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Patterns of sexual cohabitation and female ejaculate storage in the American lobster (*Homarus americanus*)

Abstract Little is known of the time and ejaculate allocation strategies during mating of American lobster, *Homarus americanus*. This study investigated sexual cohabitation and female ejaculate accumulation patterns in a laboratory mating experiment, as well as female seminal receptacle load in exploited populations in the waters of the Magdalen and Anticosti Islands, in eastern Canada. In the laboratory experiment, the length of sexual cohabitation was proportionate to female size for large but not for small males. Also, large males cohabited with pre- and postmolt females longer than small males. These different time investment strategies can be explained by different mutual benefits. In the field and laboratory, larger females accumulated more ejaculate than smaller ones. This suggests that male lobsters tailor ejaculate to female size, a reliable index of her reproductive potential. Moreover, similarly-sized females accumulated more ejaculate when mated with large compared to small males. Comparison of receptacle loads between wild-mated and laboratory-mated females suggests that the former were mating mainly with smaller males, although some evidence of positive size-assortative mating existed, especially at the less exploited Anticosti site. The results are discussed in the context of evolutionary theory and of proposed management measures to increase egg production in exploited populations.

Keywords Lobster · Sperm allocation · Sexual cohabitation · Mating system

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Introduction

Males of many species have evolved the ability to use their sperm reserves judiciously (review in Wedell et al. 2002), because they are costly to produce (Dewsbury 1982; Pitnick and Markow 1994). Depending on the mating system, male ejaculation strategies in terrestrial and marine animals can generate sexual conflicts that affect female fitness (Nakatsuru and Kramer 1982; Birkhead and Fletcher 1992). One such case is sperm limitation, which arises when the number of sperm is insufficient to fertilize all eggs produced by a female population (Pennington 1985; Pitnick 1993). In polyandrous species, intensity and risk of sperm competition may favor males who carefully partition their ejaculate among females based on anticipated frequency of mating opportunities and female reproductive output (review in Wedell et al. 2002). In polygynous species, favorite or dominant males that mate at high frequencies sometimes become depleted of sperm because of limited production and time to replenish reserves (Warner et al. 1995; Kendall et al. 2001, 2002; Preston 2001).

Even if conditions favorable to sperm economy and limitation occur in many marine invertebrates with direct sperm transfer, especially crustaceans, the issue has received little attention until recently. In decapod crustaceans, males of the spiny lobster *Panulirus argus* tailor their ejaculate to female size, a reliable indicator of fecundity (MacDiarmid and Butler 1999). In the blue crab *Callinectes sapidus,* ejaculate size and reserve are affected by male size and mating history, which can be modified by fishing, which selectively harvests large males (Jivoff 1997; Kendall and Wolcott 1999; Kendall et al. 2001, 2002). In the snow crab *Chionoecetes opilio*, males modulate ejaculate allocation to the availability and size of females (Rondeau and Sainte-Marie 2001). In the course of a more than 100-year-long history of research into the mating behavior of the American lobster, *Homarus americanus*, females have monopolized attention and to date very little or nothing is known of male time and ejaculate investments. American lobster (hence-

forth 'lobster') is heavily exploited (Anonymous 1995) and because of natural lobster behavior and fishery regulations, landings may be biased to males (Templeman 1936a; Skud and Perkins 1969; Dubé and Grondin 1985; Campbell 1992; Gendron and Savard 2000)—a context that might promote sperm limitation.

The mating system of lobster is quite unique among the Crustacea, although there is some commonality with the shelter-based mating of fiddler crabs (Christy and Salmon 1984; Goshima et al. 1996) and stomatopods (Hatziolos and Caldwell 1983; Christy and Salmon 1991). Sexual dimorphism emerges very gradually in lobster and is barely apparent in adults of less than about 100-mm carapace length (CL). For a given CL, the female abdomen is slightly broader and male claws are slightly larger compared to the opposite sex (Herrick 1911; Templeman 1935). In crustaceans exhibiting strong sexual dimorphism, males use their chelae for courting, grasping and/or caging the female during sexual activity (Hartnoll 1969; Stein 1976; Christy and Salmon 1984; Shine 1989). In contrast, male lobsters do not (Templeman 1934). Both field and laboratory observations suggest that female lobsters initiate courtship and compete for access to dominant males and their shelters (Atema et al. 1979; Karnofsky et al. 1989; Cowan and Atema 1990; Bushmann and Atema 1997). Chemical advertisement and shelter defense by males, as well as partial courtship role reversal (Atema et al. 1979; Atema 1986; Karnofsky et al. 1989), may have evolved as a time investment strategy used by males to obtain more mates. Males of many insect species trade food for sex by combining nutrients with sperm and seminal fluid in the spermatophore (Thornhill 1976; Marshall and McNeil 1989; Vahed 1998). Male lobsters may potentially trade protection for sex by sheltering the female during her vulnerable molt, which immediately precedes mating.

Once accepted by a male lobster, the female is allowed to enter the male's shelter where she eventually molts and is mated. In general, oviposition does not occur until several months later (Herrick 1911; Aiken and Waddy 1980; Comeau and Savoie 2002). Coercive "mate guarding" by the male is limited to the brief period of time after molting when the female cannot resist male behavior (Atema et al. 1979). Postmolt females eventually become sufficiently strong to leave the shelter to feed, yet the sexual association between the male and female may continue for some time in the form of intermittent shelter sharing (Atema et al. 1979). Females may continue to benefit from male shelter-based protection and males may benefit by reducing the risk of sperm competition, a real possibility because females can mate with more than one male (Templeman 1934; Dunham and Skinner-Jacobs 1978; Snyder et al. 1992) and have clutches sired by multiple males (Nelson and Hedgecock 1977; Gosselin 2003; Jones et al. 2003). A seminal receptacle allows females to store spermatophores during intermolt, but the chitinous receptacle and its contents are shed at molting (Templeman 1934, 1936b). Pre- and postmolt guarding or association is widespread and well documented in crustaceans and typically larger males guard longer than smaller males (Smith 1984; Jormalaimen 1998), as in snow and blue crabs (Jivoff 1997; Rondeau and Sainte-Marie 2001). The relationship between mate size and length of sexual association, however, is unknown for lobster.

The present paper reports on a laboratory experiment and field observations to investigate time and ejaculate allocation/accumulation patterns in lobster. In the laboratory, we tested the hypothesis that the quantity of ejaculate accumulated by females and the time invested in sexual cohabitation are independent of both female and male size. In the field, we compared the quantity of stored ejaculate between females from the Magdalen and Anticosti Islands, two fishing grounds in the Gulf of St. Lawrence (eastern Canada) with contrasting lobster exploitation rates (75% at the Magdalens and 20% at Anticosti) and size structures (lobsters >100-mm CL are rare at the Magdalens and common at Anticosti, see Gendron and Savard 2000). We tested the hypothesis that the quantity of stored ejaculate is independent of female size within sites and independent of site (exploitation rate). The results are discussed in the context of evolutionary theory and management of this important fishery resource.

Methods

Collection of females and males

During June 2000 at the Magdalen Islands (47°23′N; 61°48′W), and during early July 2000 at Anticosti Island (49°18′N; 62°00′W), fishermen collected female lobsters carrying ripe eggs. About 100 ovigerous females of 70–120 mm CL were trapped at each site and shipped by plane or truck to the Maurice Lamontagne Institute. On reception, the CL was measured to the nearest 0.1 mm using a vernier caliper (Attard and Hudon 1987) and individuals were marked with coded tags glued to the right side of the cephalothorax. Females were then placed in communal holding tanks, where they hatched their eggs, until the mating experiment was initiated.

In August 2000 at the Magdalen Islands, size frequency and sex ratio of lobsters were obtained by hand-collecting via scuba along 12 randomly chosen transects over an area of 3.8 km^2 in five popular fishing grounds. Water depth ranged from 8 to 18 m. All lobsters were sexed and CL of females and males was measured to the nearest 0.1 mm. Because the onset of maturity for male lobsters cannot be detected by morphometry of their claw and gonadal maturity does not imply behavioral maturity (Conan et al. 2001), we used the alternative and indirect approach proposed by Conan et al. (2001) of inferring the size at behavioral maturity for males based on minimum female size at maturity. The smallest ovigerous female caught by divers or fishermen was 70 mm CL, so we established this limit for both females and males. Thirty mature males, 30 females with recently extruded eggs and 30 recently-molted females were randomly selected within predetermined equal size classes, covering the full size range, for determination of male reproductive potential and female insemination level. The male's two vas deferens, which are fused except in their most proximate part at the junction with the testes (Talbot and Helluy 1995), and the female's seminal receptacle were extracted and preserved in 4% seawater-diluted formalin.

Mating experiment

This experiment was conducted to determine whether there is a relationship between the quantity of ejaculate accumulated by a female and (1) her size, (2) her mating order and (3) her mate's size. We used the ovigerous females collected by fishermen in early summer 2000 because they were known to be mature and were expected to molt and become receptive during the late summer, based on the accepted pattern of alternating years of oviposition/brooding and molting (see Comeau and Savoie 2002). Sexes were maintained separately until the beginning of the experiment. Experimental males were purchased from a local fish market well before the annual mating season was initiated and were maintained individually and fed to satiety for 3 weeks until the beginning of the experiment.

The mating experiment consisted of two treatments with five replicates each. The first treatment was composed of a small (S) male (81–84 mm CL) with five females distributed equally over a range of 72–117 mm CL. In the second treatment, a large (L) male (116–131 mm CL) was tested. Each female was clearly identified on the basis of her rank in the size hierarchy, from one for the smallest to five for the largest female (F1, F2, F3, F4, F5). Males were identified with the mark "M" associated with the tank section number (e.g. tank section 3=M3).

The mating experiment was carried out in eight tank sections 1.2 m wide×1.8 m long×0.6 m deep, and 2 tank sections of 1.8 m on each side and 0.6 m deep. Each tank section was independently supplied with fresh unfiltered seawater heated between 12 and 15°C with salinity varying naturally from 29 to 32 ‰. Photoperiod followed the natural light cycle. Lobsters had continuous access to live natural food (mussel, *Mytilus edulis*; whelk, *Buccinum undatum*; and various echinoderms) and were also fed with excess thawed shrimp (*Pandalus borealis*) and rock crab (*Cancer irroratus*) twice a week. Each tank section was landscaped with rocks, gravel and sand to mimic natural lobster habitat and was provided with five small (20×33×20 cm) and two large (38×50×30 cm and 53×50×30 cm) numbered shelters made of sections of PVC agricultural drain cut lengthwise into halves. Each shelter had one opening.

The experiment began on 17 August 2000 and ended on 21 December 2000. Salinity, temperature and flow rate were recorded daily. The tanks were inspected twice daily to determine the position of each lobster, associations between individual lobsters and shelters, and to detect molts. Dead lobsters were replaced by lobsters of similar size and molt status (i.e. pre- or postmolt) from the reserves. Dead females and males were dissected to extract the seminal receptacle and vas deferens, respectively, which were preserved in 4% seawater-diluted formalin. The exuviae of molted females were removed from the tank and the seminal receptacle was extracted and preserved as above. A week following the molt, females were retagged and remeasured to determine postmolt CL. After the experiment, the lobsters were killed to extract and preserve the vas deferens or the seminal receptacle (as previously described).

Processing of vas deferens and seminal receptacle

All preserved seminal receptacles and vas deferens were rinsed in freshwater, blotted and weighed to the nearest 0.1 mg. Prior to rinsing, any excess tissue was dissected away from the seminal receptacle, leaving only the thin sheath. The ejaculate forms a dense, cohesive mass that was not lost during the process. After weighing, the seminal receptacles were opened to assess the presence or absence of ejaculate.

Data analysis

Statistics presented in the text for raw or transformed data are the mean and standard error (SE). Fisher's exact test was used to test for differences between the two treatments in the overall frequency of female molting and mating. The Pearson chi-square (χ^2) test was performed to test for differences in the overall frequency of female molting and mating among female size classes. To respect conditions of homoscedasticity and normality, we transformed pre- and postmolt mean cohabitation time ($\log_{10} y+1$) and vas deferens weight ($\log_{10} y$) for individual males before treatment comparison using a *t*-test. The degree of association between cohabitation time and female size class, as well as between seminal receptacle load (SRL) and female molt rank in the two treatments was measured with Spearman's rank correlation coefficient (r_s). The relationship between seminal receptacle weight (SRW) or load and female CL was determined by linear regression after logarithmic ($\log_{10}$) transformation of the data. We could not directly measure SRL because the receptacle's wall adhered tightly to ejaculate and could not be peeled away without loss of ejaculate. Therefore, we estimated SRL by substracting the size-specific mean weight of an empty receptacle determined by regression from the recorded value of SRW for individual females. Seminal receptacle load of females mated with small or large males were compared by nested analysis of variance (male identity nested into male size category with female CL as a covariate). The slopes and intercepts of the regressions of $\log_{10}$ SRL on $\log_{10}$ CL of wild-mated females were contrasted among field sites by analysis of variance (ANOVA) and analysis of covariance (ANCOVA), respectively. Post hoc comparisons of adjusted mean SRL were performed using Tukey's least significant difference (LSD) test. Fisher's exact test was used to test for differences in the frequency of empty seminal receptacles between pre- and postmolt wild-mated females. For assessing the potential limits to effective polygyny in decapod crustaceans it is useful to express SRL (an index of sperm allocation) as a percentage of vas deferens weight (Jivoff 1997). Indeed, in decapod crustaceans and in *Homarus* specifically, a large part of the vas deferens is a storage area for spermatozoa and seminal fluids (Krol et al. 1992; Talbot and Helluy 1995) that may be depleted through intensive mating (Jivoff 1997; Rondeau and Sainte-Marie 2001; Kendall et al. 2002). The resulting percentages were transformed (arcsine *y*) before treatment comparison using a *t*-test. Statistics were performed with Systat10® software (Richmond, CA, USA).

Results

Molting, cohabitation and mating in the experiment

More than half (58%) of the females molted (Table 1) and there was no significant correlation between female premolt size and molt date in either the small (r_s=0.481, *df*=11, *P*=0.096) or large (r_s=0.004, *df*=14, *P*=0.988) male treatments. The proportion of females that molted was independent of the size class of males (Fisher exact test, *P*=0.567) and females (Pearson χ^2=4.43, *df*=4, *P*=0.351). Only females that molted cohabited with males, but they were not necessarily inseminated; 3 out of 25 cases were not (Table 1). One large female molted, mated and 54 days later extruded eggs. The proportion of females that mated successfully (i.e. received ejaculate) was not significantly different in the small-male compared to the large-male treatment (Fisher's exact test, *P*=0.154) and was independent of female size class (Pearson χ^2=1.30, *df*=4, *P*=0.862). Successful matings occurred at both extremes of mate size differences encountered in our experiment: a male 29% smaller than the female and a male 67% larger than the female. Only four lobsters died during the experiment (Table 1) and deaths always occurred after a molt. One female molted while cohab-

Table 1 American lobster size classes used in the mating experiment and incidence of mortality, cohabitation and mating. Male size groups are *S* for small and *L* for large

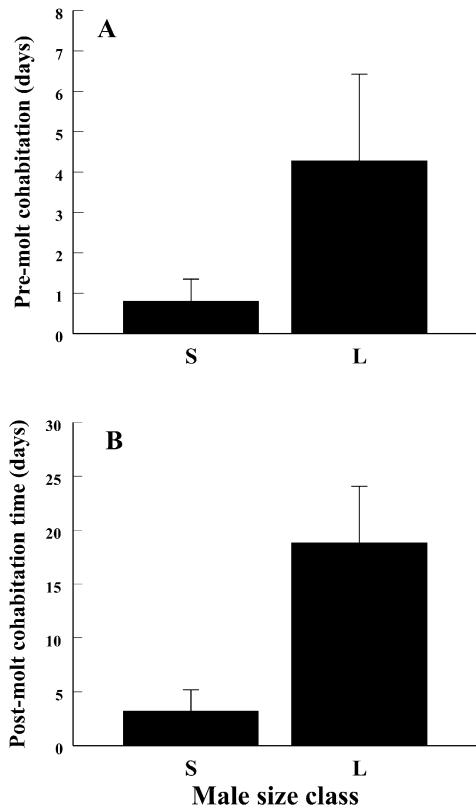| Group	n	Carapace length (mm)		No. dead	Female		
		Range	Mean ±SE		Molting n (S/L)	Cohabiting n (S/L)	Matings n (S/L)
Male							
S	5	81–84	82.4±0.7	1	–	–	–
L	5	116–131	123.8±2.5	0	–	–	–
Female							
F1	10	72–79	76.7±0.8	1	8 (4/4)	6 (3/3)	5 (2/3)
F2	10	80–87	84.7±1.4	0	4 (1/3)	4 (1/3)	4 (1/3)
F3	10	88–97	92.7±1.0	0	7 (3/4)	6 (2/4)	5 (2/3)
F4	10	97–105	101.6±1.7	1	5 (2/3)	5 (2/3)	5 (2/3)
F5	10	106–117	111.4±1.3	1	5 (3/2)	4 (2/2)	3 (1/2)
Sum					29 (13/16)	25 (10/15)	22 (8/14)

Fig. 1 Mean±SE of premolt (**A**) and postmolt (**B**) cohabitation times in days for *Homarus americanus* as a function of male size class [*S*=81–84 mm carapace length (CL); *L*=116–131 mm CL] in mating experiments. *n*=5 for each treatment. Note different scales for the *ordinate axes*

iting with a small male and was cannibalized after the male left; however, we know she was inseminated as we retrieved part of the seminal receptacle.

Pre- and postmolt cohabitation time

Total time spent cohabiting with females varied from 0 to 23 days for individual small males, and from 15 to 92 days for individual large males. In general, postmolt cohabitation was >75% longer than premolt cohabitation and on average large males cohabited 83% longer than small males. Although premolt females cohabited longer with large than with small males (Fig. 1A), the difference was not significant (t=2.27, df=8, P=0.053). The cohabitation period of postmolt females was significantly longer with large than with small males (t=3.10, df=8, P=0.015, Fig. 1B), as was the combined pre- and postmolt cohabitation time (t=2.97, df=8, P=0.018). There was no significant correlation between female size class and average time of cohabitation with small males (r_s=0.46, df=3, P=0.434). However, the correlation between female size class and average time of cohabitation with large males was significant and positive (r_s=0.90, df=3, P=0.037).

In all but one mating occurrence, males hosted females in their shelter. However, one small male, which initially stayed in the same shelter for 57 days, and was visited occasionally by females F1 and F2, left his den and after moved from shelter to shelter for 4 h, and eventually settled in the shelter of female F4. He remained with her until she molted 1 week later. In the following 2 weeks, the other four females all occupied and molted within this shelter. Over a period of 5 days, four females of which three were newly molted cohabited all together with the male. This is the only small-male treatment where all females molted. In one large male replicate all five females molted and the male seldom left his shelter (10 out of 127 observations). For a period of 9 days, he had a minimum of two females cohabiting within the shelter and females unable to enter remained at the entrance of the shelter. Around 2 weeks elapsed between successive female molts in this particular replicate.

Ejaculate accumulation by females

The weight of empty seminal receptacles was positively related to female CL (Fig. 2). Estimates of SRL for laboratory-mated females, derived by subtracting size-specific mean weight of an empty seminal receptacle from recorded seminal receptacle weight, were all positive (Fig. 3). The nested analysis of variance (Table 2)
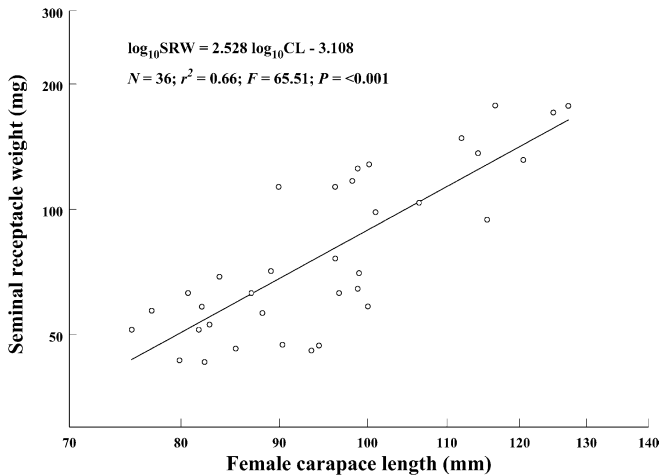
Fig. 2 *Homarus americanus*. Relation between empty seminal receptacle weight (*SRW*) and carapace length (*CL*) of females. Note that both *axes* are log₁₀ scale

In the figure: $\log_{10}SRW = 2.528 \log_{10}CL - 3.108$
$N = 36; r^2 = 0.66; F = 65.51; P = <0.001$

Table 2 Nested analysis of variance of seminal receptacle load of female *Homarus americanus* mated with small or large males. Male identity is nested into male size category and female carapace length (*CL*) is a covariate

Source	df	MS	F	P
Male size category	1	0.129	20.015	0.001
Female CL	1	0.322	50.056	<0.001
Male identity (male size category)	4	0.009	1.327	0.308
Error	14	0.006		

showed that the amount of ejaculate accumulated by laboratory-mated females was dependent on male size category ($P=0.001$) and female CL ($P<0.001$), but was independent of male identity within categories ($P=0.308$). Within each size category of males, SRL was positively related to female CL (Fig. 3). Mean SRL adjusted to overall mean female CL was 240 mg for females mated by large males and 157 mg for females mated by small males. Thus, on average, similarly-sized females stored 53% more ejaculate when mated by larges males compared to small males. Two cases of below-average ejaculate accumulation in the presence of large males involved a small (82 mm CL) and a large (117 mm CL) female (Fig. 3). The females respectively had 19% and 24% less ejaculate than predicted from regression. In both cases, the females were the first to mate in their respective tanks. However, in general, the average amount of ejaculate stored by females was independent of their mating order in the treatment with a small male ($r_s=-0.127$, $df=3$, $P=0.786$) or a large male ($r_s=0.152$, $df=3$, $P=0.604$).

Twenty six percent of ovigerous females had empty seminal receptacles at the Magdalen Islands (MI) and 24% at Anticosti Island (AI). A *t*-test for each field site showed no difference in CL between females with an empty or a loaded seminal receptacle (MI: $t=0.63$, $df=90$,
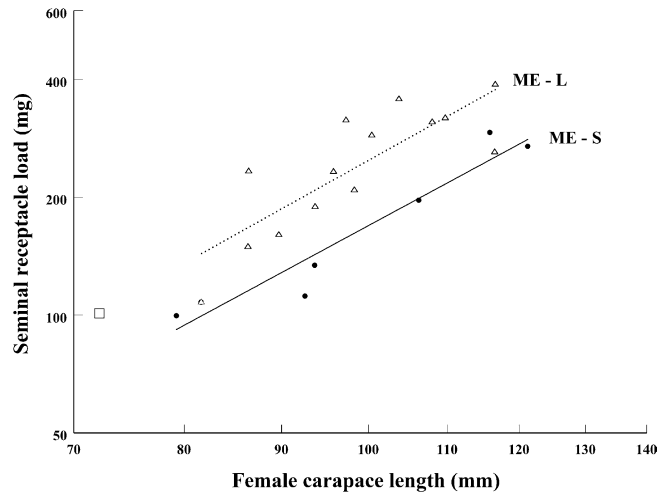

Fig. 3 *Homarus americanus*. Relation between seminal receptacle load (*SRL*) and carapace length (*CL*) of females in the laboratory experiment. Regressions of log-transformed data are significant (see Table 3). *ME–L* females mated with large males (116–131 mm CL), *ME–S* females mated with small males (81–84 mm CL). The *open square* represents the SRL of a recently molted female found cohabiting with a male of 107 mm CL during diver sampling at the Magdalen Islands. Note that both *axes* are log₁₀ scale

$P=0.529$; AI: $t=0.65$, $df=85$, $P=0.516$). The percentage of postmolt females >70 mm CL with empty seminal receptacles was 6.5% during the late summer sampling of the Magdalen Islands; the two empty females had a CL of 72 and 81 mm.

For wild-mated female lobsters with nonempty receptacles, regression analysis revealed a positive relationship between SRL and female CL at each field site and for each female status (pre- or postmolt) (Fig. 4, Table 3). Regressions did not differ in slope or intercept among field samples (Table 3). Therefore, a common regression line (pooled field sites) was fitted to the data (Fig. 4). However, it is apparent that the residuals tended to be positive for females >105 mm CL, at Anticosti Island in particular.

The slopes of SRL–CL relationships were always shallower for laboratory-mated females than for wild-mated females (Table 3). However, a comparison of regressions of SRL on CL from the pooled field sites and nonempty females from the large- (ME–L) and small-male (ME–S) laboratory treatments revealed no significant difference in slopes but a difference in intercepts (Table 3). Adjusted mean SRL for all wild-mated females (146 mg) was on average 23% less than the mean for females from the large-male (189 mg) treatment (Tukey, $P<0.05$) and 12% more than the mean for females from the small-male treatment (130 mg), although this last difference was not significant (Tukey, $P>0.05$). Note that a few wild-mated females had much larger ejaculate stores than maximum SRL recorded for similarly-sized females mated in the laboratory. For example, one wild-mated female of 105 mm CL contained an ejaculate of

Table 3 *Homarus americanus.* Regressions of $\log_{10}$ seminal receptacle load (*SRL*, mg) on $\log_{10}$ carapace length (*CL*, mm) for females in the mating experiment and pre- and postmolt females from the field. Slopes and intercepts were contrasted by ANOVA and ANCOVA, respectively. Where ANCOVA detected a significant difference, adjusted mean *SRL* was compared with a Tukey test

| Female group	Regression	n	r^2	F	P
Mating experiment (ME)					
S: mated with small males	$\log_{10}$ SRL=2.627 $\log_{10}$ CL - 3.025	7	0.93	61.75	<0.001
L: mated with large males	$\log_{10}$ SRL=2.720 $\log_{10}$ CL - 3.045	14	0.68	25.02	<0.001
Field sites					
Premolt females					
1: Magdalen Islands (June)	$\log_{10}$ SRL =3.087 $\log_{10}$ CL - 3.908	68	0.73	177.03	<0.001
2: Anticosti Island (July)	$\log_{10}$ SRL =3.527 $\log_{10}$ CL - 4.744	66	0.77	219.26	<0.001
3: Magdalen Islands (August)	$\log_{10}$ SRL =3.550 $\log_{10}$ CL - 4.822	28	0.46	22.37	<0.001
Postmolt females					
4: Magdalen Islands (August)	$\log_{10}$ SRL = 3.003 $\log_{10}$ CL - 3.709	21	0.57	25.20	<0.001
	ANOVA: Slopes	183	–	0.64	0.588
	ANCOVA: Intercepts	183	–	1.20	0.313
Pooled field sites	$\log_{10}$ SRL = 3.311 $\log_{10}$ CL - 4.334	183	0.72	460.94	<0.001
Field vs mating experiment	Pooled sites vs ME–L vs ME–S				
	ANOVA: Slopes	204	–	0.59	0.554
	ANCOVA: Intercepts	204	–	4.76	0.010
	Adjusted SRL (mg):				
	189 (ME–L)$\neq$				
	[146 (pooled sites)=130 (ME–S)]				
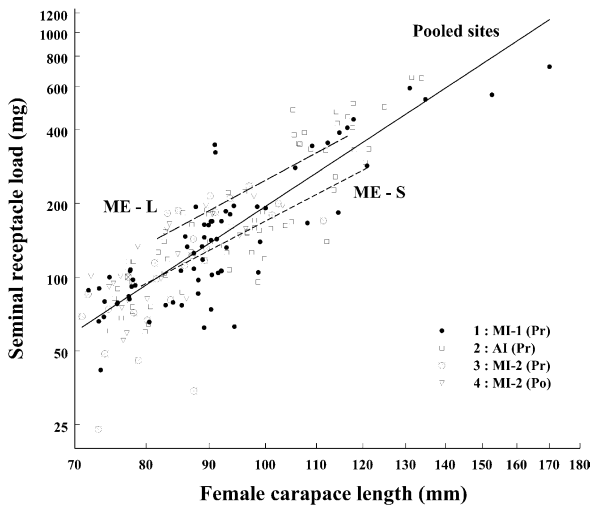
Fig. 4 *Homarus americanus.* Relation between seminal receptacle load (*SRL*) and carapace length (*CL*) of wild-mated females. ANOVA and ANCOVA indicated no difference in slope and intercept of regressions among sites or female status (see Table 3), consequently, a common *regression line* was fitted to data (*pooled sites*). *MI–1* (*Pr*): premolt females from June 2000 sampling at the Magdalen Islands. *AI* (*Pr*): premolt females from July 2000 sampling at Anticosti Island. *MI–2* (*Pr*): premolt females from August 2000 sampling at the Magdalen Islands. *MI–2* (*Po*): postmolt females from August 2000 sampling at the Magdalen Islands. *Regression lines* for laboratory females mated with small (*ME–S*) or large (*ME–L*) males are shown for comparison. Note that both *axes* are $\log_{10}$ scale

Fig. 5 Relation between vas deferens weight (*VDW*) and carapace length (*CL*) of male *Homarus americanus* collected by divers in August 2000 at the Magdalen Islands. Also shown are the mean ±1SE of VDW for small (*S* SE comprised within *open circle*) and large (*L*) males in mating experiments. The *open square* with a *plain circle* identifies the VDW of the male found cohabiting with a female during diver sampling at the Magdalen Islands

483 mg (Fig. 4); 35% more than a 104-mm-CL female mated with a large male (Fig. 3).

During diver sampling of the Magdalen Islands fishing grounds a rather large male (107 mm CL) and a small postmolt female (72 mm CL) were found cohabiting. Over an area of 800 m², and considering only lobsters >70 mm CL, the ratio of males to females was 1.86. The cohabiting male was th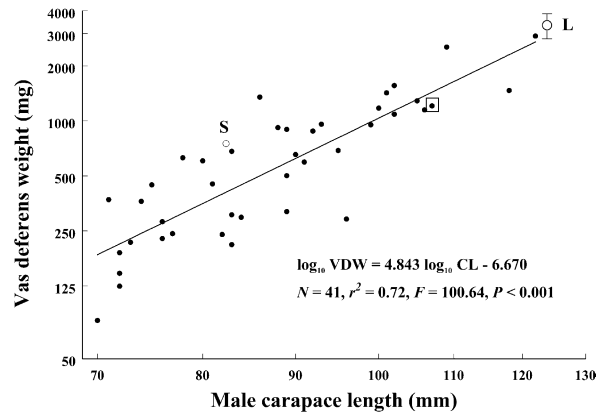e second largest in the sampled area. The female had a clean soft shell with a protruding sperm plug, indicating a recent molt and insemination, but the exuviae was not found. The SRL of this small female is consistent with extrapolation of the regression line for the large-male treatment in Fig. 3.

Male reproductive potential in the mating experiment and field

There was a significant difference in vas deferens weight (VDW) between the two treatments of the experiment (S vs L: t=9.34, df=8, P<0.001): vas deferens of large males were on average 366% heavier than those of small males. The SRL of laboratory-mated females represented on average 24% and 9% of the VDW of small and large

males, respectively. These proportions are significantly different (t=4.62, df=17, P<0.001).

A positive relationship was found between VDW and CL for male lobsters collected by divers at the Magdalen Islands (Fig. 5). In relative terms, the SRL of the female in the diver-captured mating pair represented 8% of her mate's VDW, a proportion also similar to the mean for large males in the experiment. Vas deferens of experimental males were on average 44% and 12% heavier for small and large males, respectively, than average values predicted from regression for wild males of a similar size.

Discussion

Male ejaculate allocation strategies

This is the first study to report on female ejaculate accumulation patterns in the American lobster. Our laboratory results strongly suggest that the patterns of ejaculate accumulation by female lobster result from male ontogenetic (size-dependent) and strategic allocation behaviors, even though we did not directly measure the quantity of ejaculate transferred by males. The 53% difference in adjusted mean SRL between females mated by small or large males could reflect (1) lesser ejaculate allocation by small compared to large males or (2) reduced female acceptance/retention of ejaculate from small males if they pass as much as large males. The former interpretation is more likely because ejaculate ejection is demonstrated only in species where females are coerced into mating by less desirable males (Pizzari and Birkhead 2000), whereas in the lobster courtship roles are reversed with females actively soliciting mates (Atema et al. 1979; Atema 1986; Karnofsky et al. 1989; present study). The size of ejaculates attached to females of spiny lobster *Panulirus argus* is also correlated with mate body size (MacDiarmid and Butler 1999). The difference in absolute ejaculate allocation between small and large males provides an economic basis for understanding why lobster females prefer large males (Atema et al. 1979; Atema 1986; Karnofsky et al. 1989; Cowan and Atema 1990) and why they may be more inclined to promiscuity after mating with a smaller male than after mating with a larger male (Easton 1976; Gosselin 2003).

With respect to the positive relationship between SRL and CL of females mated by small males in our experiment, it cannot be explained by limit capacity of the seminal receptacle because similarly-sized females mated by large males had more ejaculate in storage. Seminal receptacles of females mated by large males were also not filled to capacity because SRL of some wild-mated females exceeded the maximum value recorded in the laboratory for females of similar size. Therefore, the positive and nearly identical slopes of the SRL–CL relationships of females mated by either small or large males most likely reflect female-size-specific (i.e. strategic) ejaculate allocation, as demonstrated in the spiny lobster *Panulirus argus* (MacDiarmid and Butler 1999)

and a variety of other taxa (Wedell et al. 2002). The positive relationship between SRL and female CL in all our field samples and the good correspondence between laboratory-based expectations and SRL of a female from a wild-mating pair also suggest that in nature males allocate ejaculate proportionately to female size. Several proposed reasons for judicious allocation of ejaculate (Dewsbury 1982; Pitnick and Markow 1994; Wedell et al. 2002) may fit the lobster case: (1) ejaculate production may be costly; (2) ejaculate reserve can probably be depleted and therefore ejaculate may be a limited resource, especially among popular males; (3) the number of eggs is positively related to female body size; (4) females store ejaculate for an extended time before oviposition and may be promiscuous resulting in a risk of sperm competition.

As a decapod crustacean species, the average of sperm allocation by small and large male lobsters represented 16.5% of VDW. This value falls between the sperm economy of snow crabs (*Chionoecetes opilio*) and the sperm generosity of blue crabs (*Callinectes sapidus*), which are 2.5% and 47% of VDW, respectively (Jivoff 1997; Rondeau and Sainte-Marie 2001). However, the quantity of ejaculate accumulated by female lobsters represented a larger share of the VDW of small (24%) than of large (9%) males. These values may be overestimated if males did not have time to fully recharge before the end of the experiment, and the comparison between the two male size groups is justified only if vas deferens recharge rate is the same for small and large males. The difference in relative sperm allocation between large and small males could reflect a gradual change from sperm maximizer to sperm economizer as males grow bigger and become endowed with larger reproductive organs and ejaculate reserves, but are increasingly solicited by females and likely to be polygynous. However, an alternative and simpler interpretation is possible. Since the ejaculate (or spermatophore) is molded by sphinctorial contractions of the posterior vas deferens shortly before intromission (Herrick 1911; Kooda-Cisco and Talbot 1983), an innate length-based control for adjusting ejaculate size to female size, in conjunction with male body-size isometric increase of vas deferens diameter, could explain (1) the greater absolute, but smaller relative, allocation of large compared to small males and (2) the perfect congruence of relative difference of mean SRL between females mated by small or large males and of mean CL between the two size classes of males (50% in both cases). This hypothesis should be tested by analysis and simple modeling of the change in posterior vas deferens diameter and whole vas deferens volume in relation to male size.

Pair formation and sexual cohabitation

Mating of lobster appears to be based on mutual consent of female and male, and therefore mate cohabitation in this otherwise solitary animal is likely to provide benefits to both sexes. The relatively brief premolt association

may allow males to evaluate the female and prepare the appropriate ejaculate, while females benefit from male protection before and during molting. The prolonged postcopulatory postmolt association is easily explained from the female perspective: residing longer with a protective male during the vulnerable early postmolt period could allow females to allocate energy away from self-defense (against intra- or interspecific aggression) toward somatic and gonadic growth. However, it is more difficult to explain from the male perspective. Males are probably not defending their exclusive right to paternity because females fertilize and extrude eggs from several weeks up to 1 year after molting (Herrick 1911; Templeman 1934; Comeau and Savoie 2002) and in the interim may successfully mate with other males (Templeman 1934; Dunham and Skinner-Jacobs 1978; Waddy and Aiken 1990; Snyder et al. 1992). Although males may indirectly be protecting their reproductive investment—the ejaculate (Wilber 1989; Jivoff and Hines 1998; Rondeau and Sainte-Marie 2001)—and potential progeny against predators, a more likely gain is that activity of postmolt females around the male's shelter draws the attention of other females to the male, thereby increasing his mating success during a season. In a lekking species such as the fallow deer *Dama dama*, when a male is successful in building up a harem, he continues to draw extra females possibly because of female copying (Clutton-Brock et al. 1989; Balmford 1991).

Sexual cohabitation lasted longer with larger than with smaller males, and the length of association scaled to female size in mating pairs with large males, probably in reflection of mate quality and compatibility. Females may attempt to initiate premolt cohabitation more quickly and stay longer after molting-mating with a large than with a small male, due to the former's greater economic and defensive value (see previous discussion). The cannibalized female in our small-male treatment and another report of female-inflicted injury to postmolt females (Cowan 1991) clearly indicate the potential costs of molting alone or with a low rank male. Because larger females require more time to harden after molting than smaller females (Templeman 1934; own personal observation), they should attempt to reside longer with protective males. Conversely, large males may be more inclined to cohabit longer with large than with small females, due to the former's greater fecundity or attractiveness to other females (see previous discussion). Furthermore, mate size incompatibilities (small males with large females or large males with small females) could result in males ousting the female or in the female leaving on her own quickly after molting. This would contribute to weaken time assortation patterns in small-male treatments and reinforce them in large-male treatments.

The maximum size differences between mates did not limit successful ejaculate transfer in our experiment and were much greater than limit values given by Templeman (1934). He reported that males more than 6.5% smaller or more than 21% larger than a female did not mate successfully. We found that males up to 29% smaller or 67% larger than females did mate successfully. Thus, the maximum range of size differences may be quite variable but in nature it may be governed by den size, which is correlated to male size (Cobb 1971). The male's shelter may impose an upper limit on the relative size of females that can be hosted and mated, as reported for the fiddler crab *Uca tetragonon* (Goshima et al. 1996).

Ejaculate load and potential for sperm limitation in exploited populations

In most areas of the American lobster's distribution, exploitation is based on an intense recruitment fishery that severely distorts the lobster size structure and skews it towards small size relative to a virgin or lightly fished population (Anonymous 1995; Gendron and Gagnon 2001). Small males appear to be responsible for mating most females in both populations we sampled, because the ejaculate load in wild-mated females resembled that of laboratory females mated by small males more than that of females mated by large males. Nevertheless, the field data suggest a weak size assortative pattern. Indeed, the slopes of SRL–CL relationships were always steeper and the *Y*-intercepts much smaller for wild-mated females than for laboratory-mated females, while residuals about the common regression for wild-mated females tended to be positive for females >105 mm CL. This was especially true of the Anticosti population, where exploitation rate is moderate and the lobster size structure is more diverse than at the Magdalen Islands. However, the current data on SRL and frequency of empty seminal receptacles do not allow us to conclude that the different exploitation rates at Anticosti and Magdalen Islands result in a different mating success for females.

Most lobster populations are considered over-exploited and initiatives to increase egg production are being considered or promoted (Anonymous 1995; Gendron and Gagnon 2001). Some of these initiatives include additional protective measures for mature females only, such as V-notching (notching a V in the telson of ovigerous females as a conservation measure to recognize and protect mature females) or a maximum legal size, that aim to increase their abundance and mean size. However, these measures and the models to track progress toward the goal of increasing egg-per-recruit (Fogarty and Idoine 1988; Gendron and Gagnon 2001) assume that sperm supply is unlimited. Although it is clear from our data that ejaculate size varies with donor and receptor size in lobster, the question of whether ejaculate may be limiting to females is still unanswered and requires further research. In the spiny lobsters, *Panulirus argus* and *Jasus edwardsii*, females produce smaller clutches when they mate with a small compared to a large male, because of insufficient volume of spermatophore transferred (MacDiarmid and Butler 1999). A similar situation of female sperm limitation and/or increased mortality could occur in lobster if size structure and abundance of the two

sexes diverge too severely. Large or successive-year female spawners (Waddy and Aiken 1986; Comeau and Savoie 2002) might be particularly vulnerable to sperm limitation if large males are too scarce to service all females or become ejaculate-depleted through intensive mating. Moreover, large females may suffer greater molt-associated mortality if large protective males and dens are a limited resource. It is therefore conceivable that measures to increase egg production by protecting only females could be self-defeating at some point.

In conclusion, we have shown that, under laboratory conditions, American lobster sexual cohabitation and female ejaculate accumulation patterns are quite flexible, varying with male and female size. Differences in seminal receptacle load among females likely reflect male ejaculation strategies. When contrasted to the mating experiment, the pattern of natural insemination indicated that small males are probably responsible for most matings in nature, although a weak size assortative mating pattern may exist. Experimental research, further considering size assortative mating, sperm limitation and sperm competition should be pursued to assess the significance of our results concerning fishing effects.

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