Today's date: 4/1/11

Project number: R/CFR-11

Project title: Does sperm limitation take place in certain areas of the American lobster fishery and, if so, why?

Project initiation date: 2/1/2008

Principal investigator: Win Watson

Affiliation: Department of Biological Sciences

Associate investigator(s) and affiliation(s): None

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Jason Goldstein, PhD Candidate, DBS, UNH
Tracy Pugh, PhD Candidate, DBS, UNH and Mass. Div. of Marine Fisheries.
Kari Lavalli, Lecturer, Boston Univ.
Michael Clancy, Lecturer, BU

Brief project overview/Abstract:
Despite being one of the most productive and lucrative fisheries in the North Atlantic, there is continued concern that North American lobster stocks are overfished and that models used to predict fishery trends need to be recalibrated. Many of these models are based on a certain proportion of the sexually mature female lobsters contributing a large number of new recruits to the fishery each year. However, it is possible that the full reproductive potential of a portion of the sexually mature females is not being met, possibly due to skewed sex ratios and a paucity of large males. The overarching theme of this project is to determine if sperm limitation is becoming a factor in specific regions of the American lobster fishery and if so, what are some of the implications for lobster populations and the fishery that relies upon them. If sperm limitation is a factor, then we will determine if it is correlated with certain characteristics of the local populations, such as sex ratio and size differences between males and females. A final goal of this project will be to investigate one possible mechanism that might give rise to sperm limitation: the inability of small males to mate with significantly larger females.

Objectives:
1. Quantify the sex ratios and size differences between sexually mature male and female lobsters from at least 4 regions of the fishery.
2. Measure the percent of non-berried and berried females in each size class that are carrying sperm in the spring and fall of each year.
3. Determine the percentage of berried females in each region carrying fertilized eggs, as well as determining the percentage of eggs within a clutch that are fertilized.
4. Determine if small males are capable of: 1) mating with larger females and 2) providing sufficient sperm to fertilize and entire brood
5. In the laboratory, determine if eggs that are not fertilized are carried for a shorter period of time than fertilized eggs.

**Research findings/accomplishments/progress to date:**

**Objectives 1 & 2**

*Calibration Studies*

Before completing our extensive sea-sampling efforts, it was necessary to make sure that our non-invasive sperm sampling method was reliable. This goal was accomplished by sampling lobsters in various lobster pounds and then dissecting a subset of these lobsters to confirm the results. A total of 294 females were examined between June 2009 and July 2010 (Gloucester, MA, Jan 2009, n = 44; Newington, NH, March 2009 and June 2010, n = 88; Boston, MA, June and July 2010, n = 85; and Narragansett Bay, RI, June 2010, n = 77). Ninety-three percent of the females had a sperm plug, while the remainder of the lobsters did not (20). Sperm was not initially detected in fifteen of the 274 females sampled that had sperm plugs. Of these 15, 14 were dissected, and 12 (86%) had sperm in the receptacle, while two (14%) did not. Of the 20 females without a sperm plug, eight were sperm positive. It is likely that the sperm plug had yet to harden in these animals.

Based on the results of these calibration studies, we concluded that when a female had a sperm plug, but no sperm cells in the sample, the sampler did not insert the needle to a sufficient depth to collect the sperm. We also concluded that there were a few instances in which the plug was not detectable by feel, but large quantities of sperm indicated that a spermatophore was present. Thus we coded all females with either a sperm plug, or sperm in the sample even if the sperm plug was absent, as sperm positive (SP).

*Sea sampling results*

More than 70% of the females sampled in each of the eight regions either had sperm plugs or tested positive for sperm, indicating that they had successfully mated (Table 1). However, there was a considerable amount of variation between regions. For example, while approximately 85% of all female lobsters sampled in all the Gulf of Maine (GOM) regions combined were SP, 94.8% of the females in the outer Boston Harbor region were SP, while only 72.1% were SP in the Great Bay estuary (Tables 1 and 2). A higher proportion of females were SP in Rhode Island waters (94%; Table 1), and the range was smaller (min: 90.6%, Narragansett Bay, max: 98.2% in RI Sound). In the GOM a higher percentage of legal-sized females (≥ 83 mm CL) were SP compared to sublegal-sized females (< 83 mm CL), while in Rhode Island, a slightly higher percentage of sublegal-sized females (< 86 mm CL) compared to legal-sized females (≥ 86 mm CL) were SP (Table 4). Finally, more sublegal-sized females in Rhode Island were SP than in the GOM.

Nearly 98% of all ovigerous females sampled in the GOM areas were SP, while 91% of Rhode Island ovigerous females were SP (Table 2). Therefore, most ovigerous females appear to be carrying sufficient sperm to fertilize another clutch of eggs without mating again. Also, these results indicate that the only females that would not have sperm are those that never mated, either because they were sexually immature or they molted and failed to find a mate.
Table 1. Number of females sampled, size ranges for each sampling region, and percentage of those females that had a sperm plug (SP). All sampling time periods combined.

<table>
<thead>
<tr>
<th>Region</th>
<th>Total Sampled</th>
<th>Total % SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maine</td>
<td>168</td>
<td>79.2</td>
</tr>
<tr>
<td>NH - Estuary</td>
<td>229</td>
<td>72.1</td>
</tr>
<tr>
<td>NH - Coast</td>
<td>182</td>
<td>84.6</td>
</tr>
<tr>
<td>NH - Shoals</td>
<td>157</td>
<td>75.8</td>
</tr>
<tr>
<td>MA - Boston inner</td>
<td>335</td>
<td>83.9</td>
</tr>
<tr>
<td>MA - Boston outer</td>
<td>655</td>
<td>94.8</td>
</tr>
<tr>
<td>RI - S. Narr Bay</td>
<td>183</td>
<td>90.6</td>
</tr>
<tr>
<td>RI - RI Sound</td>
<td>171</td>
<td>98.2</td>
</tr>
</tbody>
</table>

Table 2. Percent of all females with a sperm plug (N sampled) by stock, and percent of all ovigerous females with a sperm plug. GOM stock includes all Maine, New Hampshire, and Massachusetts lobsters.

<table>
<thead>
<tr>
<th>Stock</th>
<th>GOM females</th>
<th>RI females</th>
</tr>
</thead>
<tbody>
<tr>
<td>All females</td>
<td>85.3 (1726)</td>
<td>94.3 (352)</td>
</tr>
<tr>
<td>Legal-sized</td>
<td>91.2 (854)</td>
<td>93.6 (249)</td>
</tr>
<tr>
<td>Sublegal-sized</td>
<td>79.6 (872)</td>
<td>96.1 (103)</td>
</tr>
<tr>
<td>Ovigerous females</td>
<td>97.6 (213)</td>
<td>90.6 (32)</td>
</tr>
</tbody>
</table>

The relationship between size and mating success

The proportion of SP females in each region generally increased with increasing size. This is to be expected because some of the smaller female lobsters examined were probably not sexually mature and therefore were unlikely to have mated. In fact, if all the female lobsters that were sexually mature successfully mated, then the proportion of SP females in each size class should match the proportion of females in that size class that were mature. To test this hypothesis we superimposed existing maturity ogives for each region with plots of the proportion of females sampled in each 1 mm CL size class that were SP. If a proportion SP data point fell below the maturity curve, fewer females of that size mated than would be expected. Conversely, if a point was above the maturity curve, more females mated than expected.

In general, in all locations, more females than would be predicted from maturity curves were SP. For example, at the Maine sampling location, more female lobsters mated than expected, particularly in the sizes ranging from the upper 70 mm CL’s to the lower 90 mm CL’s (Fig. 1). In general, there appears to be no strong evidence that sexually mature female lobsters from this region were failing to find mates. Mating success at the NH sampling sites had a similar pattern as the Maine site, with more small females mating than would be expected from existing estimates of size at maturity.
Figure 1. The relationship between size at maturity and mating success in the Friendship, ME region. The number of females sampled in each 1 mm CL size class is indicated by the gray bars (right axis). Diamonds indicate the proportion of the females in each size class that were positive for sperm or a sperm plug (left axis). The dotted line indicates the proportion of females that are mature at each size class (Boothbay Harbor maturity ogive; MEDMR data; left axis). Note that here, and in Fig. 2, if diamonds fall above the dotted line, it indicates that more females than expected successfully mated in that size class.

Smaller female lobsters in Boston Harbor also mated more often than expected (Fig. 2), and this was most extreme in the Outer Harbor area, where more than 90% of all females greater than 76 mm CL had mated, even though the existing maturity ogives for Boston Harbor suggest that only at sizes larger than 98 mm CL should more than 90% of females have successfully mated. In the Inner Boston Harbor region, only one data point fell below the maturity curve, representing one 97 mm female that was not SP. Similarly, in Outer Boston Harbor, one data point fell below the maturity curve. In neither of the Boston Harbor regions did the data indicate that a proportion of the mature females were having not mating. Rather, more females were mating that expected.

Figure 2. The relationship between size at maturity and mating success in the Boston Inner Harbor, MA region. The number of females sampled in each 1 mm CL size class is indicated by the gray bars (right axis). Diamonds indicate the proportion of the females in each size class that were positive for sperm or a sperm plug (left axis). The dotted line indicates the proportion of females that are mature at each size class (Boston Harbor maturity ogive, Estrella and McKiernan 1989, left axis).
Nearly all of the female lobsters sampled in Rhode Island Sound were SP, with only one data point below the maturity curve. However, a different pattern was observed in the Narragansett Bay sampling region. In this region, there were several data points that fell below the maturity curve, suggesting that failure to mate may be a more common event in this region than in any of our other sampling regions.

The data above suggest that either some sexually immature lobsters mate, or there has been a shift in the size at maturity in some regions since the original maturity data were obtained. To test the latter hypothesis the data from each region was used to generate a logistic model of mating success. These models were then compared to the existing maturity ogives, and the length at which 50% of females were mature (P_{mat50}) and the 95% confidence intervals around that length were examined to determine whether there was evidence for a change in the maturity schedules. Significant model fits were obtained for the data in the Maine, New Hampshire, and Massachusetts regions. However, a significant model could not be fit to the data from either Rhode Island location because an insufficient number of small lobsters were sampled. In Maine, NH, and Massachusetts sampling regions there was no overlap between the confidence intervals obtained with the sperm model and the existing maturity ogives. Moreover, in all areas the size at which 50% of females mated was smaller than the size at which 50% of the females had reached sexual maturity. Therefore, it appears that either immature female lobsters occasionally mate, or the size at maturity in all regions has shifted towards smaller size classes. Additional studies are required to decide which of these explanations is correct.

*Relationship between mating success and sex ratios*

One working hypothesis that we set out to test in this study was that the degree of sex ratio skew in a region would be correlated with the proportion of females that successfully mated and thus were carrying sperm. In fact, many of the sampling regions were specifically chosen because they had male-skewed (Great Bay, NH and Boston Inner Harbor) or female-skewed populations (all other areas, with an extreme of 78.5% females along the RI coast). However, there was no relationship between the sex ratio in a region and female mating success (percent SP) either for all size classes (r = 0.07, p > 0.05) or for only legal-sized lobsters (r = 0.24, p > 0.05).

**Objective 3**

We focused our attention in the summer of 2010 on further development of a method for determining if early stage lobster eggs are fertilized, or not. This work was very successful and we submitted a paper on the subject for publication. It has been accepted and we have submitted a slightly revised manuscript that should be published within the year.

This improved technique will allow us to have lobsters collect, and preserve, eggs for us during the normal course of their operations. This will make it possible to obtain a far larger sample of eggs than in the past. As part of our new NEC grant, we plan to complete this work in the fall of 2011.

We have also addressed this objective by sorting our sea-sampling data to examine what percentage and type of berried female lobsters (e.g., newly extruded green eggs, or late-stage eggs) in each region were carrying eggs with residual sperm reserves (Table 3).
**Table 3.** Number of egg-bearing (berried) lobsters sampled with residual sperm reserves by egg type. Green eggs are typically freshly extruded while brown and blue are considered late-stage. ‘Spent’ lobsters are those that have recently hatched out their eggs.

<table>
<thead>
<tr>
<th>Region</th>
<th>Green</th>
<th>Black</th>
<th>Brown</th>
<th>Blue</th>
<th>Spent</th>
<th>% with Sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boston (inner harbor)</td>
<td>22</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Boston (outer harbor)</td>
<td>30</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Friendship, ME</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Great Bay, NH</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>Isles of Shoals, NH</td>
<td>9</td>
<td>8</td>
<td>3</td>
<td>10</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>NH Coast</td>
<td>29</td>
<td>6</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>92</td>
</tr>
<tr>
<td>RI Sound</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td><strong>TOTALS:</strong></td>
<td><strong>120</strong></td>
<td><strong>14</strong></td>
<td><strong>27</strong></td>
<td><strong>19</strong></td>
<td><strong>7</strong></td>
<td><strong>92 % (avg)</strong></td>
</tr>
</tbody>
</table>

We chose to sample a disproportionate number of lobsters with freshly extruded egg masses to ascertain how many were still carrying sperm from a recent mating. Surprisingly the vast majority retained some residual sperm in all the regions that we sampled.

**Objective 4**

In 2010 we were able to complete a number of successful mating trials because the CML construction was complete and we received a great deal of help from Audra Chaput, an undergraduate intern. In total, 24 trials have been completed, mostly for the smaller two size difference categories. Preliminary results indicate that approximately 70% of males in the smaller two size difference treatments successfully pass a spermatophore to females, while thus far only 50% of males in the largest size difference category have been successful (Table 4, below). Video analyses are in progress for these trials.

**Table 4.** Preliminary results mating experiments with three size difference treatments. Size treatments are based on the size of the male in relation to the size of the premolt female (% smaller male). Results shown are for the proportion of trials in each treatment where a male passed a spermatophore to the female (# passed spermatophore/total # trials).

<table>
<thead>
<tr>
<th>Size treatment</th>
<th>Prop. passed spermatophore</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15%</td>
<td>0.73 (11/15)</td>
</tr>
<tr>
<td>25% - 30%</td>
<td>0.71 (5/7)</td>
</tr>
<tr>
<td>&gt; 40%</td>
<td>0.5 (1/2)</td>
</tr>
</tbody>
</table>
Objective 5
We developed a staining assay that allowed us to collect data on the fertility status of 20 different clutches of lobster eggs. The presence of egg clutches that had not been fertilized (n = 4 lobsters, size range = 78 - 100 mm CL) were likely the result of females that molted and then failed to mate. At the present time, it is not clear how common this phenomenon is in natural populations. In a separate study, we held 6 female lobsters after they had molted, so that they did not have a chance to mate. Only two of these females subsequently extruded eggs, which were unfertilized (if a spermatophore is present, it is lost when they molt) compared with the remaining lobsters (n = 4) that did not extrude eggs at all. Those egg clutches that were unfertilized typically became discolored and dropped off the female’s abdomen typically 3-4 weeks post-egg extrusion. We also found two cases (CL= 82, 85 mm) where lobsters had a mixed clutch of eggs. This situation is likely caused by a female attempting to fertilize a clutch of eggs using a spermatophore that does not contain sufficient sperm for all the eggs.

Impacts to date

1. Economic and societal benefits.
The lobster fishery is one of the most lucrative and important fisheries in New England. Our long-term goal is to provide data that will enable the industry and managers to work together to maintain a sustainable fishery. The data we have gathered to date has already shed light on the normal reproductive cycles of lobsters in the field. As a result of these data, we are beginning to question the “traditional” view of lobster reproduction and thus, the way we manage the fishery. Ultimately, when we have rigorously examined our data, we expect that it will have a strong influence on our estimates of the reproductive biomass in New England waters, and thus the way we manage the fishery.

2. Number of coastal and marine issue-based forecast capabilities developed and used for management.
As stated above, our data will be used extensively by managers. In fact, two of the scientists involved in this project are involved with managing lobsters in Massachusetts so it is likely that our data will be used in an effective manner.

   In addition, during the course of this project we have developed two new techniques. The first is a non-invasive method for determining if a female lobster has successfully mated. We are in the process of submitting this method to the Journal for Visualizing Experiments. The second is a method for staining eggs to visualize nuclei, and thus determine if they have been fertilized or not. This paper has been accepted for publication, pending minor revisions.

3. Information used by managers to improve ecosystem-based management.
A very important aspect of lobster biology is the influence of temperature on their growth and reproduction. The size at which females reach sexual maturity is strongly influenced by water temperature. This relationship is poorly understood for males. When males and females reach sexual maturity has a huge impact on fishing regulations, which are based, in part, on a minimum legal size and this is based on knowledge of when lobsters, in a given region, reach sexual maturity. Our data suggests that either lobsters are maturing at smaller sizes than expected, or immature lobsters are mating. Either situation needs will have a large impact on how the fishery is managed.

5. Education and outreach
Three graduate students (Tracy Pugh, Jason Goldstein, Tom Langley), 4 undergraduates (Kirby Johnson, Audra Chaput, Kate Masury and Casey Tobin) and a number of commercial lobstermen
have been involved in this project. One graduate student, Tracy Pugh, will use the bulk of data collected to complete her Ph.D. thesis. Many of the undergraduates have gone on to successful jobs, or professional schools, following their experiences working on this project. We continue to interact with many of the commercial lobstermen and provide them with updates about the project. Also, as stated above, we are working closely with personnel at NMFS, NOAA and other State agencies involved in regulating the lobster fishery.

Related grants and contracts:
We used some preliminary data to apply for a grant from SNECRI (Southern New England Collaborative Research Initiative) to study sperm limitation issues in Buzzard’s Bay. That proposal was successful and we are currently collecting those data, which will complement data we have already obtained.
In addition, we just recently received word that our NEC grants was approved for funding. The title is: “Biological and oceanographic mechanisms influencing lobster larvae dispersal in NH coastal waters”.

Problems encountered:
Although we have encountered some problems, most of them have been solved. As a result, we have accomplished, or will accomplish, all of our goals. Now that the CML renovations are complete, we hope to finish our mating studies in the summer of 2011 and now that we have a good method for determining if eggs are fertilized, will hope to complete objective three as well. Finally, if we can find sufficient space for holding lobsters this summer, we anticipate completing objective five as well.

Publications (please attach PDF or send a hardcopy if available)

Peer reviewed publications:

Abstract
The American lobster (Homarus americanus) is the focus of the most important commercial fishery in New England, which relies on a variety of biological monitoring programs and surveys to guide the development of appropriate management plans. One key piece of information provided by these surveys is the number of females that are carrying eggs (ovigerous) that will subsequently contribute new recruits to the fishery. A major assumption is that all eggs carried by ovigerous females are fertilized and will thus result in viable recruits. However, because some lobsters extrude, and briefly carry unfertilized eggs, this assumption needs to be re-evaluated. In particular, it is important to determine the approximate proportion of newly extruded eggs that are either fertilized, or not. The major goal of this project was to develop reliable methods for determining if early-stage lobster eggs (live and preserved) were in fact fertilized. One method involved using a nucleic stain to visualize egg DNA, after pretreatment of eggs with a proteolytic and collagenolytic enzyme solution to facilitate stain penetration through the egg membrane. With this method multi-nucleated (fertilized) eggs could be clearly distinguished from unfertilized eggs. A total of 20 egg clutches were tested to determine their fertility status using this method. Of these, 16 clutches (80 %) were fertilized while 4 were not fertilized (20 %). Of the 16 clutches with fertilized eggs, two had a mix of both fertilized and unfertilized eggs. A second method, using fluorometry to obtain measurements of total egg DNA, was also developed. There was a significant difference between the total DNA concentration in unfertilized control oöcytes and early-stage fertilized eggs (P < 0.001), and the total amount of DNA gradually increased as eggs developed. Both of these methods will make it possible to make a more
accurate assessment of the proportion of female lobsters that will actually contribute new recruits to the fishery.

Pending publications:

Other communications products (manuals, tech reports, videos, etc.):
JOVE Paper

Presentations to date, with published abstract citation if applicable:

In the fall of 2010 I was the Keynote Speaker at the College of Charleston Graduate Student Research Colloquium. I included data from this project in one of my two talks.
We will also be giving several presentations at the June International Lobster Conference. These are listed below, along with the abstracts:

Seasonal changes in the daily activity of American Lobsters (*Homarus americanus*): The influence of temperature and photoperiod

Thomas G. Langley*, Winsor H. Watson III

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Previous studies have demonstrated that the activity of American lobsters is influenced by water temperature and light:dark cycles. In general, they tend to be more active at night and have reduced activity when water temperatures drop below a certain threshold. To better understand how seasonal changes in water temperature and photoperiod altered lobster locomotion we continuously monitored the activity lobsters for two full years. Activity was recorded using accelerometers attached to the dorsal carapaces of up to 35 lobsters at a time (n=75 for the whole study). Lobsters were allowed to move freely inside 45 cm diameter enclosures that were placed inside flow-through tanks supplied with ambient water. This novel method monitoring locomotion did not restrict lobster movements and made it possible to monitor activity under a variety of temperature and lighting conditions without disturbing the animals. Lobsters were significantly more nocturnal than diurnal, and most of the animals expressed clear peaks of activity shortly after dark and before dawn. Between these peaks, lobsters expressed a less elevated level of activity in the winter, when the nights were longer; however, during the summer, animals remained fairly active throughout the night. Surprisingly, even when water temperatures dropped close to 0°C lobsters remained active throughout the winter. The results of this study demonstrate the utility of accelerometers for monitoring the activity of freely moving lobsters and indicate that lobsters are likely to be quite active in their natural habitat throughout even the coldest months of the year.
Two methods for determining if early-stage American lobster (*Homarus americanus*) eggs have been fertilized

Kirby J. Johnson, Jason S. Goldstein* and Winsor H. Watson III

Department of Biological Sciences, University of New Hampshire, 46 College Road, Durham, NH 03824 USA. email: j.goldstein@unh.edu.

The American lobster (*Homarus americanus*) is the focus of the most important commercial fishery in New England and Atlantic Canada, and the appropriate management of this fishery relies on a variety of biological monitoring programs. One key piece of information is the number of females that are carrying eggs that will subsequently contribute new recruits to the fishery. A major assumption is that all eggs carried by ovigerous females are fertilized and will thus result in viable recruits. However, because some lobsters extrude, and briefly carry unfertilized eggs, this assumption may need to be re-evaluated. The major goal of this project was to develop reliable methods for determining if early-stage lobster eggs (live and preserved) were in fact fertilized. One method involved using a nucleic stain to visualize egg DNA, after pretreatment of eggs with an enzyme solution. With this method multi-nucleated (fertilized) eggs could be clearly distinguished from unfertilized eggs. A total of 20 egg clutches were tested to determine their fertility status using this method. Of these, 16 clutches (80 %) were fertilized while 4 were not fertilized (20 %). Of the 16 clutches with fertilized eggs, two had a mix of both fertilized and unfertilized eggs. A second method, using fluormetric methods to obtain measurements of total egg DNA, was also developed. There was a significant difference between the total DNA concentration in unfertilized control oocytes and early-stage fertilized eggs (p < 0.001), and the total amount of DNA gradually increased as eggs developed. Both of these methods will make it possible to make a more accurate assessment of the proportion of female lobsters that will actually contribute new recruits to the fishery.

Influence of natural inshore and offshore thermal regimes on egg development and time of hatch in the American lobster *Homarus americanus*

Jason S. Goldstein* and Winsor H. Watson III

Center for Marine Biology and Department of Biological Sciences, University of New Hampshire, 46 College Road, Durham, NH 03824 USA. email: j.goldstein@unh.edu.

Studies of ovigerous lobster (*Homarus americanus*) movements indicate that many display seasonal inshore-to-offshore movement patterns that serve to expose eggs to developmentally-optimal thermal regimes. The overall aim for this study was to determine the impact of naturally fluctuating temperature regimes on egg development and time to hatch in lobsters exposed to natural inshore or offshore bottom temperatures over the full course of their development. We subjected ovigerous lobsters to natural inshore or offshore seasonal fluctuations in water temperature, either in the laboratory (n = 14 inshore, 8 offshore) or in the field (n = 8/each inshore/offshore). Temperatures averaged 7.08 ± 0.19 °C for inshore laboratory simulations (total degree days = 922) compared with offshore 6.40 ± 0.17 °C (total degree days = 798) ones.
There were no statistically significant differences between ambient (field) and simulated (lab) thermal regimes. While the rate of egg development between inshore and offshore animals did not differ significantly in the fall, inshore eggs developed significantly faster than eggs exposed to offshore temperatures in the spring (p < 0.001). Eggs exposed to inshore thermal regimes hatched ~ 15-20 days earlier (mean = June 26) than offshore eggs (mean = July 27), and their time of development, from extrusion to hatch was significantly shorter (inshore = 287 ± 11 days vs. offshore: 311.5 ± 7.5 days). These results suggest that seasonal movements of ovigerous lobsters have a strong influence on both the location where larvae originate and their time of hatch.

The impact of ovigerous lobster movements on egg development, larval hatch, and settlement

Jason S. Goldstein* and Winsor H. Watson III

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The American lobster (*Homarus americanus*) fishery is one of the most economically important fisheries in the Northwest Atlantic and Gulf of Maine (GoM) and its success has been attributed, in-part, to a high degree of broodstock conservation. Studies of ovigerous lobster movements indicate that some, if not most, display seasonal inshore-to-offshore patterns, and it is generally accepted that these movements serve to expose eggs to warmer offshore water temperatures, which should accelerate egg development. The overall goals of this study were to first confirm that lobsters in NH coastal waters carried out seasonal inshore-offshore movements and secondly, to determine the impact of these movements on egg development, hatch, and subsequent settlement. Ultrasonic tracking of both ovigerous (n=24) and non-ovigerous lobsters (n=26) revealed that most lobsters move offshore in the late fall and ovigerous lobsters tend to remain there until after their eggs hatch. Both laboratory and field studies demonstrated that that the eggs carried by lobsters that moved offshore actually hatched > two weeks later than eggs exposed to inshore temperatures. Finally, experimental ocean drifters were deployed in the vicinity of lobsters when their eggs were hatching to determine where these larvae might drift to and settle. Most drifters released in offshore hatching locations were carried south and were either near the coast of Massachusetts or offshore banks locations at the time when larvae would settle. Our results suggest that offshore movements of ovigerous lobsters impacts when and where eggs will hatch and, subsequently, where new recruits settle. Thus, these data have significant implications for population connectivity and management of this important fishery.
Empty inside: are ‘large’ female lobsters (*Homarus americanus*) in Southern New England failing to mate?

Tracy L. Pugh, Jason S. Goldstein, Kari L. Lavalli, Michael Clancy, and Winsor H. Watson, III.

Abstract

The Southern New England stock of the U.S. lobster (*Homarus americanus*) population is depleted and the ASMFC Lobster Technical Committee has recently declared the stock to be in a state of recruitment failure. The potential for rebuilding this stock depends, in part, on larval production and recruitment, one aspect of which is mating success. However, low abundance, coupled with female-skewed sex ratios in some areas, could set the stage for sperm limitation in Southern New England. We sampled female lobsters captured in the coastal waters of Rhode Island and southern Massachusetts and tested for the presence of a sperm plug or sperm in their seminal receptacles, as an indicator of mating success. Data (% with sperm) were compared to local size-at-maturity indices (% mature) to determine if all the potentially mature females were actually mating. Preliminary results indicate that while many smaller-than-expected females mated, some larger lobsters that should have mated did not. There are several possibilities to explain why these females failed to mate, including reduced encounter rates, an over-reliance on small males, and depleted males due to a combination of low abundance and female mate choice. Current management practices make the assumption that mature females reproduce on a predictable cycle, based on regional growth rate information. This assumption may not hold true if there are portions of the population where sperm limitation is a factor, and lobster management may need to shift its perspective from female-centric biological controls to a broader, more inclusive perspective to conserve reproductive potential.

A non-invasive method for determining mating success in female American lobsters (*Homarus americanus*)

Jason S. Goldstein, Tracy L. Pugh, Kari L. Lavalli, Michael Clancy, and Winsor H. Watson III

Despite being one of the most productive and lucrative fisheries in the North Atlantic, there is continued concern that North American lobster stocks are overfished and that the models used to predict fishery trends might need to be recalibrated. For example, recent findings suggest that the full reproductive potential of all the sexually mature females is not being met, because they are either failing to mate or not receiving sufficient sperm to fertilize an entire clutch of eggs. The purpose of this project was to determine if sperm limitation is becoming a factor in different regions of the American lobster fishery. Our first goal was to develop a reliable and non-invasive method for sampling large numbers of female lobsters at sea. The technique we settled on involved inserting a needle into the seminal receptacle of females to both determine the presence or absence of a sperm plug and to remove sperm, if it was present. In order to confirm that the method was reliable before applying it under field conditions, we conducted a series of control studies in the laboratory.
Variability in spermatophores produced by male lobsters (*Homarus americanus*), and their relationship to size at maturity

Tracy L. Pugh and Winsor H. Watson, III

The quantity (and quality) of sperm produced by a male lobster may affect the fecundity of his female partner. Additionally, spermatophores transferred by the male also contain seminal fluids, which may serve aid in preserving the spermatozoa and, when hardened into a sperm plug, block mating attempts by other males. Previous work has demonstrated variability in the size and contents of *H. americanus* spermatophores, and some contained no spermatozoa. Our goal was to extend upon this work and determine whether male lobsters of different sizes differ in the quantity or content of ejaculate produced, as this variability may impact the realized fecundity of their mates. In addition, we aimed to determine if the ability to produce spermatophores or their characteristics, could be used to determine if a male lobster was functionally and/or physiologically, mature. Spermatophore extrusion was stimulated by applying an electrical current near the gonopores of male lobsters, at the base of the male’s 5th periopods. If spermatophores were extruded they were weighed and photographed, and the images were analyzed to calculate the ratio of seminal fluid (plug material) to spermatozoa. We will review the successes and failures of a pilot study utilizing these techniques on more than 70 males and preliminary results along with a summary of an improved methodology will be presented.

**Students Supported**

**Student name:** Jason Goldstein

Institution/Department: DBS, UNH  
Duration of support: Summer 2010  
Type of support (stipend, travel, supplies, etc.): Salary  
Type of degree (undergrad, masters, PhD): PhD  
Year degree awarded: 2011  
Title of thesis (if supported by N.H. Sea Grant): The impact of seasonal temperatures on the movement, egg development, and larval release in ovigerous American lobsters (*Homarus americanus*)

**Student name:** Tom Langley

Institution/Department: DBS, UNH  
Duration of support: Summer 2010  
Type of support (stipend, travel, supplies, etc.): Salary
Type of degree (undergrad, masters, PhD): MS

Year degree awarded: 2011

Title of thesis (if supported by N.H. Sea Grant): The influence of seasonal changes in water temperature on the movements of sexually mature and immature lobsters in the Great Bay estuary

Student name: Tracy Pugh

Institution/Department: DBS, UNH

Duration of support: Summer 2010

Type of support (stipend, travel, supplies, etc.): Supplies

Type of degree (undergrad, masters, PhD): PhD

Year degree awarded: Only in First Year

Title of thesis (if supported by N.H. Sea Grant): The reproductive success of American lobster: evaluating the influence of males and the subsequent impacts to lobster fishery management.

Student name: Kirby Johnson

Institution/Department: Department of Biological Sciences

Duration of support: Portions of 2009-2011

Type of support (stipend, travel, supplies, etc.): Stipend and supplies

Type of degree (undergrad, masters, PhD): BS

Year degree awarded: 2010

Where is he/she now? Traveling.

Student name: Casey Tobin

Institution/Department: Zoology

Duration of support: 2008-2009

Type of support (stipend, travel, supplies, etc.): Helping with lab work and data analysis.

Type of degree (undergrad, masters, PhD): BS

Year degree awarded: 2009

Where is he/she now? Working at a drug company.
Student name: Audra Chaput

Institution/Department: Department of Biological Sciences

Duration of support: May-October of 2010

Type of support (stipend, travel, supplies, etc.): Helping with field work in the summer.

Type of degree (undergrad, masters, PhD): BS

Year degree awarded: 2010

Where is he/she now? Graduate School.