Video Article

A Noninvasive Method For *In situ* Determination of Mating Success in Female American Lobsters (*Homarus americanus*)

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Abstract

Despite being one of the most productive fisheries in the Northwest Atlantic, much remains unknown about the natural reproductive dynamics of American lobsters. Recent work in exploited crustacean populations (crabs and lobsters) suggests that there are circumstances where mature females are unable to achieve their full reproductive potential due to sperm limitation. To examine this possibility in different regions of the American lobster fishery, a reliable and noninvasive method was developed for sampling large numbers of female lobsters at sea. This method involves inserting a blunt-tipped needle into the female's seminal receptacle to determine the presence or absence of a sperm plug and to withdraw a sample that can be examined for the presence of sperm. A series of control studies were conducted at the dock and in the laboratory to test the reliability of this technique. These efforts entailed sampling 294 female lobsters to confirm that the presence of a sperm plug was a reliable indicator of sperm within the receptacle and thus, mating. This paper details the methodology and the results obtained from a subset of the total females sampled. Of the 230 female lobsters sampled from George's Bank and Cape Ann, MA (size range = 71-145 mm in carapace length), 90.3% were positive for sperm. Potential explanations for the absence of sperm in some females include: immaturity (lack of physiological maturity), breakdown of the sperm plug after being used to fertilize a clutch of eggs, and lack of mating activity. The surveys indicate that this technique for examining the mating success of female lobsters is a reliable proxy that can be used in the field to document reproductive activity in natural populations.

Video Link

The video component of this article can be found at https://www.jove.com/video/50498/

Introduction

American lobster (*Homarus americanus*) is one of the most productive fisheries in the North Atlantic (~ 56,000 mt in 2011, valued over $390 million)[1]. However, there is a general lack of understanding regarding the reproductive dynamics of this species in wild populations. Generating more accurate estimates of reproductive output, including the number and sizes of individuals actively participating in reproduction, may improve the stock assessment process. For example, females that are prevented from achieving their full reproductive potential due to sperm limitation have been identified as a concern for several commercially exploited marine crustaceans including: spiny lobsters[2], blue crabs[3], king crabs[4], stone crabs[5], and snow crabs[6]. The overall goal is to determine if sperm limitation might be a factor in certain regions of the American lobster fishery as well.

The first signs of a potential sperm limitation problem in lobsters were observed while conducting an unrelated tagging study with ovigerous female lobsters (with visibly intact, recently extruded eggs). Recapture reports from fishermen indicated that ~15% of these animals had dropped their egg clutches after only 1-2 months. The working hypothesis was that some lobsters were carrying eggs that had not been fertilized and, as a result, 'fell off'[7]. Preliminary data from a subsequent study have confirmed the fact that lobsters will extrude eggs even if they have not successfully mated and these unfertilized eggs are only carried for ~ 1 month[8]. Therefore, given the observation of females carrying unfertilized eggs in natural populations, we sought to determine the extent to which sperm limitation might be contributing to a submaximal level of reproduction by sexually mature lobsters. To achieve this goal, a technique was developed to detect the spermatophore deposited by males during mating.

This paper and video describe a noninvasive, simple method developed for ascertaining the mating success of female lobsters. The technique can be utilized quickly and reliably at sea, either aboard research or commercial fishing vessels. The details of this sampling method as well as some representative findings are presented to illustrate the application of the technique.

Protocol

Part A: Field Sampling Technique

1. Female Measurements

1. For each female lobster, measure the carapace length (CL) and the width of the second abdominal segment to the nearest 1.0 mm using a pair of calipers.
   NOTE: The second abdominal segment is measured because the width of the abdomen (where females carry their eggs) is an indicator of sexual maturity. In addition, it might be advantageous to ascertain the molt stage to later match these data with reproductive stage (see step 1.2).
2. Clip a small distal portion off one of the abdominal pleopods (a standard methodological protocol in lobsters) and store the pleopod in clean seawater for later viewing with a dissecting microscope[9].
   NOTE: This step might also reveal the presence of cement glands on the pleopods, which are an indication that females are preparing to extrude their eggs[10].

2. Needle Insertion

1. Insert the syringe needle (18 G, see **Table 1**) ~1 cm into the seminal receptacle, angled toward the tail at ~45° (**Figure 1**).
   NOTE: In lobsters with intact sperm plugs, the needle may meet some resistance during insertion, as it is pushed through the sperm plug prior to reaching the highest concentration of sperm at the bottom of the seminal receptacle (**Figure 1**).
   NOTE: The presence of the plug, in itself, is a reliable indicator that lobsters have mated. In some animals there may be little resistance, possibly because no spermatophore is present, the spermatophore is small, it has not hardened into a plug yet, or it may have already been used to fertilize one or more clutches of eggs.

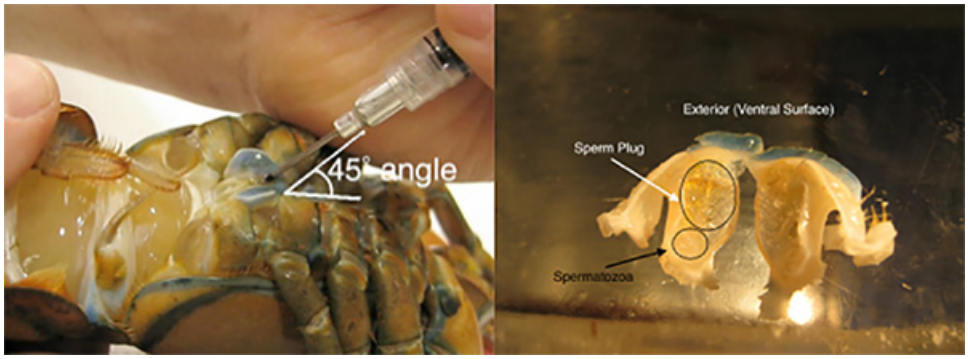

Figure 1. Needle insertion and view of lobster seminal receptacle. Inserting the blunt-tipped needle into the seminal receptacle of the lobster to extract a sperm sample. The angle and depth of the needle are both critical to obtaining consistent samples. Right: View of a seminal receptacle that has been dissected and split vertically. The sperm plug occupies the top or outermost portion of the receptacle, while the spermatozoa are located at the bottom (deepest point) of the receptacle. It is important to note that sampling below this depth may puncture the receptacle and result in a blood-contaminated sample. Click here to view larger image.

3. Penetrating the Sperm Plug

1. After the initial insertion, steepen the angle of the needle to nearly vertical (perpendicular to the body axis), and very slowly start to work it downwards into the receptacle.
2. Keep the needle tilted at an angle so that the tip is pointed slightly towards the tail. In this case the needle tip is compressing the top of the plug, until it eventually penetrates into the matrix.
3. Continue to slowly push the needle downwards until resistance is felt once more - this is the bottom of the receptacle and where the sample is taken.

4. Sperm Removal

1. Remove a sample (often solid plug material, liquid sperm and other fluid) from the bottom of the receptacle and deposit the sample into a labeled 2.0 ml plastic tube.
2. Flush the receptacle with ~0.1-0.5 ml of cold seawater.
3. Remove approximately 0.3 ml of fluid and place in the same tube.
4. Store sperm samples on ice until they can be examined in the laboratory.
   Part B: Examination of Sperm Samples in the Lab
   NOTE: Samples removed from the seminal receptacles of individual lobsters should be examined to determine if sperm cells were present or absent.
5. Remove ~50 µl of fluid from each plastic tube, place on a glass slide with a cover slip, and view at 100X with a compound microscope.

6. Score samples as: sperm scarce, numerous, or absent (this study did not quantify actual sperm numbers, **Figure 2**).

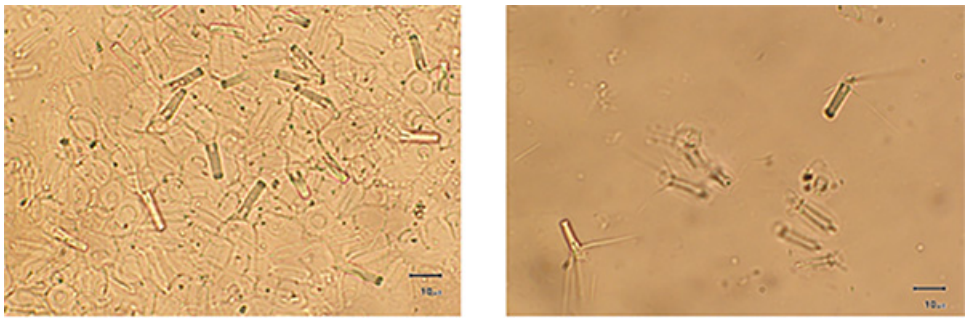

Figure 2. Lobster sperm sample images. Images of lobster sperm samples taken from a female's seminal receptacle, as viewed with a compound microscope at 40X magnification. Image on left shows a sample that contained numerous sperm while the other (right) illustrates a sparse sample (scale bar = 10 µm). Click here to view larger image.

Representative Results

The data reported here were for a subset of female lobsters that originated from both nearshore and offshore locations. The nearshore group consisted of 44 lobsters that were captured in the coastal waters near Cape Ann, Massachusetts and transported in coolers to the laboratory for examination. A total of 186 offshore lobsters were captured on George's Bank and sampled at a commercial holding facility in New Hampshire.

Sperm samples were obtained from all of the animals and immediately examined for the presence or absence of sperm according to methods described above. A total of 90.3% of all females in this subset had sperm in their seminal receptacles. Surprisingly, those that lacked sperm were not necessarily at the smaller end of the size range (**Figure 3**).

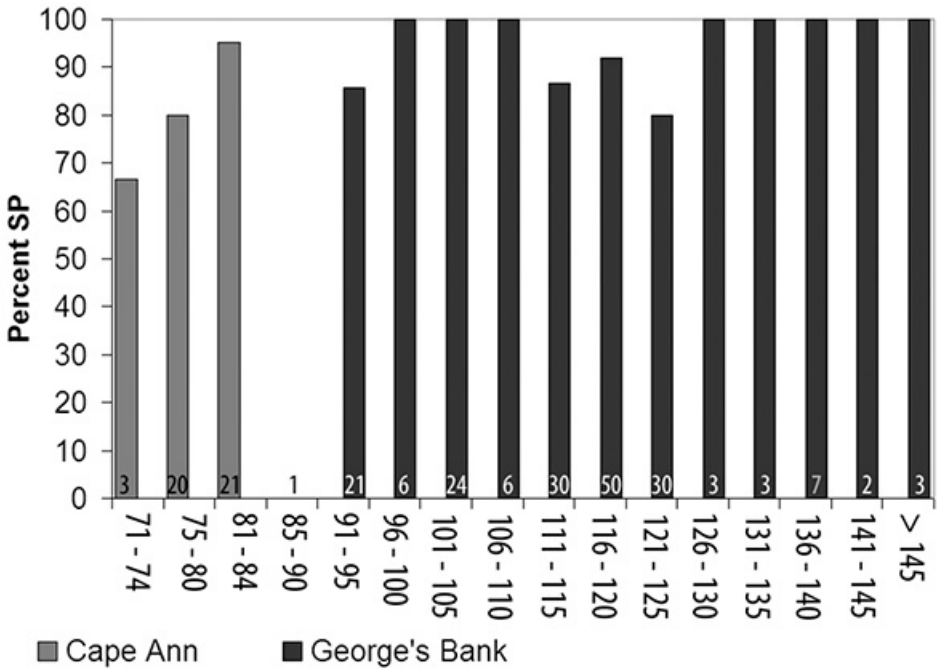

Figure 3. Sampling results for presence of sperm in lobsters. Percent of female lobsters in 5 mm CL size class bins that had sperm in their seminal receptacles (percent positive) as an indicator of mating. Females were captured from waters near Cape Ann, MA (71-83 mm CL) and from George's Bank (89-154 mm CL). Numbers inside bars represent total sample size for that bin. Click here to view larger image.

Discussion

This video and paper outline and demonstrate a method for determining if female lobsters have successfully mated. This approach is simple enough that it can be used for large-scale sampling of lobsters at sea by a variety of users. The method is based on detecting the presence of a spermatophore in the seminal receptacle that is passed from male to female lobsters during mating. These spermatophores partially harden into a sperm plug shortly after they are deposited in the receptacle; thus, the presence of a sperm plug is also indicative of mating success. In this study, as well as a subsequent larger study, it was determined that most of the sperm plugs had sperm associated with them[11]. However, while the presence of a plug is a reliable indicator of mating activity, its absence is not.

Although we cannot be entirely certain if this sampling methodology incurs a negative effect on the ability of female lobsters to spawn and successfully fertilize their egg clutch, there are aspects to this sperm sampling protocol that suggest that damage would be minimal to either the spermatophore or the receptacle. There are data (from ongoing lab studies) that indicate some lobsters, post-sperm sampling, still extrude fertilized clutches of eggs (n=3) and are capable of fertilizing more than one clutch of eggs using the same spermatophore. These eggs develop and hatch similarly to lobsters that have not been sampled. In addition, this sampling technique only serves to sample a small fraction of the total sperm. It is not intended to quantitatively sample the full contents of a receptacle. Due to the complex physical architecture of the receptacle[12,13], a different technique (*e.g.* dissection) would be required to obtain those kinds of results.

Sperm was found in a number of sampled animals that lacked a sperm plug, possibly because the spermatophore was relatively new, and had not yet hardened into a plug, or the spermatophore had been previously used to fertilize one or more clutches of eggs and had deteriorated. Therefore, in order to obtain the most accurate index of mating success it is important to both check for a sperm plug and sample for sperm.

Accurate orientation of the needle during the sampling procedure is critical for several reasons. First, assuring that the needle is parallel to the lateral body plane prevents it from penetrating the thin side of the seminal receptacle. When the technique is performed correctly, it causes no mortality, as the needle never actually penetrates the body cavity. Second, the 45° insertion angle, followed by a steepening of the needle, helps to ensure that the needle reaches the bottom of the receptacle where the spermatozoa are concentrated without penetrating the wall of the spermatophore and entering the adjacent body sinus (**Figure 1**).

There was some deterioration of the sperm samples if they were not handled appropriately. Lobster sperm undergo their acrosomal reaction quite easily, sometimes as a result of mechanical agitation[13]. Once the sperm have reacted, they begin to break down in the sample and become difficult to identify. For this reason, agitation of the sample vials should be kept to a minimum. Keeping the samples cool also appears to help preserve the integrity of the sperm. Finally, it is suggested to use sterile seawater when sample processing. This may act to minimize the growth of bacteria and other microorganisms (over 36-48 hr) that might damage the sperm.

Most (90.3%) of the female lobsters sampled had mated successfully, based on the presence of sperm in their seminal receptacles. The remainder of the animals might not have been sperm positive because either they were sexually immature, their sperm plugs had deteriorated over time or had been fully used to fertilize one or more clutches of eggs, or they had never mated, possibly because they never encountered an appropriate male at the right time in their molt cycle[11]. Female lobsters in the George's Bank region have a relatively large size-at-maturity, with only 50% of females mature at 100 mm[14]. Cape Ann females mature at a slightly smaller size than do the offshore lobsters, with 50% of females mature at 90 mm CL[15]. Therefore, given that the smallest females were 71 mm CL, it is not unreasonable to suggest that some of the females lacking sperm plugs were likely immature and thus, would not have mated. Many large female lobsters, such as those sampled in our offshore subset, may spawn in consecutive years without molting and remating[16].

Multiple fertilizations using the same spermatophore may eventually result in a breakdown of the plug material and may use up the sperm contained in the receptacle, yielding a negative result. However, the mechanical breakdown of the sperm plug over time has not, to our knowledge, been described. Finally, it is possible that those females without sperm in their receptacles were unable to locate a mate. Skewed female sex ratios have been documented in the George's Bank lobster stock[17], and the possibility that this leads to sperm limitation deserves further attention due to the potential consequences for the reproductive output of the lobster population.

Disclosures

The authors declare no conflicts of interest.

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