miller, E.H., Haarr, M.L., and Rochette, R. 2016. Using temperature-dependent embryonic growth models to predict time of hatching of American lobster, *Homarus americanus*, in nature. Can. J. Fish. Aquat. Sci.73: 1483-1492.

Conference Presentations:
Miller EH, Haarr ML, Rochette R (2013) Can the Perkins Eye Index be used to Predict Hatch Time of American Lobster (*Homarus americanus*) in the Field? Poster Presentation at the annual meeting of the Fishermen & Scientists Research Society, Truro, NS. February 20, 2013.

Miller EH, Haarr ML, Rochette R (2013) Can the Perkins Eye Index be used to Predict Hatch Time of American Lobster (*Homarus americanus*) in the field? Poster presentation at Science Atlantic Joint Aquaculture & Fisheries, Biology, and Environment Conference, Wolfville, NS. March 15, 2013.

Miller EH, Haarr ML, Rochette R (2014) Predicting time of hatch of American lobster using data collected during the fishing Season. Poster presentation at the Canadian Fisheries Research Network Lobster Node General Assembly, Halifax NS. March 26, 2014.

Miller EH (2015) Validating a method of predicting timing of hatch of American lobster,
Homarus americanus, in nature. Oral presentation at the US-Canada Lobster Symposium,
Charlottetown PEI. November 5, 2015.

Miller EH (2015) Validating a method of predicting timing of hatch of American lobster,
Homarus americanus, in nature. Oral presentation at the Canadian Fisheries Research Network Final Lobster Node Meeting, Halifax NS. November 18, 2015