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Mate limitation in an estuarine population of copepods

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Abstract

We determined the probability of mating as a function of population density in the estuarine copepod *Acartia hudsonica* by combining experimental measurements with a simple model. Pairs of unmated copepods were confined in containers of various volumes to simulate variable population density, and experiments were run for 8, 16, and 24 h. Mating frequencies indicated that males search for females at an effective search volume rate of 0.34 ± 0.15 L h^{-1} or 8.2 ± 3.5 L d^{-1} and that males become ready to mate only after ~15 h exposure to the females. We applied these parameters in a simple population model to determine the critical density for zero population growth. With high egg production, zero mortality, and residence time of 60 d, the critical density for *A. hudsonica* was 0.01 m^{-3}, at the low end of the range of observed population densities. Critical densities for less favorable conditions were well within the range of observed population densities, even allowing for the effects of aggregation. Thus, mate limitation in sexually reproducing organisms, or Allee effects, can cause negative density dependence in growth rate of these populations at low but realistic population densities. We applied these results to the introduction of exotic zooplankton via ships' ballast water under various scenarios of initial dilution in harbors and subsequent conditions for reproduction and survival. Inocula resulting from the discharge of postexchange ballast water were often high enough to establish new resident populations.

Mate limitation at low population levels can result in negative or depensatory density dependence, a form of positive feedback by which reproductive success declines as the population shrinks (Allee 1931). When reproductive success is low, mortality and dispersive losses can lead to local extinction. Therefore, mate limitation is an important factor for species that are rare, that undergo seasonal minima in abundance, or that invade a new habitat. Until recently, mate limitation has been a neglected aspect in studies of population dynamics of marine organisms (Kiørboe 2006, 2007).

We focus here on invasion success, although these concepts and methods apply as well to rare species. Successful invasions require a sufficient number of founding organisms that are physiologically robust enough to develop and reproduce (Williamson 1996; Kolar and Lodge 2001). Invasions by sexually reproducing organisms also require successful mating and reproduction following initial release. Few studies have examined the probable range of critical population density above which successful invasions are possible (e.g., Drake et al. 2005).

Copepods are major invaders in estuarine ecosystems (Ferrari and Orsi 1984; Cordell and Morrison 1996). Concern over invasions by copepods centers on their ecological role in altering aquatic food webs and their possible role as vectors for potentially harmful microorganisms (e.g., *Vibrio cholerae,* Huq et al. 1983). Many marine bioinvasions have been mediated by ships' ballast water, in which copepods are often the most abundant zooplankton and the genus *Acartia* is common (Levings et al. 2004; Choi et al. 2005). Copepods reproduce only sexually and have a well-described life history (Mauchline 1998). Thus, copepods are ideal model organisms for investigating invasion processes and mate limitation.

The behavioral aspects of mating and particularly mate detection have been described for several copepod species. Generally, male copepods search for females, increasing detection range by tracking pheromone trails in some species or hydrodynamic disturbance in other species (Katona 1973; Bagøien and Kiørboe 2005; Kiørboe and Bagøien 2005). Most studies of mating behavior have focused on detection and sometimes pursuit, but few have addressed the other components of successful mating, namely, capture and copulation (Buskey 1998; Kiørboe 2007). Therefore the available data are inadequate to predict the population density at which Allee effects occur.

We examined the influence of population density on mating success of *Acartia hudsonica,* an abundant omnivorous copepod. Members of this genus are usually biomass dominants in temperate estuaries. The ecology and behavior of *Acartia* species have been well studied (Mauchline 1998), and these species are easy to maintain in the laboratory. We combined a series of laboratory mating experiments with a model of the mating process to determine search volume rate (Kiørboe and Bagøien 2005) of the male copepods. These results were then used in a simple population model to determine how the critical density for population maintenance depends on reproductive rate and mortality.

Methods

The experiments were designed to determine mating success in pairs of copepods confined in containers of

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different volumes; i.e., volume was varied to alter population density. Mating success was determined by observing at least one spermatophore attached to the female. The data were then fit to a probabilistic model of mating to determine the "effective search volume rate." This is the volume searched daily by a fertile male based on observations of successful mating; it may be less than the search volume rate determined behaviorally if males sometimes pursue a female but fail to mate. The effective search volume rate was then used in a simple population model to determine the critical density of copepods required for population maintenance.

Main experiments—We incubated pairs of copepods for one of three durations (8, 16, and 24 h) in containers of 10 volumes: 2 mL, 30 mL, and by factors of two from 0.125 to 16 liters. The 2-mL treatment, in well plates, was used only for the 24-h duration. Thirty replicate containers were used for each combination of time and volume, and the statistic of interest was the fraction that mated as indicated by the presence of one or more spermatophores on the female. Supplemental experiments were conducted to examine the process by which the copepods become ready to mate.

Copepods were collected from San Francisco Bay by gentle tows with a plankton net and maintained with aeration in a large container of water amended with mixed phytoplankton (*Chlamydomonas* sp., *Rhodomonas salina,* and *Skeletonema costatum*). Male copepods for the 24-h experiment were collected directly from the bay, whereas males used for the 8-h and 16-h incubations were collected as juveniles to better control the age of the males. This may have introduced differences in males' readiness to mate, but the error appears to have a negligible effect on the estimate of effective search volume rate given the model fit (*see* Results). Immature males (copepodite stages IV–V) were isolated and grown to maturity in well plates (2–5 mL) at ambient temperature and salinity under approximately 8 h artificial light exposure. Upon reaching adulthood, the males were transferred and maintained for at least 2 d in containers ($\geq$1 liter). Females were grown from juveniles and were maintained in containers as for males.

Containers for mating experiments were prepared by filling them with San Francisco Bay water strained through a 53-μm mesh to remove mesozooplankton and amended with *Chlamydomonas* sp. (approximately 80 μg C L^{-1}). One adult male and one adult female copepod were then randomly assigned to each container. All containers were maintained in the dark to minimize aggregation due to phototaxis. The experiment could not be conducted all at once, so two or four sets of containers of each volume were set up at a time. All of the experiments were performed at ambient temperature and salinity from which the copepods were collected (10–16°C and salinity of 20–31). Mating success was not significantly different across incubation periods among different dates (χ^2 test, df = 10–14, $p > 0.12$ for all incubations).

To determine whether settling of particles including phytoplankton had an effect on experimental results, small Nalgene polycarbonate bottles (125 to 500 mL) were split into two groups: half of the bottles were rotated at 1 rpm on a plankton wheel to minimize settling, and the other half were not rotated. Containers (polycarbonate, polypropylene, or polyethylene) larger than 500 mL were chosen to have a similar ratio of dimensions (water depth to width) to the smaller containers. Because the larger containers were too large for the plankton wheel, half were gently stirred with plastic paddles driven by electric motors to minimize settling of particles, and the other half were not stirred. Neither rotation nor stirring had a detectable effect on mating success (χ^2 test, df = 10–14, $p > 0.25$ for all analyses).

At the end of the incubation periods, copepods were gently strained from the water, and we verified that both copepods were alive. Females were examined microscopically for attachment of a spermatophore. The mortality of the copepods during the experiments was <2%, and supplementary incubations were made to compensate for the loss.

Supplemental experiments—We determined the time lag of mating for females. Female copepodite stages IV–V were isolated in well plates and examined every 6 h. Each female that had molted to the adult stage was then randomly combined (29 replicates) with four males. We periodically examined females for attachment of spermatophores for up to 24 h. Nearly all females (28 out of 29 replicates) mated within 24 h of incubation.

Similarly, we determined the time lag of mating for males. Each adult male was randomly combined with four adult females (25 replicates), and each female was examined for the attachment of a spermatophore every 3–4 h for 48 h. The earliest mating was observed around 18 h, with the median at 22 h. In three cases a second spermatophore was attached 6–20 h after the first mating.

To compare mating ability of males of different ages, males of age 1 d and 3–4 d since reaching adulthood were introduced to four females in well plates as described above. Mating success of older males was significantly higher than that of young males ($\chi^2 = 12.8$, df = 1, $p = 0.0001$, $n = 22$). In the same manner, four males were combined randomly with a female of age 1 or 3–4 d. No significant difference was found in the mating ability of females of the two age groups ($\chi^2 = 0.09$, df = 1, $p = 0.76$, $n = 23$).

Since we detected mating by observing one or more spermatophores on a female, we were concerned that some females could drop spermatophores resulting in a false negative outcome. We examined the duration over which mated females carried spermatophores by holding two females carrying spermatophores in each of 22 well plates for 24 h. All of the females except one that had died were carrying spermatophores after incubation.

The potential effect of food limitation on mating success was tested by incubating pairs of copepods in 1-liter containers either with or without added *Chlamydomonas* sp. (approximately 80 μg C L^{-1}). The effect of food concentration on mating was not significant ($\chi^2 = 0.18$, df = 1, $p = 0.67$, $n = 21$).

Mating model design—The mating model was designed to predict the probability of at least one successful mating during an experiment and, thereby, to determine the effective search volume rate of the male copepods. We generally

Table 1. Definitions of symbols used in equations and text, indicating the equation number where the symbol is first used and the dimensions of each parameter.

Symbol	Equation	Definition
Mating model		
V_s	1	Effective search volume rate of males (volume time^{-1})
d	1	Reaction distance (length)
u	1	Swimming speed of a male copepod (length time^{-1})
v	1	Swimming speed of a female copepod (length time^{-1})
w	1	Modal speed of isotropic turbulence (length time^{-1})
P	2	Probability that a female will encounter a male at least once during a time interval
N_m	2	Number of males in volume V
V	2	A volume of water (e.g., an experimental container)
c	2	V_sN_m/V, simplification for the calculations (time^{-1})
t	2	Any time during an experiment
P_R	4	Probability that a fertile male will be ready to mate by time t
b	4	Time constant for males' readiness to mate (time^{-1})
μ	4	Proportion of males that eventually become ready to mate
P_m	6	Probability of at least one mating during a time interval
T	6	Duration of experiment (time)
Population model		
Φ	8	Fraction of males in the population that are ready to mate
c'	9	$= c\Phi$ (time^{-1})
N_{fm}	9	Number of females in population that have mated
R_{0f}, R_{0m}	9	Rate of molting to adult female or male (time^{-1})
a	9	Age of adults in a population (time since terminal molt)
m	9	Mortality rate of adults (time^{-1})
R_{1f}	10	Rate at which offspring of current adults will molt to adult female (time^{-1})
E	10	Egg production rate (time^{-1})
S_e	10	Survival of eggs to hatch (fraction)
S_j	10	Survival from hatching to adult (fraction) $= e^{-mD}$, D the development time and m the mortality rate
F_f	10	Fraction of the adult population that is female.
A	12	Population density of adult copepods (volume^{-1})
A_{crit}	14	Critical population density of adult copepods (volume^{-1})

ignored multiple matings in favor of a simple binary selection: the copepods mated or they did not. Although it is possible that mating more than once enhances a female's reproductive output, any enhancement would be small compared with that resulting from the first mating. Therefore we designed a binary model of mating success.

The mating model fits the data from our experiments in which single pairs of copepods are placed in a container whose volume is the experimental variable. The model then extends these results to the field situation in which there are numerous prospective mates. Symbols used in the mating model are defined in Table 1.

Our mating model is based on the exponential model of Gerritsen (1980). The probability that a receptive female copepod will mate during a time interval depends on the nearby density of males and the duration of the interval. This probability is zero at zero population density and approaches 1 asymptotically as density of males increases, with the rate of asymptotic increase being steeper for a longer experimental duration. For this model it is assumed that (1) males search for adult females through constant swimming; (2) copepods do not learn, so detection range and swimming pattern do not change over time; (3) copepods are randomly distributed within a local environment (though not necessarily throughout their range—in the experiments this environment is the container); (4) upon detection of a potential mate, the male pursues her and attempts to copulate, with a constant probability of success, and a negligible time spent in pursuit, mating, and recovery; and (5) all males that are ready to mate have an equal probability of mating given an encounter with a female, and all females are equally receptive. Some violations of these assumptions are likely but would be difficult to test.

Under assumptions 1 and 2, males search with some fixed radius of detection, and therefore their search pattern defines a volume per unit time. The encounter rate model for predation of Gerritsen and Strickler (1977) was amended by Rothschild and Osborn (1988) to allow for the influence of turbulence. Evans (1989) derived a straightforward version of the relative velocity, by which the search volume rate of the male copepods is

$$V_s = \pi d^2\sqrt{u^2 + v^2 + 2w^2} \tag{1}$$

For simplicity, we neglect the turbulent contribution, swimming speeds of both sexes are assumed to be the same, and the net swimming speed $\sqrt{u^2 + v^2}$ is assigned to the male. None of these simplifications reduces the generality of the results. If swimming speeds of both sexes are on the order of 1–10 mm s^{-1} (Buskey 1998), and reaction distance is on the order of 1–20 mm (Katona 1973; Watras 1983; Uchima and Murano 1988), then the volume

swept per unit time would range from 4×10^{-4} to 1.5 m^3 d^{-1} without turbulence.

The probability that an individual female will encounter a male in volume V during a short time interval dt is

$$P(dt) = \frac{V_s N_m dt}{V} = cdt \quad (2)$$

where N_m is the number of males in volume V and c is introduced to simplify the subsequent calculations. Under assumptions 2 and 3, this is a Poisson process with a time constant c. Therefore the probability that an individual female will have encountered at least one male during any interval (0, t) is

$$P(0,t) = 1 - e^{-ct} = 1 - e^{-\frac{V_s N_m t}{V}} \quad (3)$$

Under the above assumptions any encounters will occur at random times within (0, t). Note that this model satisfies the stated properties of an encounter model: encounter probability is zero if the density of males N_m/V is zero and approaches 1 as either the time interval or the density of males increases.

Mating success depends on a male's readiness to mate at the time of an encounter. In our main experiments, there was only one male ($N_m = 1$) and we varied the duration of the experiment and the volume V. However, some males may have been infertile, and others may have become ready to mate later in the experiment, but we could not distinguish fertile from infertile males. In our mating model we assume that fertile males become ready to mate at some random time, a Poisson process. Therefore the probability that a male will be ready to mate by time t is

$$P_R(0,t) = \mu(1-e^{-bt}) \quad (4)$$

where b is a time constant and μ is the proportion of males that eventually become ready to mate. According to Eq. 4, half of the males that eventually became ready to mate would be ready in (ln 2)/b h. The probability that a male will become ready to mate in a short time interval during the experiment (t, $t + dt$) is the probability density corresponding to Eq. 4, i.e., the first derivative of P_R with respect to time, as dt approaches 0

$$P_R(t,t+dt) \cong dP_R = \mu b e^{-bt} dt \quad (5)$$

The probability of a successful mating by a male that becomes ready to mate during the interval (t, $t + dt$) is then the joint probability that a male will become ready to mate in this short time interval and that there will be at least one encounter between that time and the end of the experiment at time T. This is the product of these probabilities, from Eqs. 5 and 3, respectively,

$$P_m(t,t+dt) \cong dP_R P(t,T) = \mu b e^{-bt}(1-e^{-c(T-t)})dt \quad (6)$$

Integrating Eq. 6 over the duration of the experiment gives the probability of at least one successful mating during the experiment

$$P_m(0,T) = \mu\left[1-e^{-bT} - \frac{b}{b-c}\left(e^{-cT} - e^{-bT}\right)\right] \quad (7)$$

To estimate the parameters in this mating model, we fitted the model to the experimental data for *A. hudsonica*. Because the data comprised replicate samples in which a pair of copepods either mated or did not, the data for each treatment should have a binomial distribution. Therefore, we used a general optimization procedure, adjusting the parameters b, c, and μ in Eq. 7 to maximize the log likelihood function for a binomial distribution (function *optim* in Splus, Insightful; Venables and Ripley 2003). The Hessian matrix from the optimization procedure was inverted to determine the variance of the parameters and their correlations.

As a check on the suitability of the model, we compared the residual error distribution with that expected from a binomial distribution around the model's predicted probability of mating. We scaled the observed error to the expected error and determined the fit of the scaled errors to a normal distribution using normal probability plots and a goodness-of-fit chi-square test. We also analyzed data and residuals graphically as a check against violation of assumptions and to ensure the mating model gave a suitable fit to the experimental data.

The model in Eq. 7 had a high correlation (−0.93) between the parameter estimates for b and μ, which inflated confidence limits around each parameter. To refine estimates of these parameters, both dealing with male fertility, we determined the parameters of Eq. 4 as described above using only data from volumes of 0.25 liters or less. At these small volumes, encounters are expected to occur frequently and mating success depends solely on the male's readiness to mate. Fitted values at these volumes were within 2–8% of maximum fitted values for the same experimental duration in Eq. 7, so $P_m \approx P_R$ at these volumes. This analysis revealed that the confidence limits on μ included 1, suggesting that all males would ultimately become ready to mate (Table 2). Furthermore, likelihood ratio tests between the models in Eqs. 4 and 7 and the same models with the parameter μ fixed at 1 showed no significant differences ($\chi^2 = 0.05$, df = 1, $p > 0.1$ for Eq. 4, $\chi^2 = 2.95$, df = 1, $p \sim 0.09$ for Eq. 7). Therefore, including μ did not improve the fit of either equation. For the remainder of the analysis, μ was fixed at 1 in the model in Eq. 7.

Population model—We developed a simple population model similar to that of Kiørboe (2006) to determine the critical population density, i.e., the population density at which the rate of population growth is zero for a given set of demographic parameters. Input to this population model included the effective search volume rate from the mating model together with assumed values of egg production of mated females and mortality. At population densities above the critical density the population would be expected to grow, while at densities below the critical density the population would shrink because reproduction was restricted by low mating frequency. With this population model we explored the critical density for net population growth in a copepod population either extant in a waterbody or released in ballast-water discharge. Symbols used in the population model are defined in Table 1.

Table 2. Statistics for model fits for the experimental data in Figure 1. Data presented are parameter estimates with 95% confidence limits, and correlation coefficient r. The "small V" models were fit to data for experimental volume of 0.3 liters or less.

Model form	Equation	Parameter			
		V_s (L h^{-1})	b (h^{-1})	μ	r
Three parameters	7	0.30±0.14	0.09±0.06	0.70±0.21	0.94
Two parameters	7 with μ set to 1	0.34±0.15	0.05±0.008	—	0.94
Small V, two parameters	4	—	0.04±0.05	1.1±0.96	0.96
Small V, one parameter	4 with μ set to 1	—	0.05±0.004	—	0.96

Additional assumptions for the population model are (6) mortality is not changed by processes involved in mating, including search and copulation, or by the aggregation that may allow successful mating; (7) the population is at or near steady state; (8) mortality through all developmental stages can be modeled as a single value; (9) adult males and females have a constant mortality rate and no fixed longevity; and (10) the sex ratio is constant. We assume that the initial population is close enough to steady state that the age distributions are not changing rapidly. We then determine the critical unstable equilibrium density corresponding to zero population growth as an output of the model. Assumptions 8–10 simplify the problem, enhancing clarity without reducing the generality of our results. Assumption 8 has no consequences for this model, and for exploring the consequences of mortality we use the same mortality for all postembryonic life stages. Assumption 9 is not strictly true, but realistic field mortality rates would constrain longevity (Kimmerer and McKinnon 1987). Differential mortality may skew adult sex ratios (Svensson 1997; Kiørboe 2006), but this can be addressed explicitly by changing the sex ratio in the model.

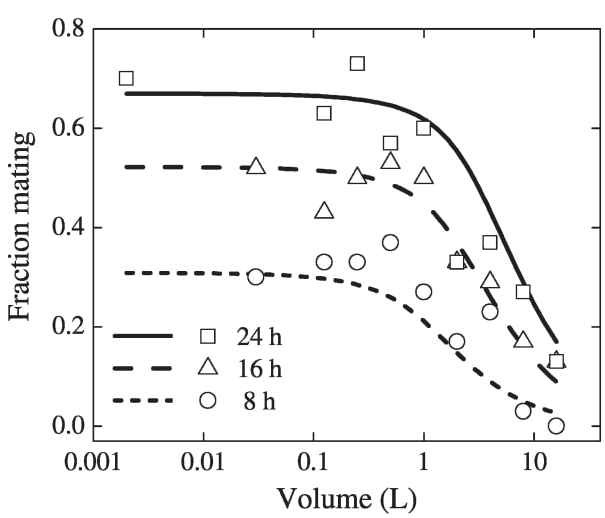

Fig. 1. *Acartia hudsonica.* Experimental results showing the fraction of copepod pairs that mated as a function of volume for three different experimental durations. Points are the fraction mating out of 30 replicates from the experiment, and lines are predictions of the model (Eq. 7) fit to the entire data set assuming that all males eventually are ready to mate ($\mu = 1$).

In a field population, encounters can occur between any male and any female, so the process of becoming ready to mate (Eq. 4) and the encounter process (Eq. 3) are independent. Therefore Eq. 3 can be used to calculate encounter rates, provided the density of males N_m/V is corrected for the fraction that is ready to mate, so c in Eq. 3 is replaced with $c' = \Phi c$, where Φ is the fraction ready to mate. The number of males in volume V that are alive and ready to mate at any given time can be determined by multiplying the number of adult males that survive to age a, e^{-ma}, by the probability that males are ready to mate by age a from Eq. 4 (substituting age a for time t) and integrating over all possible ages of adult copepods. Dividing this result by the total number of copepods gives the fraction ready to mate

$$\Phi = \frac{\int_0^\infty R_{0m} e^{-ma}\left(1-e^{-ba}\right)da}{\int_0^\infty R_{0m} e^{-ma} da} = \frac{b}{m+b} \tag{8}$$

where R_{0m} is the rate at which adult males are produced by molting from copepodite stage V, m is the constant mortality rate, and b is the time constant from Eq. 4.

In a population of female copepods within a volume V, the probability that an individual will have mated in an age interval (0, a) is the same as in Eq. 3 for time interval (0, t). Thus, the number of females that are alive and have mated at any given time can be determined by multiplying female survival to age a by the probability of mating by age a from the right-hand side of Eq. 3 (substituting c' for c), and integrating over all possible ages of adult copepods

$$N_{fm} = \int_0^\infty R_{0f} e^{-ma}\left(1-e^{-c'a}\right)da = R_{0f}\frac{c'}{m(m+c')} \tag{9}$$

where symbols are as in Eq. 8 but for females.

The rate at which the female offspring of the current generation will molt to adult after development, R_{1f}, is the product of egg production of the mated females, survival over subsequent egg and juvenile life stages, and the fraction of the offspring that are female

$$R_{1f} = N_{fm} E S_e S_j F_f \tag{10}$$

Substituting N_{fm} from Eq. 9 into 10 and solving for c' gives

$$c' = \frac{m^2}{\frac{R_{0f}}{R_{1f}} E S_e S_j F_f - m} \tag{11}$$

From Eqs. 2 and 8, c' can be expressed in terms of the local population density of adult male copepods that are ready to mate

$$c' = \frac{V_s N_m}{V}\frac{b}{m+b} = V_s A\left(1-F_f\right)\frac{b}{m+b} \tag{12}$$

Combining Eqs. 11 and 12 and solving for density A gives

$$A = \frac{m^2}{V_s\left(1-F_f\right)\left(\frac{b}{m+b}\right)\left(\frac{R_{0f}}{R_{1f}}ES_eS_jF_f - m\right)} \tag{13}$$

At the lowest population density allowing persistence of the population, the number of females molting to adulthood is the same in each generation, i.e., $R_{0f} = R_{1f}$. Thus the critical density for population maintenance A_{crit} is

$$A_{\text{crit}} = \frac{m^2}{V_s\left(1-F_f\right)\left(\frac{b}{m+b}\right)\left(EF_fS_eS_j - m\right)} \tag{14}$$

If density within aggregations is greater than A_{crit} the population will grow; if less, the population will collapse. This equation applies strictly only at the critical density and neglects compensatory density dependence implied in several of the parameters (e.g., egg production and survival). This is acceptable because we are concerned with dynamics only at low population densities. In addition, the derivative of population density A with respect to the rate of increase of the population per generation, R_{1f}/R_{0f}, must be positive according to Eq. 13, implying an unstable equilibrium with positive feedback between population density and the rate of population growth, a consequence of the Allee effect.

To determine critical population density, we applied the mating results from our experiments to population growth in field situations using the population model (Eq. 14). Inputs to the model were daily effective search volume rate attributed to males (V_s) and demographic parameters that were varied to produce the output: egg production rate of mated females and mortality of egg, juvenile, and adult life stages, with water residence time included explicitly in mortality of juveniles and adults. Egg production rate was set to either 34 (high) or 8 eggs ♀$^{-1}$ d^{-1} (low), respectively, the asymptotic and median egg production rates of *A. hudsonica* in the San Francisco estuary (Kimmerer et al. 2005). Development time was set at 22 d and sex ratio at 0.5. Daily mortality of all stages except eggs was set equal to zero or to 0.03 or 0.1 d^{-1}, the lower and mean values from Hirst and Kiørboe (2002, fig. 4) for 15°C. Survival of eggs was calculated assuming a tenfold higher mortality rate of eggs than other life stages (Hirst and Kiørboe 2002) and a 1-d development time.

Results

Mating success of pairs of *A. hudsonica* decreased exponentially as container volume increased above a threshold, in general agreement with the prediction of the mating model (Fig. 1). The effective search volume rate of the males, estimated from the fit of the experimental data to the mating model, was 0.34 ± 0.15 L h^{-1} or 8.2 ± 3.5 L d^{-1} (95% confidence interval, Table 2, Fig. 1). All diagnostic plots and analyses indicated a good fit and an appropriate model (e.g., Fig. 2).

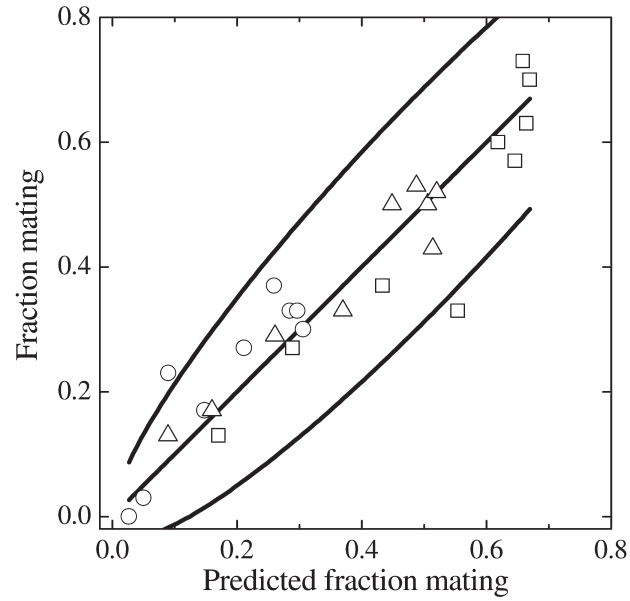

Fig. 2. Data from Fig. 1 plotted as fitted vs. experimental data. Lines are the 1 : 1 line and curves representing the 95% confidence limits around fitted values based on a binomial distribution with n = 30.

Mating success in small volumes increased asymptotically toward a maximum with duration of the experiment, indicating a delay in readiness of males to mate (Table 2). Using Eq. 7 and assuming that all males eventually are ready to mate (μ set to 1), the parameter b was 0.05 ± 0.008, so half of the males were ready to mate in 15 h (95% confidence interval 13–18 h). In a supplemental experiment, newly molted males (<6 h after molting) required 22 h for 50% to be ready to mate. Over 90% of the males that mated in the main experiment did so only once, with a maximum of three mating events as indicated by spermatophores attached to the female.

The population model shows that a self-sustaining population of *A. hudsonica* can be very dilute in the absence of in situ mortality (Fig. 3). With a residence time of 60 d, no mortality, and high egg production (35 eggs ♀$^{-1}$ d^{-1}), the population can recover from abundance lower than ~0.01 m^{-3}. Less favorable conditions result in orders of magnitude higher critical density (Fig. 3). Critical density was undefined for shorter residence times or for higher mortality rates than shown in Fig. 3.

We applied the population growth model to examine the likelihood that a population of introduced copepods could be established by a single ballast-water discharge event in a harbor. We assumed that environmental conditions in the receiving water are hospitable to the species of interest. For discharge into a harbor, it was necessary to consider initial dilution, which generally takes <1 d. A discharge of 10,000 m^3 of ballast into a harbor that is 1 km^2 in area and 10-m deep would result in a ~1,000-fold initial dilution. This dilution effectively scales the influence of residence time, mortality, and fecundity (Fig. 3). With an initial 1,000-fold dilution, adult abundance of *A. hudsonica* must exceed ~5 m^{-3} in the ballast water for positive

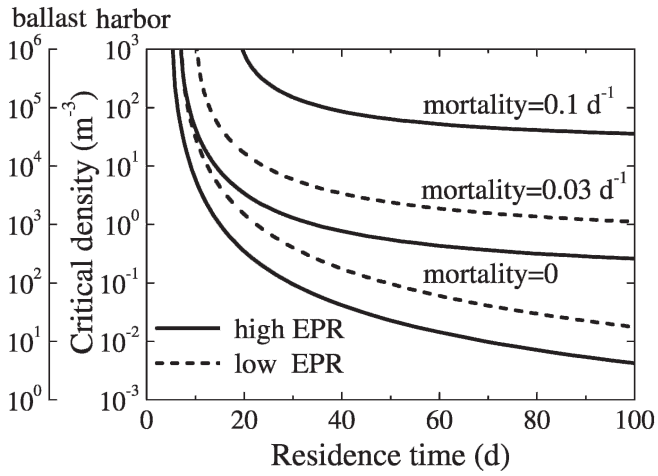

Fig. 3. Critical population density of *Acartia hudsonica* adults as a function of the receiving harbor's residence time and copepod mortality rate and egg development time. The critical density is shown for the receiving harbor (inner vertical axis) and for ballast water (outer vertical axis) assuming a 1,000-fold initial dilution in the receiving harbor.

population growth after discharge, assuming the higher fecundity value and longest residence time with zero mortality (Fig. 3).

Discussion

This is the first analysis of mating probability and consequences for population growth in an estuarine copepod. Kiørboe (2006) analyzed the relationship between mating biology and population outcomes including sex ratios and critical densities, and Kiørboe (2007) examined population consequences of mate limitation and male fertility in *Oithona davisae*. Previous analyses have focused on aspects of mating behavior, particularly mate detection using chemical (Katona 1973) or hydromechanical cues (Bagøien and Kiørboe 2005). Search volume rate, i.e., the volume sampled for the chemical or hydromechanical signal of a female copepod, has also been estimated for various copepods. Search volume rate appears to depend more on the size of the copepod than on the mode of detection, although purely hydromechanical detection seems to be limited to small copepods (Bagøien and Kiørboe 2005; Kiørboe and Bagøien 2005). Although the mating model in this paper applies to copepods that search for mates using hydromechanical cues (Bagøien and Kiørboe 2005), the same model can be applied to copepods that detect mates using pheromones provided the search volume rates are determined for those species. The population model presented here does not apply to planktonic taxa that can reproduce asexually or to planktonic larvae of benthic organisms for which mating probability depends on the distribution of settled adults.

Buskey (1998) identified four components of mating, directly analogous to the components of predation of Holling (1959): detection, pursuit, capture, and copulation. The probability of a successful mating event between two copepods that are physiologically ready to mate is the joint probability of these components, i.e., the product of their conditional probabilities. Most studies of behavior examine only the first one to three components, whereas we determined the outcome of all components combined. If pursuit, capture, and copulation all had a probability of 1, then V_s, the effective search volume rate in our experiments, should be consistent with experimental evidence on swimming speed and detection radius. Kiørboe and Bagøien (2005) reported a search volume rate for *Acartia tonsa* of 0.8 mm cephalothorax length of 48–72 L d^{-1}. Search volume rate scales by $L^{2.7–2.8}$ (Kiørboe and Bagøien 2005; Kiørboe 2007), and correcting for length the search volume rate of *A. hudsonica* (mean cephalothorax length 0.74, Kimmerer et al. 2005) should be 38–58 L d^{-1}, considerably higher than the upper confidence limit of our estimate. Thus, the conditional probability of mating given detection may be <1. If this is true for other copepods, then estimates of mating success during pursuit, capture, and the production and transfer of spermatophores are required before the results of behavioral experiments can be used to estimate the influence of Allee effects in field situations.

At the critical density in a field population, most copepods should be ready to mate by the time they encounter a suitable partner unless mortality rates are high (Eq. 8). Nevertheless, we are unable to explain the delays in readiness to mate observed in all of our experiments, which did not appear to depend on the time since the terminal molt to adult. The low frequency with which we observed >1 mating event in the main experiments was more likely due to limits on males' ability to produce spermatophores than avoidance of repeated mating with the same female. We did not determine the interval between mating events directly, but the experimental evidence is consistent with an interval similar to that reported for *O. davisae* of 0.4–1.5 times per day (95% confidence interval, Kiørboe 2007).

Zooplankton distributions are patchy, with aggregations extending centimeters to meters and with densities twofold to fivefold higher than the mean (e.g., Davis et al. 1992). Aggregation may be a mechanism to enhance mating probability (Davis et al. 1992). Thus, estimates of the critical density for population persistence from our population model should be compared with density in aggregations, not with the bulk density determined by typical plankton samples.

The population model indicated that the *A. hudsonica* population could recover from very low densities under suitable conditions. These conditions might arise, for example, if the population were distributed at low abundance throughout an estuary at the onset of a spring phytoplankton bloom. Considering that bulk density from plankton samples would be lower than density in aggregations, the critical density under favorable conditions (~0.01 m^{-3}) would result in zero reported catch in most samples in plankton surveys of estuaries (e.g., Ambler et al. 1985). Therefore, it is not necessary to invoke the presence of resting eggs (Uye 1985) to explain recovery of plankton populations from seasonal abundance below the survey's detection limit, unless the sample volume and subsampling procedures are designed to detect plankton species at

densities several orders of magnitude below typical densities of more common species.

If our model can be applied to oceanic copepod species, either effective search volume rates must be much larger than those reported here or aggregations must be much denser relative to mean population density. Densities of adults of common epipelagic species of copepod in the North Pacific gyre are <0.1 m^{-3} (McGowan and Walker 1985), with density of rare species being extremely low (<0.001 m^{-3}); mesopelagic copepods have typical densities $\ll 0.1$ m^{-3} (Deevey and Brooks 1971). With the lower availability of food in less productive oceanic waters, the egg production rate is correspondingly low most of the year. Egg development is slow in colder waters (e.g., mesopelagic environments), although slow population growth may be somewhat compensated by year-round reproduction (Yamaguchi and Ikeda 2000) and possibly by low egg mortality rates. Some oceanic copepods that are numerically scarce, especially those in mesopelagic waters, may be large and swim fast, with longer detection range of mates and consequently a larger search volume rate. Behavioral patterns that enhance the probability of mating success (e.g., release of pheromones, swarming, synchronization of vertical migration, and mate guarding; Buskey 1998) could be more pronounced in oceanic copepods, which would compensate for the lower average population density in maintaining the populations. Paradoxically, volume search rates for copepods using hydromechanical cues were similar to rates of copepods using pheromones, after correction for copepod size (Bagøien and Kiørboe 2005).

Consequences for invasions—The critical density for successful invasions from ballast water is highly sensitive to the initial dilution and residence time of the receiving harbor (Fig. 2) (e.g., Nicolini and Penry 2000). The probability that a population would become established is greatest when the initial inoculum is released into a small harbor of long residence time. This suggests that discharge of ballast water would be safer in open embayments or channels than in enclosed harbors. This is consistent with the operations of at least some ships, which generally begin discharging ballast water during transit into an estuary in order to reduce draft. Regulation of ballast-water discharge should take into account characteristics not only of likely source populations but also of receiving water.

Although the analysis of critical density is based on the mating results of a single species, upper bounds may still be drawn for densities of similar copepods in ballast water that would be unlikely to result in establishment of a population. We must assume that other species of copepod have similar probabilities of mating given detection to that of *A. hudsonica*. A copepod with a cephalothorax length of $\sim$2 mm (larger than most estuarine species, $\sim$2.5 times as large as *A. hudsonica*) would have a search volume rate about tenfold higher (Kiørboe and Bagøien 2005). The critical density for the establishment of a population under the above assumptions would decrease to 0.001 m^{-3} in harbors and 1 m^{-3} in ballast water.

Adults of individual species of copepods in ballast water following reduction through midocean exchange were usually ($>$90 %) more abundant than the critical population density determined for the most favorable conditions ($\sim$10 m^{-3}) (Choi and et al. 2005; Choi unpubl.). This suggests that the risk of invasion for similar species may be high even after midocean exchange. The probability of invasion may be even higher since not all vessels exchange ballast water, and the abundance of a single species in ballast water that has not undergone exchange can be high (on the order of 10,000 m^{-3}; Levings et al. 2004), even accounting for mortality during the voyage (Wonham et al. 2001). Moreover, the cumulative probability of a successful invasion even for a species of low abundance may be high in a busy harbor with a large volume of shipping resulting in frequent discharge events (Drake and Lodge 2004).

To estimate the likelihood of a population of copepods becoming established as a result of ballast discharge, a probabilistic model would be more suitable than our critical-density population model. However residence time, a critical component of such a model, would have to be determined for each specific harbor or bay. In addition, analysis of cumulative effects would require specific information on the frequency of discharge events. Thus, our model provides a general guide to the conditions under which populations of copepods may become established after ballast discharge, but to apply these model results the particular circumstances in a given harbor would have to be investigated. Nonetheless, the principle remains that a quantitative understanding of both mating and population growth is essential for better prediction and prevention of invasions.

References

Allee, W. C. 1931. Animal aggregations: A study in general sociology. Univ. Chicago Press.

Ambler, J. W., J. E. Cloern, and A. Hutchinson. 1985. Seasonal cycles of zooplankton from San Francisco Bay. Hydrobiologia **129:** 177–197.

Bagøien, E., and T. Kiørboe. 2005. Blind dating—mate finding in planktonic copepods. III. Hydromechanical communication in *Acartia tonsa.* Mar. Ecol. Prog. Ser. **300:** 129–133.

Buskey, E. J. 1998. Components of mating behavior in planktonic copepods. J. Mar. Sys. **15:** 13–21.

Choi, K-H., W. Kimmerer, G. Smith, G. M. Ruiz, and K. Lion. 2005. Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. J. Plankton Res. **27:** 707–714.

Cordell, J. R., and S. M. Morrison. 1996. The invasive Asian copepod *Pseudodiaptomus inopinus* in Oregon, Washington, and British Columbia estuaries. Estuaries **19:** 629–638.

Davis, C. S., S. M. Gallager, and A. R. Solow. 1992. Microaggregates of oceanic plankton observed by towed video microscopy. Science **257:** 230–232.

Deevey, G. B., and A. L. Brooks. 1971. The annual cycle in quantity and composition of the zooplankton of the Sargasso Sea off Bermuda. II. The surface to 2,000 m. Limnol. Oceanogr. **16:** 927–943.

Drake, J. M., and D. M. Lodge. 2004. Global hot spots of biological invasions: Evaluating options for ballast-water management. Proc. R. Soc. Lond., B. **271:** 575–580.

———, D. M. Lodge, and M. Lewis. 2005. Theory and preliminary analysis of species invasions from ballast water: Controlling discharge volume and location. Am. Mid. Nat. **154:** 459–470.

Evans, G. T. 1989. The encounter speed of moving predator and prey. J. Plankton Res. **11:** 415–417.

Ferrari, F. D., and J. J. Orsi. 1984. *Oithona davisae,* new species, and *Limnoithona sinensis* (Burckhardt, 1912) from the Sacramento-San Joaquin Estuary, California. J. Crust. Biol. **4:** 106–126.

Gerritsen, J. 1980. Sex and parthenogenesis in sparse populations. Am. Nat. **115:** 718–742.

———, and J. R. Strickler. 1977. Encounter probabilities and community structure in zooplankton: A mathematical model. J. Fish. Res. Board Can. **34:** 73–82.

Hirst, A. G., and T. Kiørboe. 2002. Mortality of marine planktonic copepods: Global rates and patterns. Mar. Ecol. Prog. Ser. **230:** 195–209.

Holling, C. S. 1959. The components of predation as revealed by a study of small mammal predation of the European pine sawfly. Can. Entomol. **91:** 293–320.

Huq, A., E. B. Small, P. A. West, M. I. Huq, R. Rahman, and R. R. Colwell. 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl. Environ. Microbiol. **45:** 275–283.

Katona, S. K. 1973. Evidence for sex pheromones in planktonic copepods. Limnol. Oceanogr. **18:** 574–583.

Kimmerer, W. J., and A. D. McKinnon. 1987. Growth, mortality, and secondary production of the copepod *Acartia tranteri* in Westernport Bay, Australia. Limnol. Oceanogr. **32:** 14–28.

———, M. H. Nicolini, N. Ferm, and C. Peñalva. 2005. Chronic food limitation of egg production in populations of copepods of the genus *Acartia* in the San Francisco Estuary. Estuaries **28:** 541–550.

Kiørboe, T. 2006. Sex, sex-ratios, and the dynamics of pelagic copepod populations. Oecologia **148:** 40–50.

———. 2007. Mate finding, mating, and population dynamics in a planktonic copepod *Oithona davisae:* There are too few males. Limnol. Oceanogr. **52:** 1511–1522.

———, and E. Bagøien. 2005. Motility patterns and mate encounter rates in planktonic copepods. Limnol. Oceanogr. **50:** 1999–2007.

Kolar, C. S., and D. M. Lodge. 2001. Progress in invasion biology: Predicting invaders. Trends Ecol. Evol. **16:** 199–204.

Levings, C. D., J. R. Cordell, S. Ong, and G. E. Piercey. 2004. The origin and identity of invertebrate organisms being transported to Canada's Pacific coast by ballast water. Can. J. Fish. Aquat. Sci. **61:** 1–11.

Mauchline, J. 1998. The biology of calanoid copepods. Adv. Mar. Biol. **33:** 1–710.

McGowan, J. A., and P. W. Walker. 1985. Dominance and diversity maintenance in an oceanic ecosystem. Ecol. Monogr. **55:** 103–118.

Nicolini, M. H., and D. L. Penry. 2000. Spawning, fertilization, and larval development of *Potamocorbula amurensis* (Mollusca: Bivalvia) from San Francisco Bay, California. Pac. Sci. **54:** 377–388.

Rothschild, B. J., and T. R. Osborn. 1988. Small-scale turbulence and plankton contact rates. J. Plankton Res. **10:** 465–474.

Svensson, J. E. 1997. Chaoborus predation and sex-specific mortality in a copepod. Limnol. Oceanogr. **42:** 572–577.

Uchima, M., and M. Murano. 1988. Mating behavior of a marine copepod *Oithona davisae.* Mar. Biol. **99:** 39–45.

Uye, S. I. 1985. Resting egg production as a life history strategy of marine planktonic copepods. Bull. Mar. Res. **37:** 440–449.

Venables, W. N., and B. R. Ripley. 2003. Modern applied statistics with S, 4th ed. Springer-Verlag.

Watras, C. J. 1983. Mate location by diaptomid copepods. J. Plankton Res. **5:** 417–423.

Williamson, M. 1996. Biological invasions. Chapman and Hall.

Wonham, M., W. Walton, G. Ruiz, A. Frese, and B. Galil. 2001. Going to the source: Role of the invasion pathway in determining potential invaders. Mar. Ecol. Prog. Ser. **215:** 1–12.

Yamaguchi, A., and T. Ikeda. 2000. Vertical distribution, life cycle, and developmental characteristics of the mesopelagic calanoid copepod, *Gaidius variabilis* (Aetideidae) in the Oyashio region, western North Pacific Ocean. Mar. Biol. **137:** 99–109.

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