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Evidence of mating by sexually immature female American lobsters *Homarus americanus* (H. Milne Edwards, 1837) (Decapoda: Nephropidae)

Winsor H. Watson III[1], Jason S. Goldstein[1,2], Elizabeth M. Morrissey[1,3], Hannah A. Cole[1] and Tracy L. Pugh[3]

[1]*Department of Biological Sciences and School of Marine Science and Ocean Engineering, University of New Hampshire, Durham, NH 03824, USA;*
[2]*Maine Coastal Ecology Center, Wells National Estuarine Research Reserve, 342 Laudholm Farm Road, Wells, ME 04090, USA; and*
[3]*Massachusetts Division of Marine Fisheries, 30 Emerson Ave., Gloucester, MA 01930, USA*

Correspondence: Winsor H. Watson III; email: win@unh.edu

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ABSTRACT

Recent findings indicate that female American lobsters, *Homarus americanus* (H. Milne Edwards, 1837), are either reaching sexual maturity at smaller sizes than in the past, due to ocean warming or fishery-induced selection, or they are mating before they are sexually mature. To test the second hypothesis, we determined the mating status (i.e., presence of sperm in seminal receptacles) and physiological maturity (i.e., ovary stages) of 208 females captured off the coast of New Hampshire, USA. We found that 27.8% of the females with immature ovaries had sperm in their seminal receptacles, indicating that some females mate while still immature. We proceeded to record the behavior of immature females that were about to molt after placing them in tanks with mature males to determine if mating was consensual. A mature female that is about to molt typically approaches a male in a den, exchanges chemical signals (pheromones) with the male, enters the den to cohabitate with the male, and then molts, mates, and remains with the male while her soft, post-molt, exoskeleton hardens. Previous studies have demonstrated that in order for this sequence of behaviors to take place normally, the exchange of chemical signals is important, and probably necessary. In this study, 11 of 14 immature females exhibited normal mating behavior and successfully mated. It therefore appears that some female lobsters become sexually active before they are physiologically capable of reproducing. Moreover, these females might use "dishonest signals" to trick males into mating to gain protection from them while their exoskeleton hardens.

Key Words: lobster fishery, pheromones, sexual maturity, spermatophore

INTRODUCTION

The American lobster, *Homarus americanus* (H. Milne Edwards, 1837), is found from inshore coastal waters to offshore depths of more than 600 m in the northwestern Atlantic Ocean from North Carolina, USA to Newfoundland and Labrador, Canada. The American lobster is commercially harvested from Newfoundland, Canada to Virginia, USA, and it is one of the most valuable single-species fisheries in the northeastern United States. According to the Maine Department of Marine Resources (MEDMR), more than 56.2 million live kg (124 million pounds) were landed in Maine in 2014, with an ex-vessel value of more than US$458 million (http://www.maine.gov/dmr/commercial-fishing/landings/documents/11-15LandingsBySpecieswithBonus.Table.pdf).

There are currently three biologically based regulations in place to help maintain a sustainable American lobster fishery. Females with eggs must be released, lobsters above a certain size cannot be harvested in some areas, and lobsters below a certain size (83−89 mm carapace length (CL), depending on the region) must also be released. This minimum size has been established based in part on the size at which 50% of female lobsters are mature, with the goal of ensuring sufficient reproduction takes place to sustain the resource. Understanding the reproductive dynamics of the American lobster, including size at maturity and mating behavior is therefore a critical aspect of the underlying science behind lobster management.

The mating behavior of the American lobster has been studied extensively, starting as early as the 1930s (Templeman, 1934, 1936). Although molting can take place during any molt stage (Waddy & Aiken, 1990a), mating activity is typically initiated immediately after a female molts (Atema *et al.*, 1979; Atema, 1986; Atema & Steinbach, 2007), which typically takes place once a year in late spring and summer. Pre-molt females seek out males that are defending mating shelters, then cohabitate with the male for up to several days prior to, and after, molting. During mating, the male places a spermatophore (i.e., gelatinous package of sperm) into the female's seminal receptacle, a pouch-like structure on the female's underside (Talbot *et al.*, 1983). The female typically uses the sperm contained in the spermatophore in late summer or early fall to fertilize her eggs as they are extruded, and then they are cemented to the setae on the abdominal pleopods. If she molts prior to spawning, the stored spermatophore is lost along with the rest of her old exoskeleton. The cohabitation period that follows mating can be considered mate guarding by the male, a common characteristic in crustacean mating systems (Thiel & Duffy, 2007). Cohabitation aids in paternity assurance for the male, whereas the female gains protection during the vulnerable post-molt period while her exoskeleton is hardening. Chemical communication is a critical component of lobster mating behavior, because pheromones released by females reduce male aggression and allow cohabitation (Atema & Engstrom, 1971; Atema *et al.*, 1979; Atema, 1986; Atema & Cowan, 1986; Bushman & Atema, 1997).

We sampled over 1,600 female American lobsters in the Gulf of Maine and southern New England, U.S.A. (Pugh *et al.*, 2013), and an additional 3,851 females in Buzzard's Bay, Massachusetts (Pugh, 2014) to examine mating activity in females. We found that more than 75% of the females were carrying sperm in their seminal receptacles and had therefore successfully mated. Surprisingly, in many of the smaller size classes, significantly more females had mated than would be expected based on existing size at maturity data (Pugh *et al.*, 2013). There are two possible explanations for this finding. One is that female size at maturity is very closely related to water temperature (Aiken & Waddy, 1980; Little & Watson, 2003, 2005) and thus ocean warming due to climate change could be causing a downward shift in the size at maturity. A second hypothesis is that a portion of females mate before they reach sexual maturity (having undeveloped ovaries that are not capable of producing viable eggs; Waddy & Aiken, 2005), even though they are not capable of using the sperm received to fertilize their eggs. The goal of this study was to test the second of these two hypotheses by determining if some of the females carrying spermatophores are actually sexually immature, as well as pairing immature pre-molt females with males to see if they exhibit "normal" (consensual) mating behaviors.

MATERIALS AND METHODS

Determining the mating and maturity status of lobsters

Lobsters for all experiments were captured in New Hampshire coastal waters (Southern Gulf of Maine) by commercial lobstermen (New Hampshire Fish and Game Permit MFD 1520 to WHW) in the spring and early summers of 2011–2014 and held communally with other females in flow-through ambient seawater systems at the University of New Hampshire (UNH) Coastal Marine Laboratory. After being held for about 2–3 weeks, the following information was obtained for all 208 female lobsters used in the first experiment: 1) carapace length (CL) to the nearest mm; 2) abdomen width (AB), to the nearest mm; 3) molt stage; 4) presence of a sperm plug and/or sperm in the seminal receptacle (spermatophore positive, "SP"; Goldstein *et al.*, 2014); 5) cement gland stage (see Aiken & Waddy, 1982), and 6) ovary size and color (see Aiken & Waddy, 1982). These procedures provide information about the reproductive and maturity status of female lobsters and they have been described in detail in a number of previous manuscripts (Aiken & Waddy, 1980, 1982; Little & Watson 2003, 2005; Waddy & Aiken 2005). Lobsters with ovaries that were at developmental stages 1–3 (based on the color and size of individual eggs), and which had the appropriate AB:CL ratios (less than the ratio of berried females captured in the same area), and/or cement gland stages (less than stage 2), were considered to be immature (Aiken & Waddy, 1982). To obtain ovary samples we used the technique described by Little & Watson (2003, 2005). A 1 cm diameter hole was cut with a fine pair of scissors in the dorsal carapace just above the region where one of the anterior extensions of the ovaries is located, and a small group of about 50 oocytes were removed for examination. The portion of exoskeleton that was removed was then replaced and covered with a piece of duct tape, which was secured in place with superglue. Lobsters were then returned to a seawater tray to recover for several days, and then the duct tape was removed and the individuals were released. In previous studies (Little & Watson, 2003, 2005), as well as in this study, no mortalities occurred because of this procedure. Ovary indices (i.e., "ovary factor"), which require the removal and weighing of the complete ovary (Waddy & Aiken, 2005), were also determined for a subset (n = 30) of the lobsters that were assessed as being immature, according to the aforementioned criteria. All of the lobsters we considered to be immature based on the measurements stated above were also immature based on their ovary indices (Aiken & Waddy, 1982; Waddy & Aiken, 2005) indicating that the less invasive method for ovary staging was sufficient for assessing the maturity status of the lobsters that was used in our study.

Statistical analyses

Each female lobster was then assigned a value of 0 or 1 (negative or positive, respectively) for mating (SP) and maturity status. Logistic regressions were then fit to the SP and maturity status data by size in the R statistical package using the GLM function, with family defined as binomial (R Core Team, 2014). Model fits were assessed using a goodness-of-fit test as well as examination of residuals (Faraway, 2006). To estimate the size at which 50% had mated (belong to SP) and at which 50% were mature, we used the "p.dose" function from the MASS software package in R, which returns the predicted value (estimated by the logistic model) at the specified proportion (0.5 in this case).

Mating behavior

To determine if mating was "consensual," we placed individual pre-molt females in one of four replicate 1 m × 1 m Plexiglas aquaria containing a PVC "mating shelter." A single mature male lobster was also placed in each of the aquaria, which was always the same size or larger than the female. Continuous time-lapse recordings of courtship, mating, and other associated behaviors were obtained for each trial using digital time-lapse video cameras (BrinnoTLC200 Pro, Walnut, CA, USA) that captured one image every five seconds. Mating data were collected using seven males having a mean size of 81.9 ± 1.4 mm CL, CL_{range} = 78–88 mm CL (values expressed as averages ± SEM) and 17 females with a mean size = 62.8 ± 0.84 mm CL, CL_{range} = 54–70 mm CL. To determine if adult male lobsters could distinguish between males and females, we also paired six pre-molt males (mean size = 64.7 ± 1.9 mm CL, CL_{range} = 60–72 mm CL) of approximately the same sizes as the pre-molt females (student's t-test for size difference; P = 0.4123) with mature males. We observed female lobsters for at least 36 h post-molt to determine if the males cohabitated with them after mating. These females were then removed and their maturity status was assessed as described above.

RESULTS

Do immature females mate in the field?

In contrast to previous studies, we determined both the maturity status (ovary condition) and mating status (sperm in seminal receptacle, or sperm positive, SP) for each of the 208 females examined, which made it possible to document that immature females did indeed mate. Of the 208 lobsters examined, 149 were sexually immature (size range = 53−86 mm CL) and of these 28.2% were SP (Table 1). Out of the 59 sexually mature females examined, (size range = 65−105 mm CL), 91.5 % had mated (Table 1). Logistic regressions were fit to model the relationship between size (CL) and maturity, and between size (CL) and mating status (SP), and model diagnostics indicated a good fit to the data in both cases (maturity: $\chi^2 = 28.9$, P = 0.88, R^2= 0.80, SP: $\chi^2 = 32.9$, P = 0.74, R^2= 0.82). The resulting ogives showed that a higher proportion of females had mated (were SP) at a given size than were sexually mature based on the aforementioned physiological and morphological criteria (Fig. 1). The estimated size at which 50% of females had mated (75.4 mm CL; LCI = 73.7 mm, UCI = 77.1 mm) was significantly smaller than the size at which 50% were mature (82.0 mm CL; LCI = 79.8, UCI = 84.2). All these data support the hypothesis that some immature females mate in their nature habitat.

Observations of immature lobsters mating

The aforementioned data, which was obtained from lobsters in their natural habitat, suggest that some females become sexually active before they are physiologically ready to successfully spawn (i.e., extrude and fertilize their eggs). Because the lobster's mating system is generally characterized by female choice, it seems likely that these immature females voluntarily participated in mating and, in turn, the male lobsters recognized them as potential mates. To determine if the mating of immature females with mature males was, in fact, consensual, and followed the same paradigm that has been described previously for mature females and males (Atema, 1986), we observed the behavior of 17 females individually placed in an aquarium with a mature male. Of the 17 females, 14 had immature ovaries (stage 3 or less; Supplementary Material Fig. S1), one had mature ovaries (Supplementary Material Fig. S2; excluded from subsequent analyses), and two were consumed by the male immediately after they molted, which prevented us from determining their maturity or mating status. The mature female mated, as did 78.6% (11 of 14) of the immature females. The remaining five females (three immature and two of unknown sexual condition) did not mate. Lobsters that successfully mated cohabitated 72.8 ± 9.6% of the time they were in the tank before molting and mating (average duration they were observed = 47.5 ± 0.37 h, range = 2.0−107.5 h), and 100% of the time after copulation (average duration they were observed = 31.4 ± 0.1 h, range = 5.5−36.0 h). In contrast, immature females that did not mate also did not cohabitate (see Supplementary Material Video S3), with the exception of one female that was in the shelter for several hours before she molted and was eventually eaten.

Table 1. The number of female *Homarus americanus* sampled that were immature and mature (summarized in 5 mm CL bins) and the percentage of each that had mated (% SP).

CL bin	Immature females		Mature females	
	Number	% SP	Number	% SP
51–55	1	0.0	0	
56–60	5	0.0	0	
61–65	31	3.2	1	100.0
66–70	42	11.9	2	0.0
71–75	26	53.8	7	71.4
76–80	24	29.2	10	100.0
81–85	16	75.0	14	92.9
86–90	3	66.7	14	100.0
91–95	1	100.0	4	100.0
96–100	0		4	100.0
>100	0		3	100.0
TOTALS/AVE.	149	28.2%	59	91.5%

Mating behavior was considered "normal" if: 1) there was cohabitation (lasting > 2 h pre-copulation and > one-day post-copulation) and 2) it appeared to be consensual (females were cooperative and males did not require multiple attempts; see Supplementary Material Video S4). The behavior of immature females that mated did not deviate from normal mating behavior, although on at least one occasion the male required two attempts to complete the process. Such behavior has nevertheless also been observed on several occasions with mature females and males (Pugh, 2014). The size of the females had some impact on mating success. Immature females that mated successfully were significantly larger (64.6 ± 1.4 mm CL) than those that failed to mate (59.8 ± 1.6 mm CL; unpaired t-test, P = 0.045). In contrast, there was no significant difference in the size of females that expressed normal mating behavior (63.9 ± 1.4 mm CL) vs those whose behavior was not normal (60.4 ± 1.9 mm CL; unpaired t-test, P = 0.16).

One of the clearest indications that females probably provided some type of signal in order to elicit normal mating behavior from males was the fact that three of the females that failed to mate were eaten by the males after they molted (one was also killed before she molted). This was also the case in 50% of the males that molted in the presence of a mature male. Given the importance of pheromones in lobster mating, our working hypothesis is that a major effect of the pheromone released by females is to reduce the aggressiveness of males (Atema & Engstrom, 1971) so the male does not consume them during the particularly vulnerable post-molt period.

DISCUSSION

The results of this study clearly demonstrate that some physiologically immature female American lobsters exhibit normal mating behavior and successfully mate, even though they will most likely fail to produce offspring. Other investigations have also reported finding small, apparently immature, females carrying spermatophores in their seminal receptacles (Templeman, 1936; Krouse, 1973; Waddy & Aiken, 1990b). To our knowledge, however, this is the first study that specifically examines the co-occurrence of stored sperm and immature ovaries over a range of sizes, along with assessing the mating behavior of immature females in the laboratory. The range of lobster sizes here examined represents the range over which they are transitioning from immature to mature in this particular region. Specifically, examination of the SP vs maturity ogives, suggests that the immature females that mate are typically one molt away from becoming sexually mature. Atema *et al.* (1979) noted that some lobsters in the 58−70 mm CL size range exhibited incomplete formation of the typical pair bond and proposed this size range represents a transitional period during which appropriate sexual behavior develops. Because water temperature strongly influences size at maturity, however, this size range likely varies by geographical location. Both our data on sperm and observations on behavior appear to support this hypothesis and demonstrate that some females are capable of completely normal mating behavior even though they are unlikely to be capable of utilizing the sperm they receive, because they will probably molt and lose the spermatophore before their ovaries become mature.

Several authors had previously stated that the presence of sperm in the seminal receptacle of a female lobster is not a reliable indicator of the ability to spawn (Waddy & Aiken, 2000; 2005). While unlikely, however, it is possible that some females that are considered immature based on ovarian condition can complete ovarian development prior to their next molt and spawn successfully. For

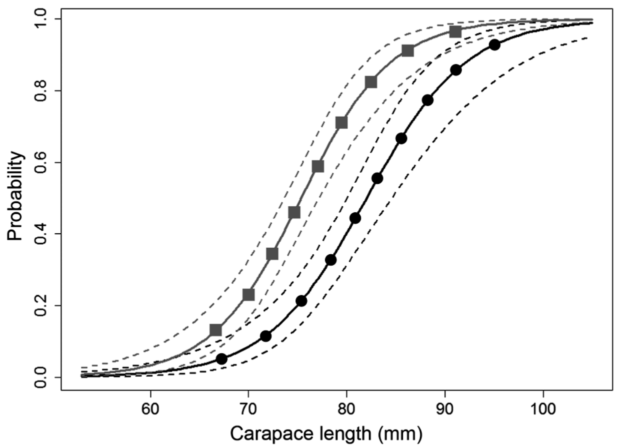

Figure 1. Logistic regression models showing the predicted proportion of female *Homarus americanus* in each 1 mm CL size bin that were physiologically mature (black line with circles, model parameters: $\beta_0 = -16.14 \pm 2.3$, $\beta_1 = 0.20 \pm 0.03$) and that had successfully mated (SP; gray line with squares, model parameters: $\beta_0 = -16.46 \pm 2.2$, $\beta_1 = 0.22 \pm 0.03$). Dotted lines indicate upper and lower 95% confidence intervals (UCI, LCI) for each curve.

logistical purposes, we obtained the lobsters used in this study in the summer and fall and it is possible that, by the following summer, the ovaries of some immature lobsters would have finished maturing and, rather than molting, they could have used the stored sperm to fertilize their eggs. This is nevertheless unlikely for those females with stage 1 and 2 ovaries based on published accounts of the time course of typical ovarian development and maturation, especially at the water temperatures typical in the Southern Gulf of Maine (Waddy & Aiken, 2005). Large mature female lobsters will occasionally skip a molt either to recover from spawning (Waddy & Aiken, 2000) or to fertilize a second batch of eggs using the sperm remaining in their seminal receptacle (Templeman, 1936; Waddy & Aiken, 1986). We are nevertheless not aware of any data indicating that an adolescent, or sub-adult, lobster will forego molting in order to use sperm obtained the previous year to fertilize a clutch of eggs. Nonetheless, some of the lobsters with stage 3 ovaries that were on the large end of the size frequency distribution might have exhibited this behavior and were thus conveying "honest" signals with respect to their ability to reproduce.

Recent increases in ocean temperatures, along with an intense and extensive lobster fishery, may also have selected for a small decrease in female size at maturity (Gaudette *et al.*, 2014). The maturity ogive generated in this study was similar to the maturity ogive reported in a previous 2003 study that used lobsters captured in the same vicinity (Little & Watson, 2003), but the estimated size at 50% maturity was 82.0 mm CL, compared with 86.0 mm CL in 2003. This suggests that the size at maturity of female lobsters may have shifted downwards in the last eight years, perhaps due to the factors cited above. As in previous studies (Pugh *et al.*, 2013), there was approximately a one-molt interval difference between the size of immature females that had mated and those that were mature (for example, about 40% of females mated by 74 mm CL, while about 40% of females were mature and capable of spawning at 80 mm CL; Fig. 1). It appears that New Hampshire coastal lobsters may be maturing at a smaller size than when they were previously assessed due to climate change or fishing pressure (Landers *et al.*, 2001). Our results strongly suggest that this would only explain a portion of the small lobsters that were sperm positive in this investigation and in Pugh *et al.* (2013). Moreover, we clearly demonstrated that some immature females do mate in a normal manner. It is noteworthy that Templeman (1936) made similar observations as early as 1936, and a recent re-examination of historical data suggests that immature female mating might be common in Canadian waters as well (Gaudette *et al.*, 2014).

While there are a number of examples of immature insects mating (Reif *et al.*, 2002; Tuni & Berger-Tal, 2012), in many of these cases the sperm obtained remain viable until the female becomes mature, and therefore she is able to eventually use it to fertilize her eggs. If the female ovaries do not fully mature prior to the next molt in the American lobster, however, the contents of the seminal receptacle are shed with the molt and thus they lose the stored sperm (Waddy & Aiken, 2000). Even though immature lobster mating will not lead to successful reproduction, there are several possible reasons why they might mate. It could be in their best interest to "trick" the male, so that he will protect her during the post-molt period when she is most vulnerable to predation. While the use of "dishonest signals" by insects has received considerable attention (Funk & Tallamy, 2000; Barry, 2015), much less is known about this phenomenon in crustaceans (Steger & Caldwell, 1983; Wilson *et al.*, 2007). Mate guarding is common in decapods and other crustaceans and we did observe post-copulatory guarding (cohabitation) in this study. Atema & Steinbach (2007) noted how post-copulatory/post-molting mate guarding is an important adaptation, both for vulnerable females and so that males can protect their reproductive investment. A second reason why immature females might mate is that this type of behavior might allow them to "practice" mating. This appears to take place in some spiders, and while male spiders do not pass sperm to the female while "practicing," non-conceptive "practice" sex serves to increase reproductive success for those individuals who express this behavior (Pruitt & Riechert, 2011). Despite the extended lifespan for American lobsters (> 20 years), and the capacity for long-term fecundity, continued fishing pressure means that many female lobsters are likely harvested either before they ever reproduce, or after just one mating opportunity. The size at which 50% of female lobsters were sexually mature is about 83 mm CL in our study area (this varies regionally due to differences in water temperature). The legal size limit in New Hampshire waters is also 83 mm CL. It might therefore be adaptive both for females to reach sexual maturity at a smaller size, and for them to "practice" mating when they are approaching sexual maturity, so success will be more likely when they do become mature. The onset of courtship and associated reproductive behaviors may not be inextricably linked to the development of the ovaries in the same way that ovary development is not tightly linked to somatic growth in lobsters (Aiken & Waddy, 1980). Some lobsters may thus become sexually active and capable of releasing the appropriate pheromones one molt before they are capable of using the sperm they receive to fertilize their eggs and this trait may be maintained in the population because there are no apparent disadvantages to the females of this behavior.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Figure. Image of a partially dissected, immature *Homarus americanus* female showing ovaries *in vivo*.

S2 Figure. Image of a partially dissected, mature *Homarus americanus* female showing ovaries *in vivo*.

S3 Video. Video shows the absence of appropriate mating behavior in *Homarus americanus*.

S4 Video. Video shows the normal mating behavior in *Homarus americanus*.

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