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ORIGINAL ARTICLE

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Evidence for seasonal mate limitation in populations of an intertidal amphipod, *Corophium volutator* (Pallas)

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Abstract Potential rates of reproduction (PRR) differ between the sexes of many animal species. Adult sex ratios together with PRR are expected to determine the operational sex ratio (OSR) defined as the ratio of fertilizable females to sexually active males at any given time. OSR is expected to determine the degree to which one sex competes for another—the limiting sex. We explored the potential for mate limitation in an intertidal amphipod, *Corophium volutator* (Pallas). Males have higher PRR than females, but males may be limiting because of extreme female-biased sex ratios observed in this species. Consistent with this idea, late season females were less likely to be ovigerous and had smaller size-specific clutches, both of which were associated with seasonal declines in availability of males of reproductive size. Seasonal changes in ovigery could not be explained by seasonal changes across sites in other factors (e.g., female body size or phenology of breeding). Smaller females were less likely to become ovigerous later in the season at three of four sites. Seasonal reductions in clutch size also occurred among small females expected to be reproducing for their first time. In complimentary laboratory experiments, reduced likelihood of ovigery and reduced fecundity occurred when the number of receptive females was increased relative to availability of a reproductively active male. Our results suggest male mate limitation can occur seasonally in this species and that male limitation is regionally widespread and may affect recruitment.

Keywords Amphipod · *Corophium* · Mate limitation · Operational sex ratios · Recruitment

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Introduction

Operational sex ratio (OSR), defined as the ratio of fertilizable females to sexually active males at a given time (Emlen and Oring 1977), is often biased in many species because there are many more sexually active males than there are fertilizable females. Researchers also have become interested in how the potential rate of reproduction (PRR) differs between the two sexes and influences OSR (Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjo 1996; Correa and Thiel 2003). In many animal species, females do invest more in reproduction and have a lower PRR than do males (Clutton-Brock and Vincent 1991). Males are thus expected to compete for access to receptive females, provided that adult sex ratios are not extremely female-biased.

In sex-role-reversed species, males may become limiting and females can compete for males (e.g., to obtain male-provided resources, Gwynne 1993). Similarly, in species with parasitic sex ratio distorters, females in populations with high prevalence of infection may actually assume male roles such as lekking (Jiggins et al. 2000). Some recent theoretical work suggests that male limitation due to parasitic sex ratio distorters can even transcend into population effects (e.g., Hatcher et al. 1999, 2000). However, even under strong female biases in adult sex ratios, receptive females may still be limiting relative to reproductively active males because females spend more “time out” of reproductive readiness (Moreau and Rigaud 2000).

Here, we describe the potential for male mate limitation of female reproduction in the amphipod, *Corophium volutator* (Pallas), at sites located in the intertidal ecosystem of the Bay of Fundy, Canada. In terms of the behavioral ecology of this amphipod, males of *C. volutator* appear to provide only ejaculates to females and can mate with many females (see below). In comparison, females mate only following their parturial molt, and females brood their young in a marsupium (McCurdy 1999). Thus, there is a lower PRR in females than in males. However, juvenile and adult sex ratios are highly female-biased in this species (Schneider et al. 1994). At present, we do not know the causes of female-biased sex ratios in adults of *C. volutator*, but recent evidence shows that sex-biased predation by birds and higher mortality of males are contributing factors (McCurdy 1999). Additionally, the recent discovery of an undescribed microsporidian parasite in females, but not in males of this species, suggests the potential for parasitic sex ratio distortion (S.I. Mautner, personal observations).

Our rationale for examining the potential for mate limitation in populations of this amphipod followed from field observations and previous laboratory studies. Sex ratios of adults are female-biased in this species, ranging from 3:1 to 10:1 at our study sites (Boates and Smith 1989; Matthews et al. 1992; Peer et al. 1986). Further, McCurdy et al. (2000) showed that female *C. volutator* have a receptivity window of 3 days following a molt. In studies with another amphipod, Borowsky (1988) demonstrated that a delay between a female's parturial molt and mating resulted in reduced fertilization success and smaller clutches (cf. McCurdy et al. 2000). Such delays may be expected in the field for *C. volutator* females because females outnumber males, and females at some sites also show synchrony in their molting over spatial scales (i.e., patches) relevant to a male's mate searching ability (Forbes et al. 1996; McCurdy et al. 2000). In other words, there could be more receptive females in a patch than there are males available for mating.

We had several specific objectives related to assessing whether male mate limitation can occur in this species. First, we examined whether the proportion of females of reproductive size that were ovigerous (hereafter, proportion of females that were ovigerous) was related to the availability of males of reproductive size, over four mudflat sites sampled over two periods (early June and late July). To do this, we first examined whether there was significant variation in proportion of females that were ovigerous over the four sites across the two sampling periods. We then examined whether the relative availability of males of reproductive size also varied in a way that could explain variation in proportion of females that were ovigerous. We then examined whether other factors such as size of adult females and phenology of breeding at sites could explain proportion of females that were ovigerous. In the former case, it is expected that larger females should provide more egg gains per unit time of male investment in mating than would smaller females. We might expect higher rates of ovigery for samples with larger females. In the latter case, we may expect that females at sites where breeding is advanced may have lower rates of ovigery because many females have already brooded and released young.

We had three other objectives. We examined whether size-specific clutches of females were lower when reproductively active males were less available for mating, as expected based on previous work with other intertidal amphipods (Borowsky 1988). We also examined whether females expected to be reproducing for their first time had smaller clutches if reproductively active males were less available. Finally, we assessed whether male limitation of female reproductive output could be reproduced in the lab for this species under conditions where the availability of receptive females was manipulated relative to the availability of reproductive males. This experiment was meant to emulate conditions that can occur in nature (e.g., few males and many newly receptive females available for mating).

Methods

Study species

C. volutator is found in intertidal zones on both sides of the North Atlantic (Fish and Mills 1979; Peer et al. 1986) and in Japan (Omori and Tanaka 1984). This amphipod ranges in length from 1 to 11 mm (rostrum–telson length, Peer et al. 1986). In the Bay of Fundy, *C. volutator* is sexual and sexually dimorphic (McCurdy 1999): males have elongated second antennae (Schneider et al. 1994). The females construct and live in U-shaped burrows, whereas males do not construct burrows but can cohabit with females (Gratto et al. 1983). Mate guarding has not been described for this species. As mentioned, reproductive females carry fertilized eggs, and later developing embryos, in a marsupium (Fish and Mills 1979; Schneider et al. 1994). Reproductively active males crawl on the mud surface searching for females (Boates and Smith 1989; Forbes et al. 1996). At our study sites, breeding typically begins in late May to early June and there are two generations yearly: offspring produced in late summer grow and then overwinter (Gratto et al. 1983; Peer et al. 1986; Wilson 1989). Based on surveys, overwintering is a time of high amphipod mortality (up to 70%, Gratto et al. 1983; cf. Wilson 1991).

Field survey

The present study is part of a larger ongoing investigation on population dynamics of *C. volutator* in the Bay of Fundy ecosystem. We sampled this species from each of the following four mudflats over 9–12 June 1997 and again over 21–23 July 1997: Starr's Point (45°08′N, 64°22′W), Blomidon (45°13′N, 64°22′W), Grande Anse (45°49′N, 64°31′W), and Peck's Cove (45°45′N, 64°29′W). On each visit to each mudflat, we sampled from three patches (each about 5×5 m) located at high, mid, and low tidal lines. In each patch, we took five randomly placed 86.5-cm^2 circular cores that were 5–10 cm deep, depending on the depth to the anoxic layer. Samples were sieved (0.85-mm mesh); individuals were retrieved from sieves using forceps and placed into 0.5-L bottles filled with equal parts seawater and 5% ethanol. Samples were later preserved in 70% ethanol until processed.

As mentioned, we collected amphipods during two time periods that corresponded to production of the first and second generations. We sexed and measured each amphipod (following Schneider et al. 1994) and counted all amphipods in each sample (total n=7585). Females were categorized as either ovigerous or non-ovigerous. Some individuals were intersexes having both penial papillae and oostegites (cf. Schneider et al. 1994). For our study, males were classified as of reproductive size if they were at least 7.0 mm, as it was known from earlier work that mate-searching males are usually this size or larger (Forbes et al. 1996). Females of reproductive size were at least 5.5 mm (see "Results").

We knew that females synchronize their reproduction at the patch level at one study site (McCurdy et al. 2000). The patch level is also relevant because males spend over 1 h mate searching on each tidal cycle. Thus, males in a patch could encounter many females in that patch (Forbes et al. 1996) over several tidal cycles. We obtained the following data per patch for each site-by-time period combination: the number of females and males of reproductive size and the number of females of reproductive size that were ovigerous.

For ovigerous females, we counted the number of eggs or embryos and scored their stage of development. Females scored as stage A had whitish yellow, opaque eggs indicating recent fertilization, whereas other females had embryos at later stages of development (B–D, following classification outlined in Peer et al. 1986). For direct comparisons of fecundity in relation to size of females, we used only females with broods at stage A of development, as brood loss often occurs during later development (McCurdy et al. 2001). Additionally, in our analysis, we considered small females that were expected to be reproducing for their first time (5.5–6.5 mm in length). Such females were collected in both surveys. Because the smallest ovigerous female was about 5.5 mm in length, these females are expected to be reproducing for their first time. Furthermore, small females in June were unlikely to still be small in July. Even small ovigerous females in early June would be able to release their broods and should grow more than 1 mm in the intervening 4 weeks before sampling again in late July (brood development takes about 2 weeks, Gratto et al. 1983).

Laboratory experiment

For the laboratory experiment, we collected 102 adult male and 600 non-ovigerous adult female amphipods at Blomidon. Males were collected with live specimen forceps while they were crawling on the mud surface; females were collected with forceps by removing them from their burrows in the substrate. Each amphipod was housed individually in 20×1.5 cm diameter test tube containing 30 ml of artificial seawater and lined to 1 cm with mud from Blomidon that had been autoclaved. Streptomycin sulfate and penicillin G (100 μg L^{-1}) were added to each tube because these antibiotics reduce mortality in the lab (Pelletier and Chapman 1996). The ambient temperature in the lab was 15–17°C, similar to the average substrates of nearby mudflats (Piccolo et al. 1993). We also added the prey diatom *Thallassiosira* spp. (200,000 cells ml^{-1}).

After a female molted (within 24 h), she was placed in a 300-ml plastic cup lined with mud and containing 150 ml of seawater with antibiotics (either by herself or with four other females that molted on the same day). Female *C. volutator* appear unable to store sperm across their molts, similar to other amphipods (of over 2,000 females housed alone after molting, none produced eggs in the absence of a male; D.G. McCurdy, personal observations). We retained only recently molted females for use in this experiment because female *C. volutator* are only able to mate 3 days following their molt (McCurdy et al. 2000) and because we wanted to control for receptivity across treatments. We then added a male to each cup (randomized) and housed each male with the female(s) for 3 days (52 replicates of one male with one female and 47 replicates of one male with five females). After 3 days, we recorded the number of ovigerous females and counted the number of fertilized eggs of each female that was ovigerous.

Statistical analyses

Using a two-factor ANOVA, we first examined whether site and time of sampling (as factors) influenced the proportion of females that were ovigerous. For this analysis, we arcsine (√) transformed the data following the recommendation of Zar (1996) for proportional data. We also performed a two-factor ANCOVA to assess whether time of sampling and site, and number of females of reproductive size (as the covariate), was related to the number of males sampled that were at least 7.0 mm, when breeding activity was underway. We also assessed the extent to which clutch sizes of females with recently fertilized eggs were dependent on either time of sampling or site and also body length of females as a covariate. We ln-transformed clutch sizes to meet assumptions of parametric tests (Zar 1996).

We also examined whether other factors varied significantly among sites and between sampling times. Of particular importance was breeding phenology. As indicated, we were interested in proportion of females that were ovigerous. Fewer females might be ovigerous at a given site or sampling time if the breeding period was well advanced such that some females already brooded and released their young. Thus, we compared the proportion of females that were ovigerous with recently fertilized eggs (stage A) across sites and between sampling periods, using a two-factor ANOVA. We again arcsine (√) transformed these proportional data. Another factor that is expected to affect likelihood of a female becoming ovigerous is her size (as well as her time to receptivity, Forbes et al. 1996). Thus, we compared size distributions of females (at least 5.5 mm or of minimum reproductive size or larger) across sites and between sampling times using a two-factor ANOVA. We then explored the relation between size class and proportion of females that were ovigerous for each site-by-sampling time combination, where the following five size classes were used: 5.5–6.4, 6.5–7.4, 7.4–8.5, 8.4–9.5, and $\geq$9.6 mm. This allowed us to explore the extent to which female size was a predictor of her likelihood of being ovigerous and whether this was consistent among sites and between sampling times. Using ovigerous females from the smallest size class, we also explored the extent to which either site or time of sampling influenced clutch size (ln-transformed numbers of eggs). As mentioned, females in the smallest size classes should be reproducing for their first time.

For the lab experiment, we assessed whether males that were housed with one receptive female were more likely to mate than males housed with five receptive females (Fisher's exact test). We also assessed whether clutch sizes of ovigerous females that were housed singly with a male differed from mean clutch sizes of ovigerous females that were housed with a male, but in the company of other females, some of which also became ovigerous. Again, a number of eggs were ln-transformed, and we assessed whether mean clutch sizes changed when males housed with five females mated with one to four females, using Pearson correlation.

Results

General

Of 7,585 amphipods collected, 5,571 were females, 1,826 were males, and 188 were intersexes. Of the females, 4,163 (74.7%) were of reproductive size defined here as at least 5.5 mm (only 3 of 1,408 females less than 5.5 mm were ovigerous). Of the females of reproductive size, 2,962 (71.2%) were ovigerous, and of these, 1,200 (40.5%) had clutches at stage A of development. For males, 551 (30.2%) were at least 7.0 mm or of reproductive size. None of the intersexes, including all those more than 5.5 mm, were ovigerous (cf. McCurdy et al. 2004).

Proportion of females that were ovigerous

As mentioned, our primary interest was to assess whether female potential for reproduction and reproductive output was related to seasonal availability of males of reproductive size. We first explored seasonal changes in the proportion of females that were ovigerous. We found no significant interaction between time of sampling and site in explaining variation in arcsine (√) transformed proportion of females that were ovigerous ($F_{3,16}$=0.41, P=0.75). Similarly, site also did not account for variation in proportion of females that were ovigerous ($F_{3,16}$=0.37, P=0.77). In contrast, time of sampling did account for significant variation in proportion of females that were ovigerous ($F_{1,16}$=37.01, P<0.0001). On average, about 81% (78.3–84.1%) of females were ovigerous in the first time period compared to 52% (48.5–55.8%) of females collected in the second sampling period (mean percentage and ranges from standard errors are based on back-transformed values). In fact, these differences in rates of ovigery were quite consistent across sites (Fig. 1).

Availability of reproductively active males

None of the four interactions between site, time of sampling, and/or the covariate of female abundance accounted for significant variation in the availability of reproductive males (F values ranged from 0.29 to 2.1, P values ranged from 0.17 to 0.59). Additionally, site did not influence availability of reproductive males ($F_{3,18}$=0.62, P=0.60). We found that availability of reproductive males was related to abundance of females of reproductive size ($F_{1,18}$=10.1, P<0.01), which is expected simply because high and low density patches are expected to have high and low numbers, respectively, for each class of amphipods. Additionally, availability of reproductive males was strongly related to time of sampling ($F_{1,18}$=13.0, P<0.005), with far fewer reproductive males being found in the later sampling periods, after factoring out numbers of females of reproductive size (least-squares mean±SE for early June: 30.6±2.7 males; late July: 15.3±2.7 males). In other words, for a given abundance of females of reproductive size, half as many males of reproductive size were available later in the season.

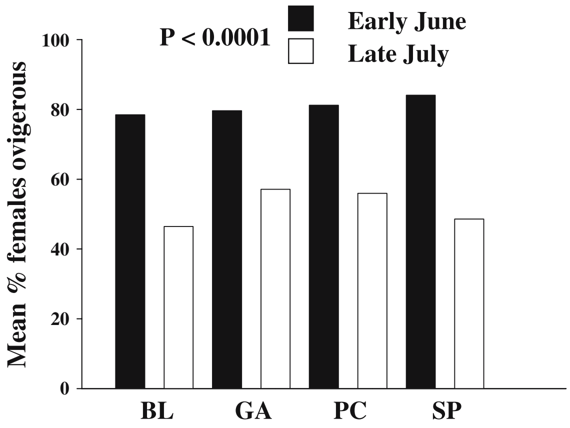

Fig. 1 Mean proportion (mean percentage) of females that were ovigerous (PFO) in relation to time of sampling (early June vs late July) at each of four sites. Proportions represent back-transformed means of arcsine (√) transformed data. At all four sites, fewer females were ovigerous in the second sampling period than in the first. *BL* Blomidon, *GA* Grand Anse, *PC* Peck's Cove, *SP* Starr's Point

Clutch size

Size-specific fecundity (only females with stage A eggs were considered, see above) declined from the first to the second sampling periods at all four mudflat sites. In all four analyses (one per site), female body length and time period each accounted for significant variation in egg number, whereas their interaction did not (see overall variance explained in Table 1). At all sites, females averaged five to ten eggs fewer for a given body size in the second sampling period. Thus, proportion of females that were ovigerous and size-specific fecundity showed the same temporal pattern, being reduced as the season advanced and when relative availability of reproductively active males was lower.

For small stage A females expected to be reproducing for their first time, there was no significant interaction between site and time of sampling in explaining ln-transformed egg numbers ($F_{2,107}$=0.70, P=0.49). In this analysis, only three sites were included because there were no small stage A females collected at Grand Anse in the first sampling period. There was no significant effect of site ($F_{2,107}$=2.59, P=0.079) and a significant effect of time of sampling ($F_{1,107}$=10.55, P<0.005). Females collected later in the season had significantly reduced least-squares mean clutch sizes (back-transformed mean was 17.6±1.1 SE eggs) as compared to small females collected earlier (26.1±1.1 SE eggs).

Phenology of breeding

As mentioned, we also reasoned that if some samples had a higher proportion of females showing advanced brood development, then we might expect other females at those sites to have already brooded and released their young (thereby resulting in lower proportion of females that were ovigerous for those samples). We used proportion of all ovigerous females that had stage A eggs as an index of how advanced breeding was at a particular site and time. We found a significant interaction between site and time of sampling in explaining proportions of ovigerous females with stage A eggs ($F_{3,16}$=4.44, P<0.025, r^2=0.47). Back transformation of the data indicated that this interaction was likely due to females from Blomidon being much more advanced in the first sampling period than in the second sampling period, whereas females from Peck's Cove were much more advanced in the second sampling period than in the first (Fig. 2). The other two sites showed a similar relation between breeding advancement and time of sampling as Blomidon, although less strong (Fig. 2).

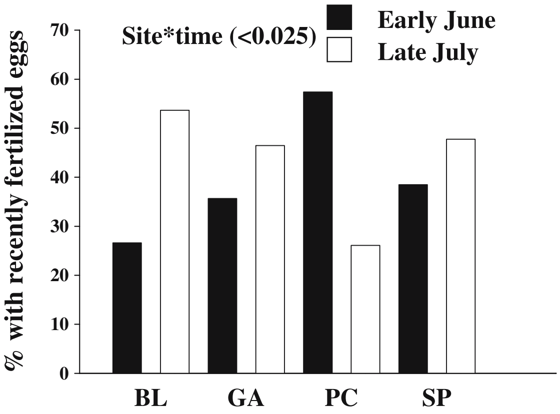

Fig. 2 Proportion of ovigerous females (%) with recently fertilized eggs indicating the degree of advancement of breeding at a particular site and time. Proportions represent back-transformed means of arcsine (√) transformed data. The degree to which breeding was advanced with respect to time of sampling (early June vs late July) differed among sites. *BL* Blomidon, *GA* Grand Anse, *PC* Peck's Cove, *SP* Starr's Point

Table 1 The numbers of ovigerous females (*N*) for each time period at each site

Site	Time	*N*	Mean (±SE)	r^2	Factor	*F* (*P*)
BL	1	199	54.0 (1.02)	0.65	Length	529.23 (<0.0001)
	2	93	43.8 (1.03)		Time	7.60 (<0.01)
					Int	1.13 (>0.25)
GA	1	83	40.0 (1.02)	0.70	Length	80.67 (<0.0001)
	2	66	30.7 (1.06)		Time	4.42 (<0.05)
					Int	0.038 (>0.80)
PC	1	238	46.5 (1.06)	0.62	Length	390.13 (<0.0001)
	2	35	36.5 (1.07)		Time	19.32 (<0.0001)
					Int	1.53 (>0.20)
SP	1	322	55.4 (1.02)	0.54	Length	252.0 (<0.0001)
	2	164	50.6 (1.03)		Time	20.95 (<0.0001)
					Int	0.047 (>0.80)

The mean number of eggs and associated standard errors (SE) are presented. These means are back-transformed from least-squares means based on relations between body length of females (Length) and ln-transformed numbers of eggs. The associated r^2 values for each ANCOVA are given. The *F* values for female body length (Length), time period (Time), and the interaction (Int) between these two factors are given with associated *P* values

Time period: *1* early June, *2* late July; site: *BL* Blomidon, *GA* Grand Anse, *PC* Peck's Cove, *SP* Starr's Point

Thus, despite breeding being advanced at three sites in the first sampling period (and strongly so at one of those sites), proportion of females that were ovigerous was still higher at that time.

Size of females and proportion of females that were ovigerous

We found a significant interaction between site and time of sampling in explaining variation in female body size ($F_{3,\ 4,155}=271.9$, $P<0.0001$). This was due to the fact that, on average, size of females increased seasonally at Blomidon (least-squares mean±SE for early June: 7.15± 0.032 mm; mid-July: 7.49±0.050 mm) and Peck's Cove (early June: 7.03±0.046 mm; mid-July: 8.24±0.062 mm). In contrast, female size declined seasonally at Grand Anse (early June: 8.08±0.061 mm; mid-July: 6.95±0.062 mm) and Starr's Point (early June: 7.93±0.031 mm; mid-July: 6.88±0.036 mm). Thus, consistent seasonal reductions in proportion of females that were ovigerous cannot be explained by seasonal declines in size of females.

We next examined associations between female body size and proportion of females that were ovigerous across sites. Using a log-linear model, we found that number of females was influenced significantly by an interaction between size class and the ovigery class variable ($F_{4,52}=2.60$, $P=0.04$). Thus, we could not interpret main effects. We thus examined relations between proportion of females that were ovigerous and size class on a site-by-site basis and with reference to time of sampling. At three sites, females of one or more of the smaller size classes were less likely to be ovigerous in late July than in the early June sample (Table 2). There were no seasonal differences in proportion of females that were ovigerous for the larger size classes of females at those same sites. At Grand Anse, the smallest females were actually more likely to be ovigerous in the second sampling period, but here only 12 females from 5.5 to 6.4 mm were sampled in early June. Overall, 6 of 20 Fisher's exact tests were significant in showing smaller females less likely to be ovigerous at the time when males of reproductive size were less available.

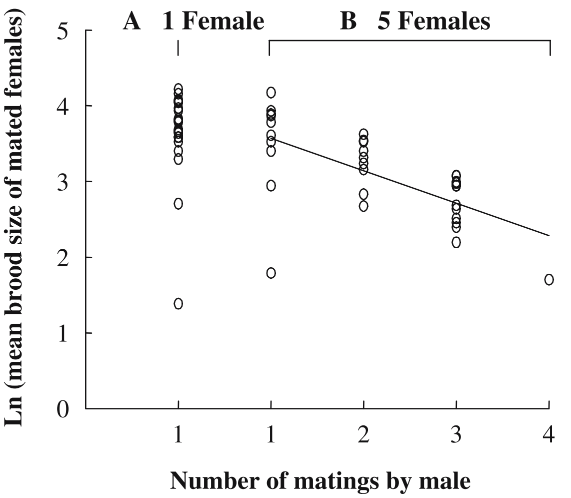

Fig. 3 Numbers of fertilized eggs produced by single females that mated when housed singly with a male (*A*—one female, 22 replicates or 22 females in total). Mean fecundity of females that mated when five females were housed with a single male (*B*—five females, 32 replicates or 160 females in total). Note that in the five-female treatment, there was no instance where all five females mated, as indicated on the *x*-axis. For the five-female treatment, there were 11 replicates of a single female mating, eight replicates of two females mating, 12 replicates of three females mating, and only one instance where four females mated with the male for a grand total of 32 replicates involving 67 females

Table 2 Proportion of females that were ovigerous (PFO) in each of five size classes at each of four sites across the two sampling periods (early June: PFO1; late July: PFO2)

	Female size class (mm)				
	5.5–6.4	6.5–7.4	7.4–8.5	8.4–9.5	>9.5
BL					
N1/N2	207/116	459/72	196/102	36/71	4/15
PFO1/PFO2	67.6/11.2^{a}	83.8/59.7^{a}	79.6/77.4	72.2/81.7	100/80
GA					
N1/N2	12/164	63/30	99/4	71/11	8/40
PFO1/PFO2	8.3/51.8^{a}	73.0/76.7	77.8/75.0	91.5/90.9	100/77.5
PC					
N1/N2	115/39	226/7	78/72	19/106	1/18
PFO1/PFO2	56.5/7.7^{a}	96.9/71.4^{b}	92.3/73.6^{a}	84.2/68.8	100/33.3
SP					
N1/N2	49/61	285/308	434/62	181/27	14/31
PFO1/PFO2	48.9/19.6^{a}	69.4/67.2	91.0/87.1	96.1/92.6	92.8/96.7

N1 refers to number of females in each size class sampled in early June; N2 refers to numbers of females in each size class sampled in late July

BL Blomidon, *GA* Grand Anse, *PC* Peck's Cove, *SP* Starr's Point

[a] Significant at $P<0.01$ (Fisher's exact test)

[b] Significant at $P<0.05$ (Fisher's exact test)

Laboratory study

The laboratory experiment showed that male mate limitation is possible when availability of receptive females is manipulated. If density was important in determining clutch size, then we would expect ovigerous females in the one-female treatment to have higher clutch sizes compared to single ovigerous females in the five-female treatment. That, in fact, did not happen (Fig. 3). As mentioned, we were interested in clutch sizes of females when many females mated vs instances where just a few females mated to the male. Mean clutch size declined when one to four females mated with the male in the five-female treatment (Fig. 3, r^2=0.26, P<0.01). In the one male–one female treatment, only 22 of 52 replicates resulted in a mating, whereas in the one male–five female treatment, at least one female mated with the male in 32 of 47 replicates. This result was significant (Fisher's exact test, P=0.015).

Discussion

Our main findings in this study are consistent with male mate limitation for an intertidal amphipod. This mate limitation appears seasonally and is regionally widespread and follows from several important relations. First, proportion of females that were ovigerous declined at all four sites as the season advanced, and this could not be explained by differences in how advanced breeding was at sites, nor could it be explained by consistent seasonal differences in size distributions of females among sites. Additionally, size-specific clutch size also declined seasonally as did clutch sizes of the smallest females expected to be reproducing for their first time. The only factor that consistently related to these seasonal declines in ovigery and clutch size was the availability of males of reproductive size, although there was a strong tendency for the smallest females to show reduced rates of ovigery at three sites.

Male limitation in field populations of *C. volutator* is expected, despite males having a higher potential rate of reproduction (PRR) than females, because this species shows extremely female-biased sex ratios and can show reproductive synchrony at the patch level. Both of these factors could lead to delays between a *particular* female's molt and her encountering a male that is ready to mate. It is interesting in this regard that when males are less available for mating, it appears that the smallest females are less likely to become ovigerous.

Our laboratory experiments demonstrated that females mating with males that have already mated multiply show a reduced clutch size. To understand the results from this experiment, it is necessary to first speak to some limitations of the design. Obviously, density and the relative availability of a male covaried. However, removing males and placing them sequentially with single receptive females required too much handling. We used the present design because we expected some instances where only a few females would mate with the male in the five-female treatment, as did occur (Fig. 3). More importantly, clutch size of singly ovigerous females was not related to housing density. Further, there was no reason to expect that clutch size should relate to housing density since a female's condition prior to the experiment is a more likely determinant of her energy available for reproduction. We thus expected that patches in the field with proportionately fewer reproductive males would have proportionately fewer ovigerous females: an expectation that was consistent with our field study.

Presently, the mechanism for this apparent mate limitation is unknown. Male limitation may be due to sperm depletion following multiple mating by males similar to that observed for terrestrial isopods (Rigaud and Moreau 2004). Alternatively, it could be due to reduced fertilization success for females that did mate in the multiple-female treatment, reflecting expected delays between their parturial molts and mating. The lab experiment should reflect what could occur in nature; that is, males can encounter several females over a short time period that are receptive to mating (Forbes et al. 1996). It was interesting that although males were randomized between treatments, fewer matings were observed in the one male–one female treatment than in the one male–five female treatment, despite large females being used that had recently molted. Of course, it is possible that some females were simply not receptive to mating and that no mating was most likely observed when only one female was housed with a male. Another possibility deserves mention. Forbes et al. (1996) suggested that males may search out patches of receptive females, and we may expect that males placed into suboptimal conditions with few receptive females may forgo mating and spend more time searching for better opportunities. This may occur if males can mate frequently over a short time period as the lab experiment indicates and similar to work on isopods feminized by bacterial endosymbionts (Moreau and Rigaud 2003).

This study presents useful approaches to investigating male limitation in natural populations in the absence of large and impractical field manipulations. What is interesting is that the apparent male mate limitation is not stochastic, but rather seasonal in nature. Any alternative explanation for these results would have to account directly or indirectly for this seasonal relation between proportion of females that were ovigerous and male availability. For example, there may be seasonal changes in densities of amphipods (both reproductive and nonreproductive) which might result in intraspecific competition and slower resource acquisition by females. Wilson (1989) argued that competition for resources can exist between juvenile and adult *C. volutator*. It is therefore possible that females may not obtain energy for reproduction or have fewer resources to allocate to clutch size if competition is severe (more individuals present). While competition likely exists, we do not think it can explain seasonal changes in size-specific fecundity and ovigery. In our data set, the total number of amphipods per patch did not differ between sampling periods. Furthermore, biomass of amphipods was higher, for a given number of amphipods, in the first sampling period, when proportionately more females were ovigerous (data not shown, but available on request). Biomass estimates were based on published length–weight relations for this species in the Bay of Fundy (Boates and Smith 1979; cf. Hilton et al. 2002).

It is perhaps most telling that even when many large females are present (some patches in the second sampling period), ovigery still can be reduced. Also, when female size is accounted for, there is a seasonal reduction in clutch size (Table 1). We have occasionally observed females attempting to drag males into their burrows, suggesting that male mate limitation is real. Data are needed on incidences of females attempting to coerce males in relation to time of season; however, these occurrences appear rare overall. Another possibility is that females later in the season simply have a different pattern of allocation, for a given amount of resources, than do early season females (cf. Hilton et al. 2002 for suspected earlier onset of reproduction by females at mudflats where *C. volutator* experience intense size-selective predation). We currently do not know whether success of late season females in overwintering is variable depending on whether resources are allocated to, or away from, ovigery and fecundity. However, we do know that clutch size and average egg volumes are inversely related for this species when early and late season samples are combined (data available on request). Other work on seasonal changes in reproductive physiology and allocation to cryoprotectants is also warranted as these stand as proximate alternatives to male mate limitation.

Our data are at least consistent with male mate limitation in this species. The cause may well be the extreme female biases. These biases are still not well understood, but biased sex ratios are expected to have important consequences. In other amphipods and isopods, microparasitic sex ratio distorters are present and can produce female-biased sex ratios (Dunn et al. 1993, 1995; Moreau and Rigaud 2000). Theoretically, male mate limitation may be great enough to hinder population growth and perhaps lead to extinction events (see Hatcher et al. 1999, 2000). Empirical demonstrations of male limitation at the population level are difficult to witness in nature, however, because they may be stochastic rather than seasonal events. Furthermore, populations nearing male limitation may be rescued by immigration of uninfected individuals to those populations, before male mate limitation can be observed. In other populations with extremely female-biased sex ratios, receptive females are still limiting relative to reproductively active males because females spend more time out of reproductive readiness (Moreau and Rigaud 2000). One problem that will be recurring for studies on this and other crustaceans is that a female's time in and time out of reproductive readiness may be cued by social interactions. If males are limiting, females may be assigned to time out when if fact they were fertilizable in the recent past, but had not interacted with reproductively active males.

Our study suggests that further research on male mate limitation is warranted, particularly with respect to parasitic sex ratio distorters. Such research may have important implications. In northwestern Europe, *C. volutator* is an abundant and important species in the intertidal zone (Milne and Dunnet 1972); however, sudden population declines and local extinction at some sites has prompted concern. One suggested cause for declines is infestations by trematodes (Jensen and Mouritsen 1992; Meissner and Bick 1999). In another study, the presence of macroalgal mats is associated with declines presumably because they interfere with feeding by amphipods (Raffaelli et al. 1991). Major population declines or large population fluctuations have occurred in Bay of Fundy populations (Shepherd et al. 1995). Empirical information of the importance of microparasitic sex ratio distorters to population biology of their hosts is now needed for this and other study associations.

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