Title: Resource and mate availability, and previous social experience modulate mate choice in
the copepods *Acartia tonsa* and *Acartia hudsonica*

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Abstract

Copepods, the most abundant metazoans in the oceans, exhibit sexual selection through mate choice. Mate choice may depend on resource and mate availability, mate quality, and the ability of an individual to assess potential mates. We measured the relative strength of mate choice for body size in male and female individuals of the copepod species *Acartia tonsa* and *Acartia hudsonica* as a function of food availability, encounter time, and previous social experience with the same and opposite sex. Mate choice in *A. tonsa* females depended on food availability and previous exposure to the opposite sex, whereas exposure to the opposite sex and encounter time affected mate choice in males. For *A. hudsonica*, previous social experience with the same and opposite sexes and encounter times were important for mate choice in females, and food availability and encounter time affected mate choice in males. The results suggest that mate choice responds to resource and mate availability, as well as social experience, but that the responses are species and sex-specific even within the same genus. Therefore, these copepods appear to have a flexible mate-sampling strategy that allows them to raise or lower their mate preferences in order to balance the benefits and costs associated with finding a desired mate.

Key words: mate choice, copepod, *Acartia*, social experience, resource availability, mate availability

1. Introduction

Sexual selection, a form of natural selection due to differences in mating success (Darwin, 1871), can often explain a species' morphology, behavior, and population biology (Andersson, 1994). While sexual selection has been studied extensively in numerous terrestrial and even marine taxa, there have been few studies on pelagic copepods (Titelman et al., 2007; Ceballos and Kiørboe, 2010) even though they are the most abundant metazoans in the ocean. Copepods exhibit several behaviors that may be examples of sexual selection (i.e. pre-copulatory mate guarding, copulatory dances, mate coercion, and mating avoidance) (Titelman et al., 2007); moreover, the strength of sexual selection is likely different from one copepod species to the next since mating patterns vary among species (brood vs. free-spawn, single mating vs. obligate repeated mating to remain fertilized, feeding vs. non-feeding males, etc.). Thus, copepods may be informative for studies of sexual selection (Ceballos and Kiørboe, 2010).

Sexual selection, by way of mate choice, is starting to be recognized as important in the reproductive biology of pelagic copepods. Recent work has provided evidence that copepods may choose mates based on a variety of traits including size, age, and reproductive state. For instance, both sexes of *Acartia tonsa* prefer to mate with large mates (Ceballos and Kiørboe, 2010). Since size is heritable in copepods (McLaren, 1976; McLaren and Corkett, 1978), and since larger individuals produce larger and more offspring, an individual can increase its fitness by mating with a large mate (Weatherhead and Robertson, 1979; Ceballos and Kiørboe, 2010). Similarly, both sexes of *Temora longicornis* mate preferentially with young individuals likely because offspring number and quality decrease with parental age (Ceballos and Kiørboe, 2011; Sichlau and Kiørboe, 2011). Additionally, males of many species (*A. tonsa*, *Acartia hudsonica*, *Oithona davisae*, and numerous parasitic copepods) distinguish between the reproductive states

of females, and most mate with virgin females over those females that have already mated (Anstensrud, 1990, 1992; Heuch and Schram, 1996; Heuschele and Kiørboe, 2012; Burris and Dam, 2014). In summary, members of either sex or of both sexes simultaneously can choose for a specific trait and select among available mates.

While these studies are valuable in providing information about whether or not a trait is important for mate choice, relatively little work has been done in the field where mate choice is subjected to the effect of multiple time-dependent parameters. Mate choice in natural populations can change temporally depending on different ecological conditions as either more or less of the population is being selective (Emlen and Oring, 1977; Wilson and Hedrick, 1982; Jennions and Petrie, 1997; Forsgren et al., 2004). For instance, the strength of mate choice can vary simply because the potential benefits of mating with a particular mate is context-dependent and influenced by factors like the availability of mates (Shuster and Wade, 2003), the quality and variation in quality of those potential mates, and the ability of an individual to assess potential mates (Jennions and Petrie, 1997; Kokko and Monaghan, 2001; Shuster, 2007). As these conditions vary over time, the strength of mate choice will also likely vary. In field populations of copepods, these parameters can vary significantly between and within a season (Mauchline, 1998). Availability of potential mates, for instance, can be influenced by a number of population parameters, including population density and sex ratio. In coastal copepods, encounter rates can be very high during peak densities, when up to 30 potential mates may be encountered on a daily basis (Kiørboe, 2007). Since males can only mate about once a day and females remain fertilized for up to ten days (Ceballos and Kiørboe, 2010; Burris and Dam, 2014; Burris, unpublished data), these high densities exceed the mating needs of both sexes and likely allow individuals to be selective when choosing a mate. In contrast, at the beginning and end of the

growing season for these populations, densities can be low enough that theoretically a potential mate is encountered only every few days (Heuschele et al., 2013). Here, the benefits of being selective may no longer exist, as copepods run the risk of not finding a mate, or experience increased costs as they search for another mate (Real, 1990). This inference is supported by numerous models (Hubbell and Johnson, 1987; Crowley et al., 1991; Kokko and Monaghan, 2001; Hardling and Kaitala, 2005; Kokko and Mappes, 2005; Heuschele et al., 2013).

Variation in sex ratio can also influence mate availability, and thus mate choice, by changing the density of members of the opposite sex as well as that of the same sex (Kokko and Rankin, 2006). Competition and aggression among individuals of the abundant sex for mating opportunities with the rarer sex is reported in some copepod species (see Ahnesjö et al., 1993). In this case, mate choice may be influenced by previous exposure to members of the same sex prior to mating. Moreover, copepod sex ratios vary considerably over the season and are often highly skewed toward females (review: Kiørboe, 2007). Therefore, individuals may adjust mate choice in response to the rate at which they encounter other individuals of the same sex. Many animal species across many taxa vary their choice of mate according to their previous social experience. For instance, a high-quality female that encounters a low-quality female may become more selective in her initial choice of a mate, since she may have a lower risk of failing to mate (Petrie and Hunter, 1993).

Similarly, mating preferences are influenced by the variation in trait quality between potential mates in a number of invertebrate species (Hebets, 2003; Dukas, 2005; Fincke et al., 2007; Hebets and Vink, 2007). In this case, a female's decision to mate may be influenced by the quality of the previous *male* she has encountered. When male size is variable, a female may refuse small males since the potential benefits of mating with a large male are much greater

(Parker, 1983; Getty, 1995; Real, 1990). Conversely, females may be less selective in choosing a mate when male body size varies little. There is little information on variation in mate quality in copepods, but body size has been shown to be an important trait for mate choice in *Acartia tonsa* (Ceballos and Kiørboe, 2010) and *Acartia hudsonica* (Burris, unpublished data). Copepod body size is a relatively easy trait to measure, and is variable within and between seasons in copepod populations, by up to a factor of two in field populations (Berggreen et al., 1988; Dam and Peterson, 1991; Arendt et al., 2005). Body size can be used as a proxy for mate quality since it reflects reproductive potential: female fecundity and spermatophore size are positively correlated with adult size in copepods (Kiørboe and Hirst, 2008; Ceballos and Kiørboe, 2010). Additionally, size may indicate ‘good genes’ (Andersson, 1994) by reflecting the ability to obtain food: adult size is related to juvenile feeding rates (Arendt et al., 2005). Body size can be influenced in field populations by a number of factors, including temperature and food availability (Durbin et al., 1992). Therefore, variance in body size, in addition to mate availability, may be important in determining the strength of mate choice in these copepods.

Finally, in addition to trait quality, the short-term *condition* of an individual also influences mate choice in invertebrates. For instance, hungry females mate less selectively and often more frequently than well-fed females in a number of insects (Proctor, 1991; Poulin, 1994; Simmons, 1994). Copepods likely experience instances of food limitation in the field (Berggreen et al., 1988; Durbin et al., 1992; Müller-Navarra and Lampert, 1996; Gulati and Demott, 1997; Sterner and Schulz, 1998). Therefore, changes to food availability may also influence mate choice in these copepods.

Individuals make mate choices. Yet, there have been no experimental studies on how changes to population-level parameters may impact *individual*-mate choice in pelagic copepods.

In the present study, variation in mate-choice strength was measured experimentally in the lab as the availability and quality of potential mates were manipulated in two pelagic copepod species, *Acartia tonsa* and *Acartia hudsonica*. The effect of resource (food) availability on mate choice was also studied. In particular, we manipulated four variables: the amount of time before two potential mates were allowed to encounter one another (a proxy for population density), the presence/absence of an individual of the *same* sex of either low or high quality before mating, the presence/absence of an individual of the *opposite* sex of either low or high quality before mating, and food availability. We hypothesized that both sexes would be less selective (i.e., mate more often) as the time between encounters increased. In addition, we predicted that those held with another individual will be less selective (mate more frequently) than those held alone, and that copepods held with similarly sized copepods would mate less readily than those held with smaller copepods. Finally, we predicted that under limiting-food conditions, male and female mating preferences would become weaker.

2. Methods

2.1. Maintenance of copepods

Live animals were collected by surface tows off of Groton Long Point, Long Island Sound, USA (Latitude: 41.3271 N, Longitude: 72.00150 W) using a conical plankton net with a 200- μ m mesh and solid cod end. Tows were conducted each year in October for *Acartia tonsa* and April for *Acartia hudsonica*; adults were sorted from the tows and kept at 15°C (the water temperature in the field) in an environmental chamber on a 12:12 light:dark cycle and fed on a standard diet of the diatom *Thalassiosira weissflogii* and the green flagellate *Tetraselmis* sp. (>700 μ g C L⁻¹; 50% of each by carbon). Algae were grown at 18°C as semi-continuous cultures

on F/2 medium (Guillard, 1975). Every week, eggs were separated from adults and raised to adulthood under the same conditions, so that differently aged cohorts could be obtained.

2.2. *Mate pairing of copepods*

In all experiments, individuals were sorted as juveniles by sex, placed in pretreatment conditions for up to 24 hours, then paired with an optimally sized partner for 24 hours, and finally, checked for mating (Box 1).

All individuals, whether to be tested or used in pre-treatments or as potential mates, were sorted by sex as stage C4 juveniles from the same cohort and held either individually in 70 mL petri dishes or with individuals of the same sex in 8L containers, depending upon the experiment, and fed as above. Within 24 hours of maturation, each copepod was photographed using a camera mounted on a dissecting microscope and its prosome length (a correlate for body size as per Mauchline, 1998) was measured to 1/1000 of a millimeter, using the program ImageJ. Copepods were paired so that the average body size for each sex was the same for each treatment. During the measurement phase, individuals were held alone in a well of a 12-well plate with high-food solution as above (about 6 mL). For any pre-treatment that lasted more than 2 hours (i.e. food availability treatments, density treatments), individuals were transferred to 70mL petri dishes with replete food. If pre-treatment lasted for more than 24 hours, then water and food were changed each day. Pre-treatment conditions are explained for each experimental variable in the next section.

Since copepods can likely only perceive one potential mate at a time, they must choose with each individual encounter whether to accept or reject mating advances (Ceballos and Kiørboe, 2010). Thus, for all experiments, the *final pairing* for an individual being tested was

with only **one** mate of **optimal** size. To control for size differences, the ‘optimal size’ for a mate resulted in an average 0.09 mm difference between female and male body lengths for each treatment, with females being the larger sex. These optimal mates were always held alone during the pre-treatment time of the test copepods. For all experiments, these pairs were allowed to interact for 24 hours after which they were checked for mating. Since mating in these copepods lasts for only a few seconds (Bagøien and Kiørboe, 2005), it could not be reliably observed directly, so the presence of an attached spermatophore on a female was used to indicate a mating event. These spermatophores remain attached for more than 24 hours, and so are useful proxies for mating if checked within 24 hours (Burris and Dam, 2014).

Since all treatments had the same final optimal mate-pairings, any difference in mating frequencies between the treatments could be attributed to changes in mate-choice strength as a result of the pre-treatment exposures. Thus, for each treatment, a single ‘mate choice’ value was calculated as the proportion of matings that occurred out of the total number of pairings for that treatment. Therefore, each treatment gave a single mating frequency (i.e. if there were 40 replicate pairings in a treatment and 10 pairs mated, then the mating frequency was 10/40 or 0.25). Differences in frequencies between treatments were tested using either Chi-square tests (for three or more treatments) or Fisher’s exact tests (for 2 treatments). These mate choice frequencies are relative values, and indicate whether copepods become more selective in mating (indicated by a lower mating frequency relative to the other treatments or control) or less selective (more willing to mate as indicated by a higher mating frequency) as the experimental variables are manipulated. In addition, since these experiments were conducted on both sexes at the same time, the strength of mate choice can be compared between the sexes as well as within each sex.

2.3. Pre-treatment Conditions

2.3.1. Encounter time

Changes in population density (of the opposite sex) were simulated by varying the amount of time before which two potential mates were allowed to encounter each other. After copepods from the cohort matured, we held individuals of each sex alone for 0 hours (directly after maturation, corresponding to instances of high density since a mate is encountered immediately), 24 hours, 48 hours, and 72 hours. The latter corresponds to low densities that would occur during the beginning or end of the growing season. For example, from Kiørboe and Bagøien (2005): the product of search volume rate (0.008 to $0.05\text{m}^3\text{d}^{-1}$; Choi and Kimmerer, 2009) and low male densities ($<10\text{ m}^{-3}$) would give <0.5 potential mates per day. After each pre-treatment, copepods were placed with an optimal mate that had recently matured (< 24 hours old; time 0). Thus, only the pre-treated copepods were of different ages due to the time treatments. These differences in copepod age between treatments (3 days different at the most) should not affect their mating success since “age effects” do not become apparent until 10 days for males and 20 days for females (Rodríguez-Graña et al., 2010; Ceballos and Kiørboe, 2010, 2011; Sichlau and Kiørboe, 2011). In addition, it is unlikely that the “optimal mates” can distinguish between individuals differing by only 3 days in age, for similar reasons. Therefore, any differences in mating frequency between treatments were considered to be due to mate choice by the sex that had been time-manipulated. As such, “female choice” was measured when females were the sex held alone for different times and paired with time-0 males, and “male choice” was measured when males were held alone for different times and paired with time-0 females. Finally, to determine if there was a difference in mating when **both** males and females were being selective, an additional treatment was added at each time point. Here, both males and

females that had been held alone for the same amount of time were paired together and allowed to mate. Thus, for each experimental time, we had a ‘female-choice’ treatment (i.e. female held alone for 72 hours, paired with a newly matured male), a ‘male-choice’ treatment (i.e. male held alone for 72 hours, paired with a newly matured female), and a ‘both-sexes-choosing’ treatment (i.e. female held alone for 72 hours, paired with a male held alone for 72 hours). There were between 25 and 44 replicates per treatment, and a total of 10 treatments for each species. To determine if female-choice changed as treatments varied, a Chi-square test was run using the four female-choice mating frequencies. This was also done for male-choice and ‘both-sexes-choosing’ treatments.

An additional study was performed to determine if the results in the above experiments were due to males taking a long time to produce their first spermatophore rather than just being selective. For both species, newly matured males were placed with five females and allowed to mate for 24 hours. If these time-0 males were being selective, then by increasing the number of potential optimal mates, we should see high rates of mating. In contrast, if the results of our above experiments were due to young males not producing spermatophores, then we would expect to find few males that had mated and few males with spermatophores in their bodies. Therefore, after 24 hours, females were checked for mating and males were preserved in 10% buffered formalin and immediately examined for the presence of a spermatophore in their body.

2.3.2. *Same-Sex Exposure*

Variation in the population sex ratio results in changes to both the encounter rate with the opposite sex (see density experiment above) and the encounter rate with the *same* sex. For instance, if males are the more common sex, then a male will likely encounter more males before

finding a female than he would if females were the common sex. Thus, here we are testing the effect of an encounter with an individual of the same sex on the subsequent mate choice of a copepod. In addition, because the quality of the same sex individual may influence mate choice, copepods were exposed to individuals of different sizes (large individuals are considered high quality since both sexes prefer to mate with large mates; Ceballos and Kiørboe, 2010). For each sex, copepods were placed in one of three pre-treatments for 24 hours: held with a copepod of the same sex and of smaller size than itself (average 0.1 mm difference for females, 0.085 for males), held with a similar-sized copepod, or held alone. After pre-treatment, copepods were then placed with a mate of optimal size for 24 hours and allowed to mate. Here, ‘female choice’ was measured in the treatments where females were pre-treated with other females, and ‘male choice’ was measured in treatments where males were exposed to other males. Mating frequencies were then compared among treatments. There were 37-38 replicates per treatment for each sex. At the start of the experiment, individuals were one day old.

2.3.3. Opposite-Sex Exposure

An individual may become more or less selective in mating depending upon the number and quality of other potential mates it has encountered. Thus, these experiments are the same as the sex-ratio experiments, except that instead of using an individual of the same sex during the pre-treatment period, individuals of the opposite sex are used. Therefore, for each sex, copepods were held with either a low-quality copepod of the opposite sex (females were placed with small males for an average size difference of 0.2 mm; males were placed with small females for a difference of 0.004mm), an optimal (high-quality) mate, or held alone. Since these copepods were paired with the opposite sex during the pre-treatment, to ensure that mating did not occur

during this phase, pairs were held together for 2 hours, and females were checked for mating before the final optimal pairing and removed from the trial if mating had occurred (<3% of pairs mated during pretreatment). To make sure that the pairs encountered each other during this short time period, they were held in 12-well plates (6 mL). For the final optimal mate pairings, the individuals to be tested were then placed into 70 mL petri dishes with their mate and allowed to mate for 24 hours. There were 44 replicates in each treatment and mating frequencies were then compared for each sex between the three treatments. At the start of the experiment, individuals were recently matured (day 0).

2.3.4. *Food availability*

These experiments tested the effect of food abundance on the strength of mate choice for both species of copepods. Copepods of each sex were pre-treated for 24 hours under three food regimes: Food replete: ~30 mL of $>700 \mu\text{g C L}^{-1}$; food limiting: $\sim 100\text{-}200 \mu\text{g C L}^{-1}$; no food: filtered sea water). The different conditions were chosen from functional relationships of food versus ingestion rates (Besiktepe and Dam, 2002). These copepods were then placed in high food with either an optimal mate fed at replete conditions, or one fed at the same food condition, in order to determine the effect when both sexes are selective. Mating frequencies among treatments were tested for differences using chi-square tests. At the start of the experiment, individuals were two days old.

3. Results

3.1. *Encounter time*

For both *Acartia tonsa* and *Acartia hudsonica* males, the strength of mate choice was significantly affected by being held alone for different amounts of time (Fig. 1). Males of both species were most selective directly after maturation (*A. tonsa*: 1 in 46 pairs (2.2%) mated; *A. hudsonica*: 1 in 44 pairs (2.3%) mated) compared with the other experimental times (*A. tonsa*: day 1 = 24%, day 2 = 41%, day 3 = 47%, all N=34; *A. hudsonica*: day 1 = 3.3% of 30 pairs, day 2 = 28% of 25, day 3 = 18% of 22) (Fig. 1). For *A. tonsa*, males became statistically less selective (were more willing to mate) as time held alone increased (chi-square = 25.36, $p < 0.001$, power = 1.0) (Fig. 1A). *A. hudsonica* males did not show the same increase in mating with time; instead, there was little mating the first two days and significantly higher mating the following two days (chi-square = 14.04, $p = 0.003$, power = 0.9) (Fig. 1B).

In order to measure female-mate choice, females were paired with recently matured males, which were the most selective in mating. Thus, for both *Acartia tonsa* and *Acartia hudsonica*, male-mate choice was much stronger than female-mate choice. Because of this, few pairs mated in any treatment (< 9% for *A. tonsa* and < 6% for *A. hudsonica*) and as a result any female-mate choice that was occurring was swamped out by male choice. However, data from the 'both-sexes-selecting' treatment enables us to compare time-0 females with day-1, day-2, and day-3 females. For example, mating frequencies of time-0 females that mated with day-1 males can be compared with those for day-1 females that mated with day-1 males. Since the males for both treatments have been held alone for the same amount of time, any differences in mating are due to female choice. To standardize for the effect of the male treatment (since mate choice in these males is affected by being held alone for 1, 2, and 3 days), we can calculate a proportional increase in mating for each time treatment. For example, the mating frequency of day-1 *A. tonsa* females held with day-1 males (0.143) is divided by the mating frequency of time-0 females held

with day-1 males (.235). A value of 0.61 means that day-1 females mated only about 2/3 as frequently as time-0 females after controlling for male choice (Table 1). After doing this for the other 2 treatments, we find that time-0 females mated 16.5% and 13.7% more than day-2 and day 3 females, respectively (Table 1). Therefore, time-0 females of *A. tonsa* were the least selective (most willing to mate), followed by day-3 females, then day-2 females, and ending with the most selective females after day-1. For *A. hudsonica*, proportionally, day-1 females had much higher mating frequencies than the other female treatments after correction for male-mate choice (day-1 females mated 6 times more frequently than time-0 females) (Table 1). However, day-2 females had the lowest mating frequency after correction, with time-0 females mating 55% more frequently than day-2 females. Finally, day-3 females were less selective than time-0 and day-2, but more selective than day-1 females. They mated 3.4 times more frequently than time-0 females.

When both sexes of *Acartia tonsa* and *Acartia hudsonica* were held for the same amounts of time and then mated, the strength of mate choice was also significantly affected by treatment time (Fig. 1). In *A. tonsa*, couples became significantly less selective as the time held alone increased (chi-square: 21.89, $p < 0.001$, power = 0.99) (Fig. 1A). Day-3 females and males were the least selective, with 41% of pairs mating (N=32), followed by 34% of day-2 pairs (N=32), 14% of day-1 pairs (n=35) and 2.2% of time-0 pairs mating (N= 46). While at every time point these frequencies were lower than for the male-choice treatments, the difference was not statistically different (paired t-test: = 2.846, $p = 0.065$) (Fig. 1A). This suggests that male-mate choice accounts for most of the success in mating under these conditions. For *A. hudsonica*, day-3 males and females were also the least selective, with 63% of pairs mating (N= 27) (Fig. 1B). Day-1 pairs and day-2 pairs were much more selective, with 20% (N= 45) and 13% (N=24) of

pairs mating, respectively. Time-0 pairs were the most selective, with 2.3% of 44 pairs mating (Fig. 1B). As with *A. tonsa*, the differences in frequencies between the treatments were statistically significant (chi-square: 38.45, $p < 0.001$, power = 1.0). Compared with the male choice frequencies, however, two of the three same-time treatments were significantly higher. This suggests that female-mate choice is stronger than male-mate choice in these instances.

The follow up study to determine if male-mate choice results were due to day 1 males being selective rather than unable to produce a spermatophore showed that males of both *Acartia tonsa* and *Acartia hudsonica* produced spermatophores within the 24 h mating period. In addition, by increasing the number of potential female mates, a significant number of males mated. For *A. tonsa*, 58.8% of 51 males mated, and over 45% of 50 *A. hudsonica* males mated as well. However, all but 3 of the remaining unmated males in *A. tonsa* (6 in *A. hudsonica*) had large spermatophores present in their bodies.

3.2. Same-sex Exposure

For *Acartia tonsa* female choice, in which all males were held alone and females were exposed to another female of the same size, one of much smaller size, or held alone, there were no significant differences in mating frequencies among treatments (chi-square= 0.056, $p = 0.97$) (Fig. 2A). Females pre-treated with another female of their size mated 58% of the time (N= 38), those held with a smaller female mated 57% of the time (N= 37) and those held alone mated 59% of the time (N=37) (Fig. 2A). For females of *Acartia hudsonica*, in contrast, there was a significant difference in mating frequency for females that were held with small females (N=37, 42.9% mated) compared with females held with large females (N=36, 8.3% mated) or those held alone (N=42, 14.3% mated) (chi-square= 14.6, $p < 0.01$) (Fig. 2B).

There was no significant difference in mating frequency for males of either species when held with another male of different sizes or held alone (*Acartia tonsa*: chi-square= 0.95, $p = 0.62$; *Acartia hudsonica*: chi-square= 0.23, $p = 0.89$) (Fig. 2A,B). Those *A. tonsa* males held with a large male mated 57% of the time ($N=37$) (*A. hudsonica*: $N= 37$, 13.5% mated), and those held with a small male mated 49% of the time ($N=37$) (*A. hudsonica*: $N= 37$, 10.8 %). Neither was there a difference in being held alone or with another male (Fig. 2A,B).

3.3. Opposite-sex Exposure

For *Acartia tonsa* females, there was no significant difference in mating frequencies for those exposed to a small male (0.33, $N= 42$) versus those exposed to an optimal male mate (0.27, $N= 62$) before allowed to mate (chi-square = 0.18, $p = 0.67$) (Fig. 3A). However, while size of the male did not matter, its presence prior to mating resulted in higher mating frequencies for females when compared with females that were held alone (0.07, $N = 56$) (chi-square = 9.66, $p = 0.002$) (Fig. 3A). Similar results were found for females of *Acartia hudsonica*-- the size of the male did not affect mating frequency, but the presence of a male resulted in higher mating frequencies compared with females held alone (Fig. 3B). Females held with a small male mated more frequently (0.52, $N = 44$) compared with females held with an optimal male (0.32, $N = 41$) (chi-square = 2.88, $p = 0.09$). Females held alone mated statistically less frequently (0.02, $N = 44$) than those held with a male of either size (chi-square = 20.85, $p < 0.001$) (Fig. 3B).

Male choice for *Acartia tonsa* also varied significantly according to treatment (Fig. 3A). The size of the female was important for mating; males held with a small female mated more frequently (0.37, $N= 41$) than males held with an optimal female (0.18, $N = 57$) (chi-square = 3.6, $p = 0.054$). Similarly, the presence of the female was important for mating, with fewer

matings (0.07, N= 56) when males were not exposed previously to a female, regardless of its size (chi-square = 13.52, $p = 0.001$) (Fig. 3A). For males of *Acartia hudsonica*, there was no significant difference in mating frequency when exposed to females of different sizes (Fisher's Exact test: $p = 0.71$) (Fig. 3B). Those exposed to an optimal female mated slightly more (0.12, N= 43) than those held with a small female (0.07, N= 43). There was neither a significant difference in mating when males were held alone (0.02, N= 44) or exposed to a female prior to mating ($z = 1.12$, $p = 0.26$) (Fig. 3B).

3.4. Food Availability

Mating frequencies for males of *Acartia tonsa* were not influenced by food availability (Fig. 4A). Males in the high-food treatment mated slightly more frequently (0.46, N = 95) than those in the low-food (0.4, N = 88) or no-food treatments (0.44, N = 89), but these differences were not statistically significant (chi-square = 0.81, $p = 0.67$) (Fig. 4A). Female-mating choice, however, was influenced by food availability (chi-square = 7.09, $p = 0.03$) (Fig. 4A). Females in the low-food treatment had the highest mating frequency (0.56, N = 88), followed by those in the high-food treatment (0.46, N = 95) (Fig. 4A). The lowest mating frequency occurred in the no-food treatment (0.36, N = 87). Mating frequency did not vary among food treatment when both males and females were part of a treatment (chi-square = 0.43, $p = 0.80$): 46% of 95 pairs mated in the high-food treatment, 44% of 93 pairs in the low-food treatment, and 49% of 92 pairs in the no-food treatment (Fig. 4A).

Results for *Acartia hudsonica* were exactly opposite to those for *A. tonsa* (Fig. 4). Male-mate choice was influenced by food availability, but female-choice was not (Male: chi-square = 11.14, $p = 0.004$; Female: chi-square = 3.27, $p = 0.20$) (Fig. 4B). Males in the high-food treatment

mated more frequently (0.54, N = 35) than those in the low-food (0.26, N = 34), or no-food treatment (0.19, N = 37) (Fig. 4B). Females showed the same pattern of mating frequencies by food treatments (High: 0.54, N = 35; Low: 0.48, N = 40; Starved: 0.34, N = 41), just not as extreme as males (Fig. 4B). There was also a significant effect of food when individuals of both sexes were part of a treatment, with the highest mating frequencies for the high-food treatment and the lowest for the no-food treatment (High: 0.54, N = 35, Low: 0.54, N = 39, No-food: 0.24, N = 38) (chi-square = 9.41, $p = 0.009$) (Fig. 4B).

4. Discussion

In both male and female copepods of the species *Acartia tonsa* and *Acartia hudsonica*, the strength of mate choice in laboratory experiments varied in response to changes in resource availability (food abundance), mate availability (encounter rates), mate quality (body size variation) or previous social experience (same or opposite sex encounters). This suggests that not only are both males and females being selective when mating, but also that mate choices are not fixed and uniform. Instead, our results suggest that mate choice is flexible in these two species and that the sexes are influenced differently by the factors above. A mate-sampling strategy that relies upon a fixed mate preference has advantages and drawbacks. An advantage is that individuals are not required to remember the previous potential mates encountered. The drawbacks are that, at the extremes, individuals will not mate if their preferences are set too high, or, will mate with low-quality mates if their fixed preferences are too low (Jennions and Petrie, 1997). The flexible mate-sampling strategy would allow for an individual that is at either extreme to raise or lower their mate preferences based on information from their surroundings (food availability, mate density, sex ratio, trait-quality, etc.) in order to balance the benefits and

costs associated with finding a preferred mate (Janetos, 1980; Real, 1990). Here, we summarize the general mate choice findings and then discuss which variable(s) may be most important for each sex in the field where all variables may be encountered simultaneously. In addition, we discuss some possible reasons for the differences between the two species and the two sexes in mate choice responses.

4.1. Encounter time

In both *Acartia tonsa* and *Acartia hudsonica*, the strength of male-mate choice was affected by variations in the time they were held alone, and both species were most selective (least likely to mate) during the first 24 hours after maturation (Fig. 1). This was not due to recently matured males being unable to produce spermatophores (see methods). It is not surprising that the youngest adult males in our study were the most selective. They can enhance their fitness by mating with a higher-quality mate than by mating immediately with an inferior one, since female egg production rates increase significantly with female size (Weatherhead and Robertson, 1979; Ceballos and Kjørboe, 2010). *A. tonsa* males became increasingly less selective with increasing time held alone (i.e. as mate density decreased) (Fig.1), as predicted by density-dependent models (Hubbell and Johnson, 1987; Crowley et al., 1991; Kokko and Monaghan, 2001; Hardling and Kaitala, 2005; Kokko and Mappes, 2005). This decrease in choosiness at low population densities is probably adaptive. By passing on a mating opportunity later in life in hopes of finding a higher-quality mate, an individual would risk never finding another mate, or delaying its reproductive output (Real, 1990). *A. hudsonica* males also appear to have a time-dependent reduction in mating preferences, but their threshold for finding a mate is longer than for males of *A. tonsa* (Fig. 1). Males of *A. hudsonica* mated very little during the

first two days (days 0 and 1), but significantly increased their mating by days 2 and 3. Two days of not encountering a female may be the threshold value after which males significantly alter their mate choice criteria. The difference in the response between males of the two species may be due to differences in the length of time their spermatophores remain viable. For instance, spermatophores produced by males of *A. tonsa* may go bad earlier than spermatophores produced by males of *A. hudsonica*. If so, then this may explain why *A. tonsa* males were more willing to mate after only 1 day than *A. hudsonica* males. While there is no information on spermatophore viability in copepods, it may explain the species specific response to different mate encounter rates.

Female-mate choice in *Acartia hudsonica*, but not *Acartia tonsa*, was influenced by increasing the time it took to encounter a mate. After accounting for male choice, day-1 *A. hudsonica* females mated proportionally more than any others (Table 1), possibly because the benefit of waiting longer for a high-quality mate is not worth the trade-off of delaying offspring production (Janetos, 1980; Real, 1990). Since these females continue to produce eggs even when unfertilized, it may be energetically costly to remain unfertilized for extended periods.

In both species, when females and males were held alone for the same amount of time (interaction treatment), the strength of mate choice was also affected by treatment time. While at every time point the mating frequencies for the interaction in *Acartia tonsa* were lower than for the male-choice treatments, the difference was not statistically different between treatments (Fig. 1A). This suggests that male-mate choice accounts for most of the success in mating under these conditions. In *Acartia hudsonica*, day-3 males and females were the least selective (most willing to mate) (Fig. 1B), which is what is predicted by mate choice models (Hubbell and Johnson, 1987; Crowley et al., 1991; Kokko and Monaghan, 2001; Hardling and Kaitala, 2005; Kokko and

Mappes, 2005; Heuschele et al., 2013). Compared with the male-choice frequencies for *A. hudsonica*, however, two of the three interaction treatments were significantly higher for females. This suggests that female-mate choice is stronger than male-mate choice in this species, at least for females that have been held alone for more than 24 hours.

Contrary to what was predicted by a mate-selectivity model for copepods (Heuschele et al., 2013), mate choice was stronger in males than in females for recently matured individuals of both species, and also more affected by encounter time. Males of both species are restricted by the number of times they can mate, since they produce a limited number of spermatophores over their lifetime (Burris, unpublished data) at a low rate (only about 1 spermatophore day⁻¹) (Ceballos and Kiørboe, 2010). Therefore, when population density is high, males would encounter more females per day than they can mate with (up to 30 potential mates, see Kiørboe, 2007), which favors choosy males. By being selective, a male can increase its fitness considerably since large females have more and larger offspring (Ceballos and Kiørboe, 2010). Since females of species that are not capable of storing sperm, such as *Acartia tonsa* and *Acartia hudsonica* (Ohtsuka and Huys, 2001), can re-mate immediately after having just mated, and, theoretically, an unlimited number of times over a lifetime, males appear to have the more limited reproductive period. As such, males may experience a greater fitness penalty than females by making a poor mating decision, and so should always be more selective in choosing their partners than females.

4.2. Same-sex Exposure

Exposure to another member of the same sex, regardless of its quality, did not affect the strength of mate choice for either sex of *Acartia tonsa* or for males of *Acartia hudsonica* (Fig. 2).

Mate choice in females of *A. hudsonica*, however, was affected by the quality of the previous female encountered. Large females held with small females mated significantly more frequently than those held alone or with other large females. This suggests that females may adjust their selectivity in response to assessment of their own quality (Burley and Foster, 2006). Since an individual's attractiveness to potential mates can limit its ability to obtain a willing mate, individuals may adjust the strength of their mate choice based on their own current mating quality. This may help them to avoid wasting energy courting an unwilling mate (Burley, 1977, 1981). Females without knowledge of other females (i.e. held alone) or with knowledge of similarly attractive females (held with large females) may deem themselves of low quality and decide not to waste time or energy in courtship that will may not lead to mating (Burley and Foster, 2006). Conversely, females held with low-quality females may be more willing to mate when high-quality males are encountered because they have assessed themselves to be of high-quality as well. It is not surprising that female choice is influenced more than male choice by previous same-sex social experience. In the field, there are often more females than males in the population and so both sexes will likely encounter more females than males. Thus, in the field, male-choice should be influenced more by the opposite sex, and female-choice by previous experience with the same sex.

4.3. *Opposite-Sex Exposure*

Neither *Acartia tonsa* nor *Acartia hudsonica* females showed differences in mating frequencies between those previously exposed to small or large mates (Fig. 3). However, previous exposure to a male regardless of its size increased mating significantly when compared with females held alone. It may be that females with no information about males are less

willing to mate than those that have information because they are uncertain about the quality of the male (Dukas, 2006). For both species, there was also a trend (although not significant) of increased mating frequency in females exposed previously to small males compared with large males. It is possible that by encountering a low-quality male, females are more willing to mate with the next male (of high quality) because the difference in size is more extreme and thus quality is easier to determine (Janetos, 1980; Reid and Stamps, 1997). It may be easier for females to tell that this male is of high quality after exposure to a small male. Effects of previous exposure to males are known to influence female-mating choice in a number of animals, including birds (Collins, 1995), fishes (Brown, 1981; Bakker and Milinski, 1991; Downhower and Lank, 1994), crickets (Bailey and Zuk, 2009), and beetles (Roitberg et al., 1993). Copepods now join the ranks.

While previous exposure to females had no effect on males of *Acartia hudsonica*, male mate choice in *Acartia tonsa* was significantly affected (Fig. 3). The size of the female, as well as just her presence, was important for mating. As hypothesized, males held with a small female subsequently mated more frequently than males held with an optimal female, and males held alone mated less frequently than males held with a female. This suggests, as with the female choice results, that previous exposure of males to low-quality females may make high-quality females more attractive or their quality more easily distinguished. In some flies, males are capable of assessing relative sizes of females in order to mate with larger ones (Svensson and Petersson, 1994). Therefore, it is conceivable that copepods use information obtained from a previous encounter to influence mate preferences. It is surprising that male mate choice in *A. hudsonica* was not affected by social experience, when male choice in *A. tonsa* was significantly influenced. It is possible that by allowing males to encounter multiple females over a short

period of time, we mimicked high encounter rates. From the encounter rate experiment above, we know that males of *A. hudsonica* are most selective under “high encounter rates” (when allowed to immediately encounter a female), more so than males of *A. tonsa*. This suggests that for *A. hudsonica* males, mate choice is influenced more by female encounter rates than by the actual quality of the females encountered. If we had conducted this experiment after holding males alone for several days, instead of using males at their most selective, we may not have measured a difference in the response between the two species.

4.4. Food availability

Mate choice in *Acartia tonsa* was affected by food availability in females, but not males (Fig. 4). Females were less choosy in the low-food treatment and most selective (least willing to mate) in the no-food treatment. Egg production of *Acartia* decreases as food decreases and ceases in the absence of food after 2-3 days (Dagg, 1977; Tester and Turner, 1990; Finiguerra et al., 2013). Thus, in the absence of food, mating has little or no benefit for *Acartia* females as there are few or no eggs to be fertilized. Moreover, mating decreases the longevity of both sexes even under high-food conditions (Nakatsuru and Kramer, 1982). As a result, starved females, by mating, would only incur costs and none of the benefits associated with mating. Previous work has shown that males of *A. tonsa* are much more sensitive to lack of food than females (Finiguerra et al., 2013), and are thus less likely to survive long without food. This outcome is consistent with the terminal investment hypothesis (Issac and Johnson, 2005). That is, males, instead of ceasing reproduction to increase survival (as females may do), continue to reproduce to increase offspring because their own survival is probably limited.

Contrary to what was found for *Acartia tonsa*, male-mate choice in *Acartia hudsonica* was affected by food availability, but female choice was not. Males were more selective in matings as food became less available. Females also showed the same pattern, but the differences between treatments were not significant. When both sexes were part of the same food treatment, individuals were more selective in mating as food availability decreased. It is possible that the differences observed in mate choice as a function of food availability between the two species are the result of a difference in energy requirements or tolerance to lack of food. *A. hudsonica* may be able to survive for longer periods of time under food limitation than *A. tonsa*, increasing the chance they will make it to a new food patch. If this were the case, then it may be more beneficial for males of *A. hudsonica* to delay mating now in order to invest in future reproduction.

4.5. Conclusions

Our results suggest that not only are mate choices in different species affected by different factors, but that male and female choice within the same species are also affected differently. In both sexes of the species *Acartia tonsa* and *Acartia hudsonica*, the strength of mate choice in laboratory experiments varied in response to changes in resource availability (food abundance), mate availability (encounter rates), mate quality (body size variation) or previous social experience (same or opposite sex encounters). This suggests that mate choices are not fixed in copepods, and instead, flexible. A flexible mate-sampling strategy would allow for an individual to raise or lower their mate preferences based on information from their surroundings in order to balance the benefits and costs associated with being selective (Janetos, 1980; Real, 1990). In field populations, copepods likely encounter many of these variables

simultaneously and the interaction of changing variables may be important in determining whether or not an individual is selective during mating. While our study only measured mate preferences for a single variable at a time, it is the first evidence that individual-mate preferences can vary. In addition to the variables tested in our experiments, other factors may be as important for influencing mate choice in these copepods. Predation pressure or water turbulence may have an effect on mate choice, by changing the costs associated with being selective (Jersabek et al., 2007). Copepods may attract more attention from predators during mating because of increased conspicuousness as a result of courtship and copulation behaviors (Svensson, 1995). To minimize the costs of mating during times of high-predation pressure, copepods may decrease their swimming speeds or number and length of courtship bouts by mating with inferior mates. Similarly, the search costs of finding a mate may increase with increasing turbulence (Strickler, 1975; Hwang and Strickler, 1994; Hwang et al., 1994; Kiørboe et al., 1999; Lee et al., 2011); thus, having flexible mate preferences may be beneficial in a changing environment. Likewise, mate choice for other traits (age, reproductive state, etc.) may also respond differently to changes in ecological variables. Our understanding of copepod-mating dynamics would be aided by research focusing on these questions.

Table and Figure legend:

Box 1: Schematic of experiments conducted.

Table 1: Ratios of female-mating frequency by density treatment after standardization for male-mate choice.

Figure 1: Mating frequency of males (black bars) and both sexes (grey bars) for *Acartia tonsa* (A) and *Acartia hudsonica* (B) as a function of time held alone. Males of both species (black bars) were most selective (lowest mating frequencies) directly after maturation compared with the other experimental times. *A. tonsa* males became statistically less selective (were more willing to mate) as time held alone increased (chi-square = 25.36, $p < 0.001$), whereas *A. hudsonica* males reached a peak in mating frequency (least selective) by day 1 (chi-square = 14.04, $p = 0.003$). When both sexes of *A. tonsa* (A) were held alone for the same amount of time, couples became significantly less selective as the time held alone increased (chi-square: 21.89, $p < 0.001$). Similarly, *A. hudsonica* day-3 males and females were the least selective (highest matings), and time-0 pairs were the most selective (chi-square: 38.45, $p < 0.001$). Lowercase letters above histograms represent significant differences.

Figure 2: Mating frequencies for females (black bars) and males (grey bars) of *Acartia tonsa* (A) and *Acartia hudsonica* (B) after being exposed for 24 hours to a large or small individual of the same sex, or being held alone. For *A. tonsa*, there were no significant differences in mating frequencies among treatments for males or females. For *A. hudsonica*, females held with small females mated significantly more frequently (*) than those held with either large females or

alone (47.9% compared with 8.3 and 14.3%, respectively) (chi-square= 14.6, $p < 0.01$). There was no difference between treatments for males.

Figure 3: Mating frequencies for females (left 2 bars) and males (right 2 bars) of *Acartia tonsa* (A) and *Acartia hudsonica* (B) after being exposed for 2 hours to a small or optimally sized individual of the opposite sex, or being held alone. For females of both *A. tonsa* and *A. hudsonica*, the size of the male did not affect mating frequency, but the presence of a male resulted in higher mating frequencies compared with females held alone. For males of *A. tonsa*, both female size and female presence were important for mating. Males held with a small female mated more frequently than males held with an optimal female. Similarly, there were fewer matings when males were not exposed previously to a female, regardless of her size. In males of *A. hudsonica*, neither the presence of a female nor her size was important for mating. Lowercase letters above histograms represent significant differences.

Figure 4: Mating frequencies for males (black bars), both sexes (grey bars), and females (light bars) of *Acartia tonsa* (A) and *Acartia hudsonica* (B) after being exposed for 24 hours to high, low or no-food conditions. Mating frequencies for males of *A. tonsa* were not influenced by food level, but female mating did significantly increase in the low-food treatment and decreased in the no-food treatment compared with the high-food treatment. When both males and females were part of a treatment, there was no difference in mating frequencies among treatments. In *A. hudsonica*, mating in both males and females decreased as food availability decreased, but this tendency was only statistically significant for males. When both sexes were part of a treatment, the no-food treatment had significantly lower mating frequencies than the other two.

665 **Boxes:**

666 **Box 1:** Schematic of experiments conducted.

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1. Sorted by sex as stage C4 juveniles
2. Within 24 hrs. of maturation, placed in one pretreatment condition:
 - a. Encounter time (held alone for 0, 1, 2, 3 days)
 - b. Same sex exposure (held alone or with small or large individual for 24 hrs.)
 - c. Opposite sex exposure (held alone or with small or large individual for 2 hrs.)
 - d. Food availability (held in high, low, or no food for 24 hrs.)
3. Removed from pretreatment and placed with an optimally sized partner for 24 hrs.
4. Females checked for presence of spermatophore (indicates mating) and mating frequencies calculated for each treatment.

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Tables:

Table 1: Ratios of female-mating frequency by density treatment after standardization for male-mate choice.

Male : Female	<i>Acartia tonsa</i>	<i>Acartia hudsonica</i>
Day 0: Day 0	1.0	1.0
Day 0: Day 1	0.61	6.0
Day 0: Day 2	0.84	0.45
Day 0: Day 3	0.86	3.4

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705 **Figures:**

706 **Figure 1:**

Figure 2:

Figure 3:

Figure 4:

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759 **References**

- 760 Ahnesjö, I., Vincent, A., Alatalo, R., Halliday, T., Sutherland, W. J., 1993. The role of females in
761 influencing mating patterns. *Behav. Ecol.* 4, 187-189.
- 762 Andersson, M. B., 1994. Sexual selection. Princeton University Press, New Jersey.
- 763 Anstensrud, M., 1990. Effects of mating on female behavior and allometric growth in the two
764 parasitic copepods *Lernaeocera branchialis* (L., 1767)(Pennellidae) and *Lepeophtheirus*
765 *pectoralis* (Müller, 1776)(Caligidae). *Crustaceana* 59, 245-258.
- 766 Anstensrud, M., 1992. Mate guarding and mate choice in two copepods, *Lernaeocera*
767 *branchialis* (L.)(Pennellidae) and *Lepeophtheirus pectoralis* (Müller)(Caligidae), parasitic on
768 flounder. *J. Crustacean Biol.* 12, 31-40.
- 769 Arendt, K. E., Jónasdóttir, S. H., Hansen, P. J., Gärtner, S., 2005. Effects of dietary fatty acids on
770 the reproductive success of the calanoid copepod *Temora longicornis*. *Mar. Biol.* 146, 513-530.
- 771 Bakker, T. C., Milinski, M., 1991. Sequential female choice and the previous male effect in
772 sticklebacks. *Behav. Ecol. Sociobiol.* 29, 205-210.
- 773 Bagøien, E., Kiørboe, T., 2005. Blind dating-mate finding in planktonic copepods. III.
774 Hydromechanical communication in *Acartia tonsa*. *Mar. Ecol.: Prog. Ser.* 300, 129-133.
- 775 Bailey, N. W., Zuk, M., 2009. Same-sex sexual behavior and evolution. *Trends Ecol. Evol.* 24,
776 439-446.
- 777 Bergreen, U., Hansen, B., Kiørboe, T., 1988. Food size spectra, ingestion and growth of the
778 copepod *Acartia tonsa* during development: implication for determination of copepod
779 production. *Mar. Biol.* 99, 341-352.
- 780 Besiktepe, S., Dam, H.G., 2002. Coupling of ingestion and defecation as a function of diet in the
781 calanoid copepod *Acartia tonsa*. *Mar. Ecol.: Prog. Ser.* 229, 151-164.

- 782 Brown, L., 1981. Patterns of female choice in mottled sculpins (*Cottidae, teleostei*). Anim.
783 Behav. 29, 375-382.
- 784 Burley, N., 1977. Parental investment, mate choice, and mate quality. Proc. Natl. Acad. Sci.
785 U.S.A. 74, 3476-3479.
- 786 Burley, N., 1981. Mate choice by multiple criteria in a monogamous species. Am. Nat.
787 117, 515-528.
- 788 Burley, N.T., Foster, V.S., 2006. Variation in female choice of mates: condition influences
789 selectivity. Anim. Behav. 72, 713-719.
- 790 Burris, Z.P., Dam, H.G., 2014. Female mating status affects mating and male mate-choice in the
791 copepod genus *Acartia*. J. Plankton Res. Doi:10.1093/plankt/fbu090.
- 792 Ceballos, S., Kiørboe, T., 2010. First evidences of sexual selection by mate choice in marine
793 zooplankton. Oecologia 164, 627-635.
- 794 Ceballos, S., Kiørboe, T., 2011. Senescence and sexual selection in a pelagic copepod. PloS one
795 Doi: 10.1371/journal.pone.0018870.
- 796 Choi, K.H., Kimmerer, W.J., 2009. Mating success and its consequences for population
797 growth in an estuarine copepod. Mar. Ecol.: Prog. Ser. 377, 183-191.
- 798 Collins, S.A., 1995. The effect of recent experience on female choice in zebra finches. Anim.
799 Behav. 49, 479-486.
- 800 Crowley, P.H., Travers, S.E., Linton, M.C., Cohn, S.L., Sih, A., Sargent, R.C., 1991. Mate
801 density, predation risk, and the seasonal sequence of mate choices: a dynamic game. Am. Nat.
802 137, 567-596.
- 803 Dagg, M., 1977. Some effects of patchy food environments on copepods. Limnol. Oceanogr. 22,
804 99-107

- 805 Dam, H.G., Peterson, W.T., 1991. In situ feeding behavior of the copepod *Temora longicornis*:
806 Effects of seasonal changes in chlorophyll size fractions and female size. Mar. Ecol.: Prog. Ser.
807 71, 113-123.
- 808 Darwin, C.R., 1871. The descent of man, and selection in relation to sex. Appleton, New York.
- 809 Downhower, J.F., Lank, D.B., 1994. Effect of previous experience on mate choice by female
810 mottled sculpins. Anim. Behav. 47, 369-372.
- 811 Dukas, R., 2005. Learning affects mate choice in female fruit flies. Behav. Ecol. 16, 800-804.
- 812 Dukas, R., 2006. Learning in the context of sexual behaviour in insects. Anim. Biol. 56, 125-142.
- 813 Durbin, E.G., Durbin, A.G., Campbell, R.G., 1992. Body size and egg production in the
814 marine copepod *Acartia hudsonica* during a winter-spring diatom bloom in Narragansett
815 Bay. Limnol. Oceanogr. 37, 342-360.
- 816 Emlen, S.T., Oring, L.W., 1977. Ecology, sexual selection, and the evolution of mating
817 systems. Science 197, 215-223.
- 818 Fincke, O.M., Fargevieille, A., Schultz, T.D., 2007. Lack of innate preference for morph and
819 species identity in mate-searching *Enallagma* damselflies. Behav. Ecol. Sociobiol. 61, 1121-
820 1131.
- 821 Finiguerra, M.B., Dam, H.G., Avery, D.E., Burris, Z., 2013. Sex-specific tolerance to starvation
822 in the copepod *Acartia tonsa*. J. Exp. Mar. Biol. Ecol. 446, 17-21.
- 823 Forsgren, E., Amundsen, T., Borg, A. A. & Bjelvenmark, J. 2004. Unusually dynamic sex roles
824 in a fish. Nature 429, 551-554.
- 825 Getty, T., 1995. Search, discrimination, and selection: mate choice by pied flycatchers. Am. Nat.
826 145, 146-154.

- 827 Guillard, R.R., 1975. Culture of phytoplankton for feeding marine invertebrates, in: Smith, W.L.
828 and Chanley, M.H. (eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, pp.
829 29-60.
- 830 Gulati, R., Demott, W., 1997. The role of food quality for zooplankton: remarks on the
831 state-of-the-art, perspectives and priorities. Freshwater Biol. 38, 753-768.
- 832 Härdling, R., Kaitala, A., 2005. The evolution of repeated mating under sexual conflict. J. Evol.
833 Biol. 18, 106-115.
- 834 Hebets, E.A., 2003. Subadult experience influences adult mate choice in an arthropod: exposed
835 female wolf spiders prefer males of a familiar phenotype. Proc. Natl. Acad. Sci. U.S.A.
836 100, 13390-13395.
- 837 Hebets, E.A., Vink, C.J., 2007. Experience leads to preference: experienced females prefer
838 brush-legged males in a population of syntopic wolf spiders. Behav. Ecol. 18, 1010-1020.
- 839 Heuch, P.A., Schram, T.A., 1996. Male mate choice in a natural population of the parasitic
840 copepod *Lernaeocera branchialis* (Copepoda: Pennellidae). Behaviour 133, 221-239.
- 841 Heuschele, J., Kiørboe, T., 2012. The smell of virgins: mating status of females affects male
842 swimming behaviour in *Oithona davisae*. J. Plankton Res. 34, 929-935.
- 843 Heuschele, J., Eliassen, S., Kiørboe, T., 2013. Optimal mate choice patterns in pelagic
844 copepods. Oecologia 172, 399-408.
- 845 Hubbell, S.P., Johnson, L.K., 1987. Environmental variance in lifetime mating success, mate
846 choice, and sexual selection. Am. Nat. 130, 91-112.
- 847 Hwang, J.S., Strickler, J.R., 1994. Effects of periodic turbulent events upon mechanoreception
848 and escape responses of a calanoid copepod, *Centropages hamatus*. Bull. Plankton Soc. Jpn. 41,
849 117-130.

- 850 Hwang, J.S., Costello, J.H., Strickler, J.R., 1994. Copepod grazing in a turbulent flow: elevated
851 foraging behavior and habituation of escape responses. *J. Plankton Res.* 16, 421-43.
- 852 Issac, J.L., Johnson, C.N., 2005. Terminal reproductive effort in a marsupial. *Biol. Lett.* 1, 271-
853 275.
- 854 Janetos, A.C., 1980. Strategies of female mate choice: a theoretical analysis. *Behav. Ecol.*
855 *Sociobiol.* 7, 107-112.
- 856 Jersabek, C.D., Luger, M.S., Schabetsberger, R., Grill, S., Strickler, J.R., 2007. Hang on or run?
857 Copepod mating versus predation risk in contrasting environments. *Oecologia* 153, 761-773.
- 858 Jennions, M.D., Petrie, M., 1997. Variation in mate choice and mating preferences: a review of
859 causes and consequences. *Biol. Rev.* 72, 283-327.
- 860 Kiørboe, T., Bagøien, E., 2005. Motility patterns and mate encounter rates in planktonic
861 copepods. *Limnol. Oceanogr.* 50, 1999-2007.
- 862 Kiørboe, T., Hirst, A.G., 2008. Optimal development time in pelagic copepods. *Mar. Ecol.: Prog.*
863 *Ser.* 367, 15-22.
- 864 Kiørboe, T., Saiz, E., Visser, A., 1999. Hydrodynamic signal perception in the copepod *Acartia*
865 *tonsa*. *Mar. Ecol.: Prog. Ser.* 179, 97-111.
- 866 Kiørboe, T., 2007. Mate finding, mating, and population dynamics in a planktonic copepod
867 *Oithona davisae*: There are too few males. *Limnol. Oceanogr.* 52, 1511-1522.
- 868 Kokko, H., Mappes, J., 2005. Sexual selection when fertilization is not guaranteed. *Evolution* 59,
869 1976-1985.
- 870 Kokko, H., Monaghan, P., 2001. Predicting the direction of sexual selection. *Ecol. Lett.* 4, 159-
871 165

- 872 Kokko, H., Rankin, D.J., 2006. Lonely hearts or sex in the city? Density dependent effects in
873 mating systems. *Phil. Trans. R. Soc. B.* 361, 319-334.
- 874 Lee, C.H., Dahms, H.U., Cheng, S.H., Souissi, S., Schmitt, F.G., Kumar, R., Hwang, J.S., 2011.
875 Mating behaviour of *Pseudodiaptomus annandalei* (Copepoda, Calanoida) at calm and
876 hydrodynamically disturbed waters. *Mar. Biol.* 158, 1085-1094.
- 877 Mauchline, J., 1998. The biology of calanoid copepods, in: Blaxter, J., Southward, A. (eds.),
878 *Advances in Marine Biology*. Academic Press, San Diego, pp. 710.
- 879 McLaren, I.A., 1976. Inheritance of demographic and production parameters in the marine
880 copepod *Eurytemora herdmanni*. *Biol. Bull.* 151, 200-213.
- 881 McLaren, I.A., Corkett, C.J., 1978. Unusual genetic variation in body size, development times,
882 oil storage, and survivorship in the marine copepod *Pseudocalanus*. *Biol. Bull.* 155, 347-359.
- 883 Müller-Navarra, D., Lampert, W., 1996. Seasonal patterns of food limitation in *Daphnia galeata*:
884 separating food quantity and food quality effects. *J. Plankton Res.* 18, 1137-1157.
- 885 Nakatsuru, K., Kramer, D.L., 1982. Is sperm cheap? Limited male fertility and female choice in
886 the lemon tetra (Pisces, Characidae). *Science* 216, 753-755.
- 887 Ohtsuka, S., Huys, R., 2001. Sexual dimorphism in calanoid copepods: morphology and
888 function. *Hydrobiologia*, 453, 441-466.
- 889 Parker, G.A., 1983. Mate quality and mating decisions, in: Bateson (ed.), *Mate choice*.
890 Cambridge University Press, Cambridge, pp. 141-166.
- 891 Petrie, M., Hunter, F.M., 1993. Intraspecific variation in courtship and copulation frequency: an
892 effect of mismatch in partner attractiveness? *Behaviour* 127, 265-277.
- 893 Poulin, R., 1994. Mate choice decisions by parasitized upland bullies, *Gobiomorphus*
894 *breviceps*. *Proc. R. Soc. London Ser. B.* 256, 183-187.

- 895 Proctor, H.C., 1991. Courtship in the water mite *Neumania papillator*: males capitalize on
896 female adaptations for predation. Anim. Behav. 42, 589-598.
- 897 Real, L., 1990. Search theory and mate choice. I. Models of single-sex discrimination. Am. Nat.
898 136, 376-405.
- 899 Reid, M.L., Stamps, J.A., 1997. Female mate choice tactics in a resource-based mating system:
900 field tests of alternative models. Am. Nat. 150, 98-121.
- 901 Rodríguez-Graña, L., Calliari, D., Tiselius, P., Hansen, B.W., Sköld, H.N., 2010. Gender
902 specific ageing and non-Mendelian inheritance of oxidative damage in marine copepods. Mar.
903 Ecol.: Prog. Ser. 401, 1-13.
- 904 Roitberg, B.D., Reid, M.L., Chao, L., 1993. Choosing hosts and mates: the value of learning, in:
905 Papaj, D.R., Lewis, A.C. (Eds.), Insect Learning: Ecological and Evolutionary Perspectives.
906 Chapman & Hall, New York, pp. 174-194.
- 907 Shuster, S.M., Wade, M.J., 2003. Mating systems and strategies. Princeton University Press,
908 Princeton.
- 909 Shuster, S.M., 2007. The evolution of crustacean mating systems. Oxford University Press,
910 Oxford.
- 911 Sichlau, M.H., Kiørboe, T., 2011. Age-and size-dependent mating performance and fertility in a
912 pelagic copepod, *Temora longicornis*. Mar. Ecol.: Prog. Ser. 442, 123-132.
- 913 Simmons, L.W., 1994. Courtship role reversal in bush crickets: another role for
914 parasites? Behav. Ecol. 5, 259-266.
- 915 Sterner, R.W., Schulz, K.L., 1998. Zooplankton nutrition: recent progress and a reality
916 check. Aquat. Ecol. 32, 261-279.

- 917 Strickler, J.R., 1975. Intra- and interspecific information flow among planktonic copepods:
918 receptors. *Verh. Intern Verein Limnol.* 19, 2951-2958.
- 919 Svensson, J. E., 1995. Predation risk increases with clutch size in a copepod. *Funct. Ecol.* 9, 774-
920 777.
- 921 Svensson, B.G., Petersson, E., 1994. Mate choice tactics and swarm size: a model and a test in a
922 dance fly. *Behav. Ecol. Sociobiol.* 35, 161-168.
- 923 Tester, P.A., Turner, J.T., 1990. How long does it take copepods to make eggs?. *J. Exp. Mar.*
924 *Biol. Ecol.* 141, 169-182.
- 925 Titelman, J., Varpe, Ø., Eliassen, S., Fiksen, Ø., 2007. Copepod mating: chance or choice? *J.*
926 *Plankton Res.* 29, 1023-1030.
- 927 Weatherhead, P.J., Robertson, R.J., 1979. Offspring quality and the polygyny threshold: " the
928 sexy son hypothesis". *Am. Nat.* 113, 201-208.
- 929 Wilson, D.S., Hedrick, A., 1982. Speciation and the economics of mate choice. *Evol. Theory* 6,
930 15-24.