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ORIGINAL ARTICLE

The relative importance of various mating criteria in copepods

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To produce viable offspring, organisms may assess mates via criteria that include traits, such as sex, species, age, reproductive status, population identity and individual quality. Copepods are small, ubiquitous crustaceans that live in freshwater and marine systems around the world whose patterns of mate choice have been long studied in numerous species. Herein, we synthesized decades of experiments describing sexual selection in copepods to assess the importance of mating criteria. We used formal, meta-analytical techniques and mixed modeling to quantify the likelihood of non-random mating associated with mating criteria. In our synthesis of the scientific literature, we found that copepods use several criteria when assessing mates and that these criteria are associated with different likelihood estimates. We report the strongest likelihood of non-random mating when copepods assess the reproductive status of females or when copepods select between conspecific vs. heterospecific mates. We found weak likelihood of non-random mating in studies that provide mates from different populations or that manipulate operational sex ratio. Studies that directly test assessment of individual quality are sparse in copepods when compared to equivalent studies in vertebrates, and we encourage future researchers to explore whether copepods use individual characteristics as key mating criteria.

KEYWORDS: sexual selection; zooplankton; recognition; marine invertebrate; mate choice

INTRODUCTION

Animals are hypothesized to select potential mates to improve their reproductive success. Sexual selection is often studied in vertebrates (Andersson, 1994; Schantz

et al., 1999; Smith and Harper, 2003; Andersson and Simmons, 2006), and there are potential benefits for mate assessment by essentially all animal species, including invertebrates such as zooplankton. In particular, there is

a rapidly expanding literature on non-random mating in species of Copepoda (Titelman *et al.*, 2007), a class of animals that is becoming a model for evolutionary, ecological and behavioral research (Raisuddin *et al.*, 2007). Herein, we focus specifically on the importance of mating behavior of copepods.

The criteria used by copepods to assess potential mates have been studied since the 1960s (Bozic, 1960). In copepods, mating can include discrete behaviors, such as changing swimming patterns to track potential mates, precopulatory guarding or mate clasping and copulation (Buskey, 1998), although these behaviors are not necessarily shared across all species. In most copepod species, males clasp females as a key behavior in mating coercion. Some species use pheromones to actively seek potential mates and to decide with which individual they will form a mating pair (Snell, 2010; Yen and Lasley, 2010). The opportunity for non-random mating does not stop at mate guarding, however, as males can switch to clasp different females before finally committing to copulate (Burton, 1985; Hull *et al.*, 1998). Moreover, females display mate choice behavior by escaping the clasp of undesirable males (Tsuda and Miller, 1998; Dur *et al.*, 2011), refusing male coercion attempts (Kiørboe *et al.*, 2005) or possibly selecting sperm belonging to a single male in species with spermathecae that allow for multiple matings (Blades-Eckelbrager, 1991; Mauchline, 1998). Although strategies between males and female copepods may differ, both sexes can be selective when mating.

It has been proposed that copepod mating is limited by the rate at which prospective mates are encountered (Buskey, 1998; Kiørboe, 2006). Indeed, copepod mate selection is likely sequential because most copepod species can only perceive one potential mate at a time (Kiørboe, 2007). However, a simple encounter-based model is insufficient to explain the mating behaviors of many copepods. It has been shown that in some species, only a small proportion of adults produce a majority of the offspring in a population (Sichlau *et al.*, 2015). Existing studies on non-random mating in copepods have manipulated specific criteria that individuals assess when choosing a mate. Potential criteria that copepods might use in assessment of prospective mates include species identity (Goetze, 2008; Goetze and Kiørboe, 2008), sex (Katona, 1973; Anstensrud, 1992; Kelly and Snell, 1998; Kelly *et al.*, 1998), population identity (Lazzaretto *et al.*, 1994; Palmer and Edmands, 2000), reproductive state (Burriss and Dam, 2014) and age/senescence (Ceballos and Kiørboe, 2011). In addition, researchers have performed manipulations on species-specific variables, including the type of diffusible pheromone signals that

copepods use to communicate (Ting and Snell, 2003; Seuront, 2013), the blockage of those chemical signals (Ting *et al.*, 2000; Snell, 2010), contact chemosensation (Lonsdale *et al.*, 1996; Ting *et al.*, 2000), mechanosensory stimuli (Ceballos and Kiørboe, 2010) and mate density during encounters (Dur *et al.*, 2012). Although many of these criteria are communicated by signals that are not easily detected by researchers, behavioral responses to these cues can be visually recorded using one or more of the following measures of choice: tracking (i.e. change in swim behavior for seeking a particular mate), precopulation (i.e. formation of a clasped pair) or copulation (i.e. spermatophore transfer), depending on the copepod species. However, no quantitative synthesis of non-random mating in copepods has been completed, and we do not understand fully which criteria instigate the strongest behavioral response in copepods during mating opportunities.

We conducted a meta-analysis of published studies to assess the relative importance of criteria used by copepods in sexual selection. We sought to describe the likelihood of non-random mating responses determined by the relative importance of each assessment criterion. We operated under the assumption that the likelihood would be dictated by the potential cost or benefits of a good or poor choice for each criterion. For example, sex (i.e. mating type) could be an important and influential assessment criterion on the likelihood of non-random mating, because there can be no reproduction if a same-sex partner is chosen. Recognition of conspecific over heterospecific mates may influence non-random mating behavior because mating outside of a species boundary can result in hybrid offspring of low fitness (Hill, 2019). Assuming significant genetic divergence in copepod populations (Lee, 2000; Edmands, 2001; Goetze, 2003; Garlitska *et al.*, 2012; Barreto *et al.*, 2018), recognizing a mate from within its own population versus genetically divergent populations can avoid costs imparted by genetic incompatibilities (Lynch, 1991). Selecting an individual of the appropriate reproductive state (i.e. virgin vs. gravid) may increase reproductive output and avoid wasted effort. Finally, age assessment may enable a selecting individual to gain maximum reproductive value from a chosen mate. Additionally, we considered factors concerning experimental design of the studies included in the meta-analysis to assess whether experiment duration, the density of potential mates, the type of response variable, the type of mating experiment (single vs. multiple choice) and the type of control used by authors to compare results played a role in influencing likelihood estimates of non-random mating behavior. To quantify these effects, we performed a series of mixed-model analyses.

Table I: Studies included in this meta-analysis

Study no.	Citation
1	Anstensrud, M. (1992). <i>J. Crustacean Biol.</i> , 12(1), 31–40.
2	Bagøien, E., & Kiørboe, T. (2005). <i>Mar. Ecol. Prog. Ser.</i> , 300, 105–115.
3	Barrera-Moreno, O. A., Ciroso-Pérez, J., Ortega-Mayagoitia, E., Alcántara-Rodríguez, J. A., & Piedra-Ibarra, E. (2015). <i>PLoS one</i> , 10(4), e0125524.
4	Burris, Z. P., & Dam, H. G. (2014). <i>J. Plankton Res.</i> , 37(1), 183–196.
5	Ceballos, S., & Kiørboe, T. (2010). <i>Oecologia</i> , 164(3), 627–635.
6	Ceballos, S., & Kiørboe, T. (2011). <i>Plos One</i> , 6(4), e18870.
7	Dur, G., Souissi, S., Schmitt, F. G., Cheng, S. H., & Hwang, J. S. (2012). <i>Zool. Studies</i> , 51(5), 589–597.
8	Frey, M. A., Lonsdale, D. J., & Snell, T. W. (1998). <i>Philos. Trans. R. Soc., B</i> , 353(1369), 745–751.
9	Goetze, E. (2008). <i>Limnol. Oceanogr</i> , 53(2), 433–445.
10	Goetze, E., & Kiørboe, T. (2008). <i>Mar. Ecol. Prog. Ser.</i> , 370, 185–198.
11	Griffiths, A. M., & Frost, B. W. (1976). <i>Crustaceana</i> , 30(1), 1–8.
12	Hull, M. O., Pike, A. W., Mordue, A. J., & Rae, G. H. (1998). <i>Philosophical Transactions of the Royal Society of London B: Biological Sciences</i> , 353(1369), 753–764.
13	Katona, S. K. (1973). <i>Limnology and Oceanography</i> , 18(4), 574–583.
14	Kelly, L. S., Snell, T. W., & Lonsdale, D. J. (1998). <i>Philos. Trans. R. Soc., B</i> , 353(1369), 737–744.
15	Kelly, L. S., & Snell, T. W. (1998). <i>Mar. Biol.</i> , 130(4), 605–612.
16	Lazzaretto, I., Salvato, B., & Libertini, A. (1990). <i>Crustaceana</i> , 171–179.
17	Lazzaretto, I., Franco, F., & Battaglia, B. (1994). In <i>Ecology and Morphology of Copepods</i> (pp. 229–234). Springer, Dordrecht.
18	Lonsdale, D. J., Snell, T. W., & Frey, M. A. (1996). <i>Mar. & Freshw. Behav. Phys.</i> , 27(2–3), 153–162.
19	Palmer, C. A., & Edmands, S. (2000). <i>Mar. Biol.</i> , 136(4), 693–698.
20	Ritchie, G., Mordue, A. J., Pike, A. W., & Rae, G. H. (1996). <i>J. Exp. Mar. Biol. Ecol.</i> , 201(1–2), 285–298.
21	Seuront, L. (2013). <i>J. Plankton Res.</i> , 35(4), 724–743.
22	Ting, J. H., Kelly, L. S., & Snell, T. W. (2000). <i>Mar. Biol.</i> , 137(1), 31–37.
23	Ting, J. H., & Snell, T. W. (2003). <i>Mar. Biol.</i> , 143(1), 1–8.
24	Yen, J., & Lasley, R. (2010). In <i>Chemical Communication in Crustaceans</i> (pp. 177–197). Springer, New York, NY.

METHOD

Data collection

Literature searches were performed from 19 June 2017 to 6 April 2018 using one search engine (*Google Scholar*) and three literature databases (*Web of Science*, *ResearchGate* and *ProQuest*) with the following search terms in isolation and combination: ‘copepod’, ‘mate choice’, ‘chemical communication’, ‘sexual selection’, ‘mate assessment’, ‘mate guarding’, ‘pheromone’, ‘mate recognition’ and

Effect size

The data extracted from the included studies were in the form of count data recorded as the number of copepods that exhibited a certain selective mating decision (varied study by study—see general examples below). Thus, the effect size selected for comparison among studies was the odds ratio (OR). The OR is represented as a proportion of events between two groups, as shown in Equation 1 and can be interpreted as the likelihood of a copepod exhibiting a certain mating response between two groups.

$$\frac{\text{Number of individuals making decision 1 in group A (a) / Number of individuals making decision 2 in group A (c)}}{\text{Number of individuals making decision 1 in Group B (b) / Number of individuals making decision 2 in group B (d)}} \quad (1)$$

‘glycoprotein’. Papers were downloaded ($N = 67$) and filtered for relevance with the following conditions: studies must include (i) measures of copepod mating in the form of raw count data and (ii) a control (either statistical or experimental, described below). Data collection with these parameters yielded 24 usable publications (Table I). Using data from these 24 publications, we grouped effect sizes into nine categories of mating criteria. We have organized and consolidated descriptions of these criteria in Table II.

Decisions 1 and 2 may represent any of two behavioral outcomes in a mating trial, and Groups A and B may represent any two experimental or control groups. For example, in the case of a mate choice trial where a single individual had to choose between clasping two different species of mates, one of the same species and one of a different species, we could encode the results of this experiment into the OR by making decision 1 = number of copepods that clasped a mate and decision 2 = number of copepods that ignored a mate. We could then encode

Table II: Criteria summary

Mating criteria	Independent variable	Response measured	Study no. (Table II)
Reproductive status	Virgin female vs. non-virgin with dropped spermatophore	C	4, 14
Species	Virgin vs. non-virgin with spermatophore intact	C, P, T	8, 9, 10, 13, 14, 16, 17
	Time since insemination		
Mechanosensory stimuli	Mate of different species vs. mate within species (same stage)	C, P, T	5, 13, 15
	Size difference between mates		
Age or developmental stage	Movement of mate	C, P	1, 6, 13, 14, 20, 21
	Frozen killed vs. live		
	Earlier stage vs. later stage		
Sex	Varying chooser adult age	C, P	1, 13, 14, 15
	Varying chosen adult age		
Diffusible pheromone	Young virgin vs. ovigerous virgin	T	2, 11, 14, 17, 21, 24
	Same sex vs. opposite sex of juvenile stages		
	Same vs. opposite sex of adults		
	Presence/absence of female pheromone		
	Presence/absence of conspecific pheromone		
Blockage of chemosensation	Presence/absence of heterospecific mate	P	12, 14, 15, 18, 22, 23
	Pheromone mimic of same sex		
	Pheromone mimic of opp. sex before/after reproduction		
	Pheromone mimic of conspecific mate vs. heterospecific mate		
Population	Antennule ablation	C, P, T	3, 8, 17, 19
	Protease, antibody or lectin administration		
Operational sex ratio	Heat-denatured proteases	C, P	7, 14
	Presented with mate of different population vs. same population		
	1:1 vs. 1:2 or 1:5 (male: female)		
	Chalimus 2 stage added to chalimus 2		

the heterospecific mate as Group A and the conspecific mate as Group B. The OR then becomes the ratio of copepods that either did or did not clasp a heterospecific mate over the ratio of copepods that did or did not select a conspecific mate. In this case, the more copepods that do (or do not) clasp a heterospecific mate would cause the magnitude of the odds ratio to become larger (indicating a higher likelihood that species identity influenced the copepod's mating decision in this example). In the instance that authors provided copepods only one type of mate to clasp (for example, just a single heterospecific mate) with no other options (i.e. a 'no-competition' trial), the OR could be encoded the same, as long as the author also included a no-competition trial with a 'control' conspecific mating option.

In studies where authors did not provide a physical control group, the odds ratio was encoded simply as whether a copepod positively responded to or ignored a mate in comparison to what we would expect by chance (we call this a statistical control). To continue our example of a mating trial with heterospecific mates: if an author did not include a mating trial in which copepods were presented with conspecific mates as an actual experimental control, but instead compared the number of heterospecific

mating decisions to the number chance decisions that would be expected based on the number of total replicates run (let us say 50 total replicates in this example), the odds ratio would be encoded as such: decision 1 = number of copepods out of 50 that clasped a heterospecific mate, decision 2 = number of copepods out of 50 that did not clasp a heterospecific mate, group A = numbers from the actual trial and group B = numbers split evenly based on chance to make two choices (i.e. 50/50 chance or 25 for Decision 1 and 25 for Decision 2). Effectively, the odds ratio is an effect size that represents the likelihood that a copepod exhibits selective behavior, given certain criteria to assess. The estimate from our encoded OR does not discriminate between copepod preference or avoidance, but simply represents the likelihood that non-random mating occurs. The greater the likelihood, the stronger the behavioral response to a given type of criteria.

We adapted this equation to the biology of copepod mating by defining selective behavior as a copepod either changing swimming behavior (tracking), engaging in pre-copulatory clasping (precopulation) or completing spermatophore transfer (copulation). The behavior used to calculate the OR was dependent on the life history of the copepod species in each individual study. In this way, we

preserved organism-specific behaviors since incorporating study-specific biology into a comparative effect size is important when making broader conclusions across studies when using meta-analyses (Detsky *et al.*, 1992). While meta-analyses in general may lack the resolution of the individual studies they incorporate, the ability to empirically assess patterns across very different studies is a key advantage to the approach (Hillebrand and Cardinale, 2010). However, inclusion of all relevant data can make comparison across studies difficult, which makes study exclusion criteria important (Eysenck, 1994).

Statistical analysis

All statistical analyses and graphing were completed using the log-transformed odds ratio (LOR) taken as its absolute value, calculated by the *metafor* v2.0-0 package (Viechtbauer, 2010) in R v3.6.1 (The R Core Team, 2019). In the results reported from these data, larger LOR values farther away from 0 indicate stronger likelihood of non-random mating. If the credible intervals around estimated LOR values do not include 0, they were considered statistically significant. Credible intervals that include 0 are equivalent to a *P* value of > 0.05 . All presentation and discussion of results using these data were in terms of the LOR and values were not back-transformed to the odds ratio.

The ‘MCMCglmm’ package v2.26 (Hadfield, 2010) was used to compare likelihoods between groups using a Markov chain Monte Carlo model. The MCMC model was chosen because it allowed for the incorporation of a phylogeny specified as a random effect. Our model chain ran a total of 600 000 iterations with a burn-in of 100 000 and thinning interval of 10. The burn-in of 100 000 was chosen to ensure our model estimations were unbiased by transient or extreme estimates, and the thinning interval of 10 (using only every 10th estimate) was chosen to reduce autocorrelation among estimates. This provided us with a final posterior distribution of 50 000 estimates per model run. We used a non-informative prior for each analysis to maximize the influence of the collected data on posterior distributions. For plotting of results, we used the R packages *ggplot2* v2.2.1 (Wickham, 2016), *bayesplot* v1.6.0 (Gabry and Mahr, 2018) and *extrafont* v0.17 (Chang, 2014). To calculate the amount of heterogeneity due to authorship and genus classification, we used the *rma.mv* function from the *metafor* package.

Phylogenetic relatedness as a random effect

When comparing likelihood estimates among genera of copepods, the phylogenetic relationships between the various orders of copepods from Khodami *et al.* (2017)

was included in our model. While performing all other statistical analyses, a phylogeny of copepod genera was used to account for variation in our estimates due to relationships between the various copepod genera. This phylogeny was constructed in *MEGA X* v10.0.2 (Kumar *et al.*, 2018) using Cytochrome C Oxidase subunit 1 (CO1) sequences downloaded from GenBank belonging to the copepods included in this meta-analysis. The final maximum-likelihood tree constructed using these sequences is shown in Fig S1.

Fixed effects

To quantify the degree to which copepods engage in non-random mating, we ran a MCMCglmm model on our entire dataset without fixed effects. To account for variation due to multiple effect sizes coming from the same study or from the same criteria as well as phylogenetic non-independence among copepods, we included random effects of authorship, criterion category and the phylogenetic relatedness between genera. To summarize the likelihood of non-random mating among different genera of copepods, we ran an MCMCglmm model with genus as a fixed effect and authorship and order-level phylogeny as random effects (Khodami *et al.*, 2017). We followed this analysis with a model that described the likelihood of non-random mating by copepod habitat and included random effects of authorship and genus-level phylogeny. Our primary goal in this meta-analysis was to determine the mating criteria associated with the strongest likelihood of non-random mating behavior in copepods. To do this, we ran a model with mating criteria as a fixed effect while controlling for random effects of authorship and genus-level phylogeny. This is comparable to a linear model comparing effects across experimental groups. These criteria and the specific experimental variables they represented are listed in Table I.

We also analyzed components of experimental design in copepod mating studies, including (i) the genus of the copepods, (ii) the type of mating response (e.g. tracking behavior, precopulation or copulation), (iii) the design of experimental trials (e.g. mate density, length of observation time), (iv) the type of control (e.g. statistical or experimental) and (v) the type of experiment (e.g. one choice or multiple choice experiments). A control was considered experimental if its data came from a physically completed trial and was considered statistical if it was based on the likelihood of non-random mating occurring due to chance. We fit a model for each of the five experimental design fixed effects, and each included the same random effects of authorship and genera-level phylogeny.

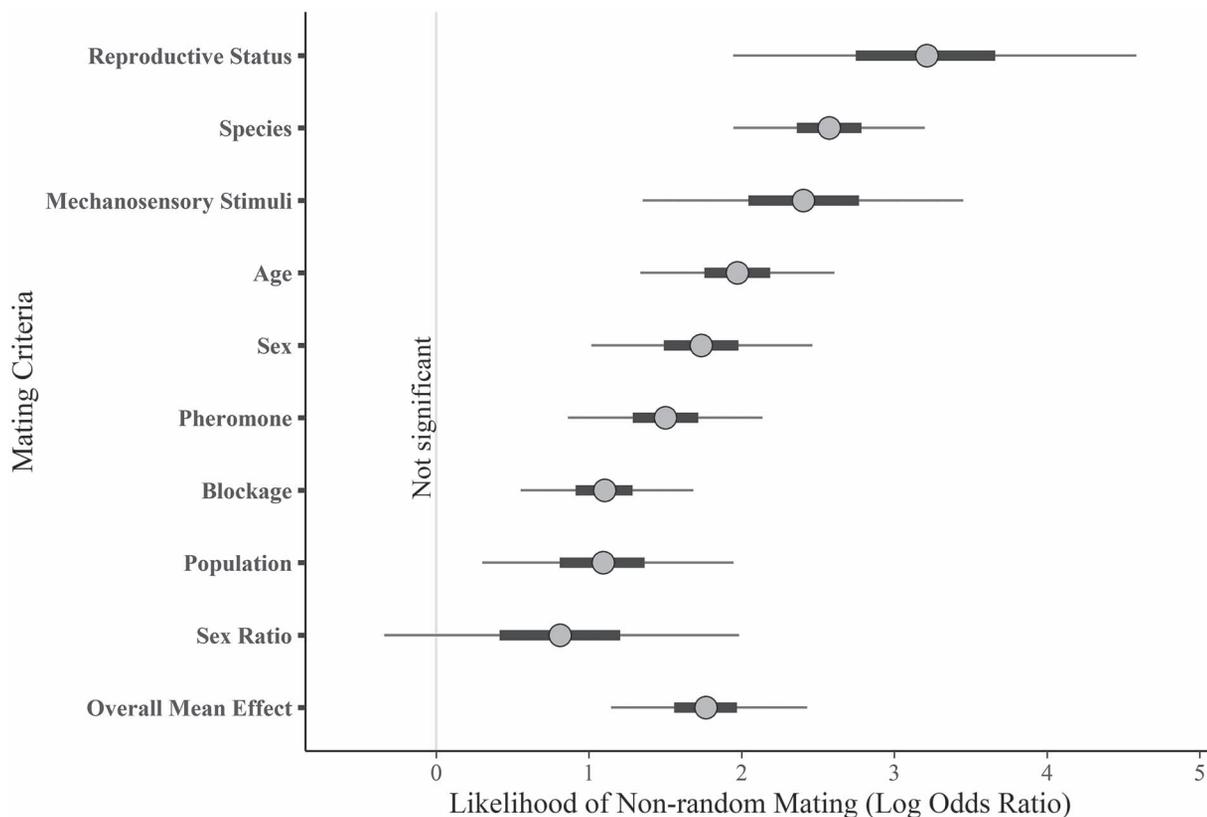


Fig. 1. Model-estimated likelihoods of non-random mating associated with each mating criterion in our MCMCglmm model. Center dots represent mean effect sizes for each group, thick blue bars represent 50% credible intervals and thin blue bars represent 95% credible intervals. Credible intervals that overlap the vertical gray line at 0 represent no significant likelihood of non-random mating.

Publication bias

Publication bias was assessed using the *funnel* function in the *metafor* package accompanied by a test for funnel plot asymmetry (Fig S2). With this method, we looked for asymmetry of the plot and points outside plot confidence intervals that would indicate that the literature on this topic is missing studies with statistically insignificant results. We also performed a statistical test for asymmetry using the *reg.test* function in the *metafor* package.

RESULTS

Non-random mating among genera

The final data set included 252 effect sizes extracted from 24 publications comprised of data across four habitats, five orders, 13 genera and 23 species. The overall likelihood of non-random mating across all experiments included in this analysis was significant (LOR = 1.77, 1.13–2.40 95% CI, $P < 0.0001$, Fig 1, Table III).

We found that four genera (*Pseudocalanus*, *Lernaecera*, *Coullana* and *Leptodiaptomus*) did not display significant,

non-random mating when modeled alongside all other genera (Fig 2, Table SII). Two genera, *Calanus* and *Centropages*, showed effect sizes that were marginally significant ($P = 0.0531$ and $P = 0.0748$, respectively). Seven genera showed estimates of effect that were significant showing non-random mating is not unique to a single genus. The likelihood of non-random mating estimated by our meta-analytical model varied by genus (Fig 2). The strongest likelihood was attributed to planktonic members of *Oithona*, while the weakest, yet still significant, likelihood belonged to the benthic group *Tigriopus* (Fig 2). However, *Tigriopus* also had the largest effective sample size (Table SII). Non-random mating was consistent across habitat types (Table SII). Pelagic and planktonic copepods (which ultimately are very similar—the ability to swim against the current is not always unequivocal) were not the most likely to engage in non-random mating, while benthic and ectoparasitic copepods were less likely to do so. The estimated overall heterogeneity in the data using our model was 83.48%. Of this heterogeneity, 44.65% was attributable to variation due to authorship and 38.83% was attributable to phylogenetic relatedness among taxa.

Table III: The model estimated effect sizes (log odds ratio) and confidence intervals (CI) representing the likelihood of non-random mating in copepods

Analysis grouping	Sample size	Effect size	Lower 95% CI	Upper 95% CI	I^2 (%)	Heterogeneity	
						Paper (%)	Genus (%)
Overall mean effect	252	1.77	1.13	2.40	80.89	73.12	7.77
Reproductive status	8	3.21	1.90	4.53			
Species identity	39	2.57	1.96	3.21			
Mechanosensory stimuli	10	2.41	1.35	3.45			
Age	32	1.97	1.33	2.60			
Sex	15	1.74	1.01	2.46			
Diffusible pheromone	27	1.50	0.87	2.15			
Blockage	91	1.10	0.55	1.68			
Population	25	1.09	0.28	1.91			
Sex ratio	5	0.81	-0.37	1.95			

Mating criteria associated with mate choice

A total of nine different criteria influencing mating (Fig. 1 and Table II) were used to compare the degree to which copepods exhibit non-random mating behavior. We measured significant likelihood estimates of non-random mating in all but a single category of criteria (i.e. sex ratio, $P = 0.1646$, Table III). Likelihood estimates in the age, blockage, diffusible pheromone, species, mechanosensory stimuli, population, reproductive status and sex categories were all significant (Fig. 1, Table III). We found variation in the likelihood of non-random mating in each individual category by our meta-analytical model (Fig. 2). The overall estimated heterogeneity in the data and the heterogeneity due to random effects of authorship and genus are shown in Table III. Total heterogeneity across the data set was high (80.89%), but only 7.77% of this was explained by differences among genera (Table III).

Reproductive status had the highest likelihood of non-random mating and was followed by experiments that manipulated species identity and mechanosensory stimuli (Fig. 1, Table III). Age and sex criteria had similar likelihoods, and estimates for these two criteria were stronger than those from the diffusible pheromone and blockage categories (Fig. 1, Table III). The weakest likelihood estimate among the significant criteria was associated with experiments that varied population identity of mates within a species.

Effects of experimental design

We found significant likelihoods of non-random mating associated with all three response behaviors, but the likelihood estimates varied among them (Table SIII). The response behavior with the strongest likelihood was copulation, while the weakest likelihood estimated belonged to precopulation. We found that there was no significant difference in the likelihood of non-random mating between

experiments that ran physical controls and those that used statistical controls (Table SIII). We found that single choice experiments (i.e. one female and one male) were on average associated with higher likelihoods of non-random mating behavior, but this difference was not significant (Table SIII).

We found no significant effect of observation time during experiments on the estimated likelihood of non-random mating (Table SIII). However, we did find a statistically significant effect of increasing the number of potential mates provided to copepods during mating trials. For every added potential mate, the estimated likelihood of non-random mating decreased slightly (LOR = -0.17 , $P < 0.05$, Table SIII). This result indicates that, in general, the likelihood of non-random mating was negatively correlated with studies in which authors chose to use a greater density of potential mates.

Publication bias in copepod mating literature

Qualitative analysis using a funnel plot (Fig. S2) showed slight asymmetry in the form of missing values near the bottom center of the plot which is confirmed using the *regtest* function in the *metafor* package ($z = -2.57$; $P = 0.0102$). This finding indicated that more studies showing significant non-random mating in copepods are being published than studies that do not. Additionally, some effect sizes fell outside the estimated confidence intervals generated in the plot and represented cases with higher leverage.

DISCUSSION

In this meta-analysis, we used Markov chain Monte Carlo simulation and mixed modeling to compare nine potential criteria that may influence copepod mating behavior. It

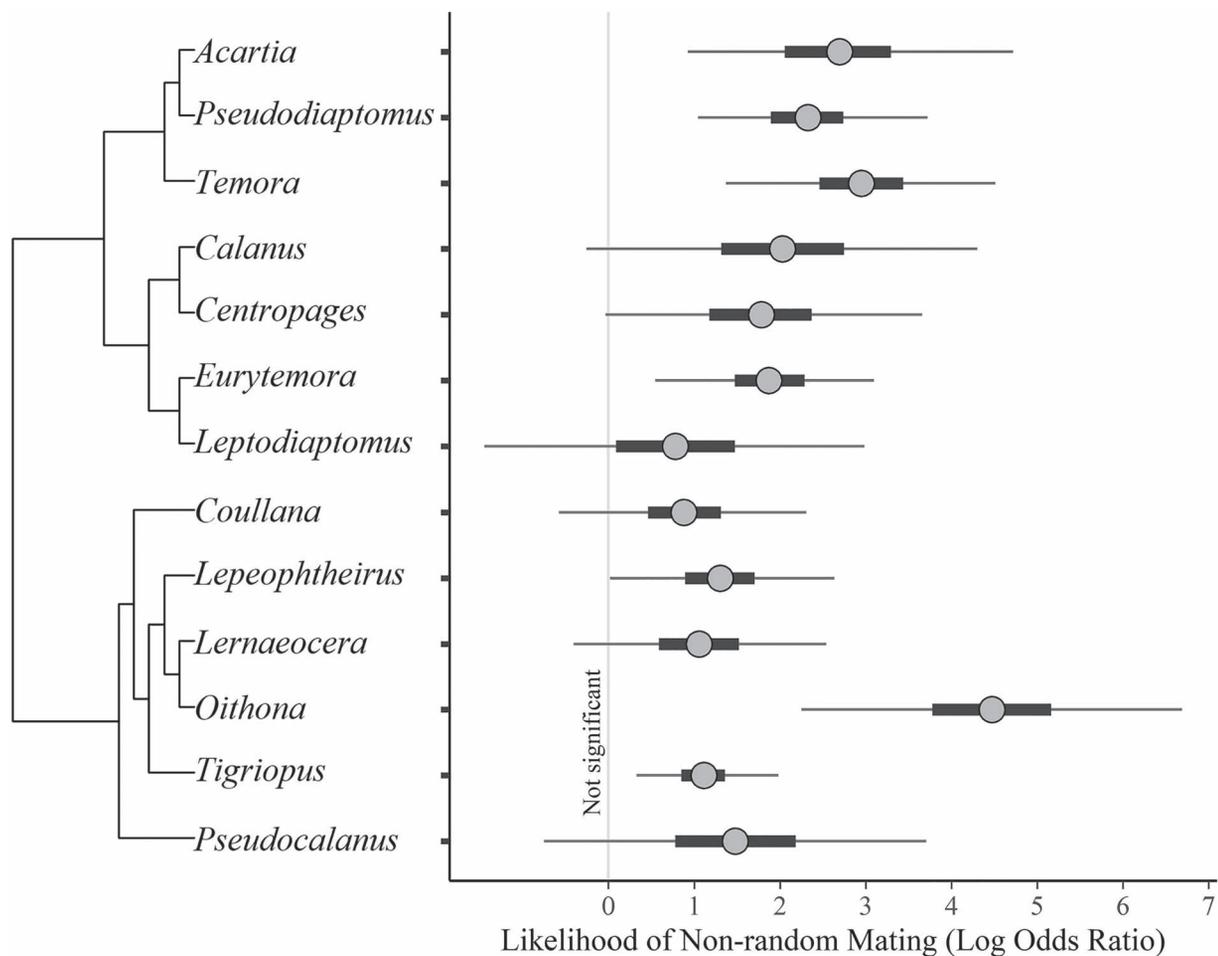


Fig. 2. Model-estimated likelihood of non-random mating associated with each genus alongside a genus-level phylogeny built using complete and partial COI sequences. Center dots represent mean effect size for each group, thick blue bars represent 50% credible intervals and thin blue bars represent 95% credible intervals. Credible intervals that overlap the vertical gray line at 0 represent no significant likelihood of non-random mating.

could be predicted that sex (i.e. mating type) would be very influential in instigating non-random mating behavior based on the need to avoid competitive encounters between members of the same sex and wasted energy expenditure; however, we saw that sex of a mate was less influential on non-random mating than reproductive status, species identity or mechanical stimulation. In theory, there may be a high cost of low selectivity for mating type, premised on the assumption that mating opportunities, energy or resources might be lost by pursuing and entering precopula with a same-sex mate. Such a high cost of pursuing a same-sex partner may not be relevant, however, if the precopulatory selection of a same-sex mate is quickly recognized and corrected by releasing or escaping an unwanted mate (Tsuboko-Ishii and Burton, 2017).

Based on our analysis, chemical cues, such as diffusible pheromones, on their own may not be as influential as

cumulative signals between mates during close interactions (Fig. 1). It has been proposed that animals may use multiple signals to enhance the accuracy of their message to other individuals (Johnstone, 1996; Loyau *et al.*, 2005). Copepods, for example, may recognize mates based on movement, diffusible pheromones and surface-bound glycoprotein cues simultaneously. In copepods, blockage of the ability to sense chemical or mechanical cues, via antennule ablation, protease digestion, lectin blockage or heat denaturation, can be expected to have a strong effect on non-random mating. It is possible that although diffusible pheromones are hypothesized to aid copepods in recognition of viable mates, they are only one trait out of many assessed during mating events. Both pheromone and blockage mating criteria were associated with significant likelihood estimates (Fig. 1), but these were not as large as estimates for more cumulative criteria, such as species identity or developmental age. Thus,

copepod mating systems may serve as important models for comparing differences in communication using single versus multiple mating signals.

Reproductive status appeared to have the most influence on non-random mating patterns in copepods. It is not surprising that reproductive status was an important determinate of mating behavior, as many copepod species can only receive a transferred spermatophore once in their lifetime (Burton, 1985; Blades-Eckelbrager, 1991; Subramoniam, 1993). If we assume that males can only mate with a set number of females in his lifetime, it becomes paramount that males select females at an optimal reproductive stage to optimize his reproductive output. Male copepods have been shown to preferentially avoid females that have already been inseminated (Kelly *et al.*, 1998; Burris and Dam, 2014), although evidence of sperm competition in copepods is described in some species (Ianora *et al.*, 1989; Ohtsuka and Huys, 2001). It is possible that female copepods also benefit from selecting ‘experienced’ males over immature males that are still virgins. For males, there may be an energetic or resource-based cost to transfer a spermatophore to an already-inseminated female.

We observed a high likelihood of non-random mating when copepods could select between mates belonging to the same or different species (Fig. 1). This criterion included studies that used pelagic species that exist in sympatry and tidepool species that are completely allopatric. We could predict different intensities in selective pressure to recognize intra- from inter-specific individuals between allopatric and sympatric groups. However, it seems important that copepods are able to recognize and avoid hybrid matings. Similar to species identity, there was also a strong likelihood for non-random mating responses based on mechanosensory stimuli (Fig. 1). The assessment of movement aids in the detection and encounter of mates, but it may also stop copepods from mating with dead, dying or unhealthy individuals. The assessment of body size within a discrete developmental stage may suggest that copepods assess aspects of individual condition, but this area of research currently lacks experimental evidence in copepods.

The likelihood of non-random mating responses to the age of potential mates was high in comparison to other criteria, such as sex and copepod pheromone signals. In experimental settings, studies found preference for copepodites that are older and closer to full adult-hood (Burton, 1985; Kelly *et al.*, 1998), as well as preferences for adults that are younger compared to adults nearing end-life senescence (Ceballos and Kiørboe, 2011). Preference for older copepodites is hypothesized to be driven by the fact that males do not have to clasp older copepodite females as long before insemination can take place (Anstensrud, 1992; Kelly *et al.*, 1998). Avoiding adult

mates near the end of their lifetimes could help increase reproductive success (Ceballos and Kiørboe, 2011). Selective behavior based on the age of mates could be interpreted as selection for mates of better condition or quality in studies where younger adults are preferred over older adults, particularly in species where senescence is clearly observed (Ceballos and Kiørboe, 2011).

The criterion with the weakest likelihood estimate of non-random mating was population identity. In the wild, mating between allopatric populations of tidepool copepods is rare and selection for intra-population mates may not have had the opportunity to evolve yet (Palmer and Edmands, 2000) despite high rates of genetic differentiation between conspecific populations. High inter-population genetic divergence has also been observed in copepod species that live in open waters (Lee, 2000; Goetze, 2003; Garlitska *et al.*, 2012). In *Tigriopus* tidepool populations where genetic incompatibilities have been rigorously documented, these incompatibilities do not manifest until the second generation of hybrids (Burton *et al.*, 2006; Burton *et al.*, 2013; Barreto and Burton, 2013). Beyond a lab setting, it is not well-studied how often there is admixture of wild *Tigriopus* populations. First generation hybrids of *T. californicus* have been shown to experience hybrid vigor (Burton *et al.*, 2006; Hui, 2018), and there is evidence for preference of population outbreeding in the face of repeated inbreeding in the *Tigriopus* genus (Palmer and Edmands, 2000). Optimal outbreeding avoiding both extreme inbreeding and extreme outbreeding has been proposed in organisms with less extreme population delimitation (Waser and Price, 1989; Lynch, 1991; Atalay and Schausberger, 2018). In contrast to the tidepool-restricted *Tigriopus* species, other pelagic copepods that coexist in waters with admixed species may be more selective of species identity. However, many of the individual effect sizes in the ‘species identity’ criterion in our meta-analysis came from experiments with *Tigriopus*.

We saw no statistically significant likelihood of non-random mating from effect sizes grouped under the sex ratio criteria (Fig. 1). In studies that purposefully changed the operational sex ratio of male and female copepods, there was often no significant change in selective behavior. However, we still considered differences in the density of mates important to investigate across all studies, regardless of whether primary authors intended to test its effect. Thus, we included this aspect independently in our analysis of experimental design.

Considerations for experimental design

When considering the effect of increasing the density of stimulus individuals to choosing copepods, it is possible that having more individuals from which to select in a

set space may have a significant effect on the outcome of a given mate choice test. Importantly, we observed that providing copepods with higher density of mates is not associated with higher likelihoods of non-random mating (Table SIII). This could be because the experiments included in this meta-analysis were on both male and female copepods, and males of many species can mate multiple times. Other work has suggested that having more available mates could allow an organism to be more selective (Kokko and Johnstone, 2002).

We did find that the type of response that authors used to document selective behavior played a role in determining the estimated likelihood of non-random mating. The largest estimates were associated with experiments that measured copulation as the mating response, while the weakest estimates were seen in studies that measured precopulation (Table SIII). Insemination is one of the final steps in mating, and therefore, it is understandable that experiments that used copulation as the final measure of non-random mating were associated with strong likelihood estimates in our analysis. Precopulatory pair formation can include mistakes by a copepod (Tsuboko-Ishii and Burton, 2017), and this may explain why the estimated likelihood on non-random mating for this type of response was lower.

According to our analysis, there was no significant difference in the likelihood of non-random mating between studies that used single versus multiple-choice designs. This is in contrast to results that show stronger mating preferences when using multiple choice experimental designs (Dougherty and Shuker, 2015). However, this does not necessarily mean that single-choice and multiple-choice experiments can be interpreted equivalently in copepods. We also found that time had no significant effect on the strength of mate choice. This is perhaps surprising, since it could be predicted that the longer a copepod can assess available mates, the more likely it will be to make an observable and recordable choice, but this seems not the case (Table SIII).

CONCLUSION

Most genera of copepods in this study show non-random mating behavior, indicating that many copepods do not indiscriminately accept the first prospective mate that they encounter (Fig. 2, Table SII). Such experimental evidence for non-random mating is corroborated by observations of swarming behavior in copepods in the wild. Copepods often have an abundance of mates available in these swarms, and this type of mating environment can even resemble pseudo-lekking behavior as studied in birds (Titelman *et al.*, 2007; Höglund and Alatalo, 2014). Diverse genera of copepods were found to engage in non-random mating (Fig. 2, Table SII). Grouping experiments

by specific copepod habitats (Table SII) only reinforced the conclusion that copepods engage in non-random mating, as all four habitat types were estimated to show significant non-random mating behavior on average. However, the likelihood of non-random mating cannot be assumed to be equal in all genera; the likelihood of the non-random behavior likely varies among the criteria assessed by different copepods, many of which have very different biology and life cycles.

The broad spectrum of studies on different aspects of copepod mating behavior covers a wide variety of topics and mating criteria. In summary, we have found that copepods show the strongest behavioral response for reproductive status, followed by recognition of conspecific mates versus heterospecific mates (Fig. 1). When presented with individuals from the same versus different populations, the likelihood of non-random mating is low (Fig. 1). However, as shown by our literature search and the final set of extracted data, we are lacking experimental studies describing whether copepods assess mates for individual quality (Figs. 2 and 3). Studies on mate choice for individual characteristics that communicate quality are well-represented in literature for many vertebrate animals (Andersson and Simmons, 2006; Weaver *et al.*, 2017). However, similar studies in copepods are scarce and descriptive rather than experimental (Titelman *et al.*, 2007; Ceballos and Kiørboe, 2011). As copepods become an important model with which we can study speciation and sexual selection, it is critical to assess whether copepods inspect individual quality of potential mates.

DATA ARCHIVING

Raw data can be found online at *Mendeley Data*. Scripts used in R to analyze this data can be found online at *Journal of Plankton Research*.

SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Plankton Research* online.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest in the analysis and presentation of these results.

REFERENCES

- Andersson, M. (1994) *Sexual Selection*, Princeton University Press, New Jersey.
- Andersson, M. and Simmons, L. W. (2006) Sexual selection and mate choice. *Trends Ecol. Evol.*, **21**, 296–302.
- Anstensrud, M. (1992) Mate guarding and mate choice in two copepods, *Lernaeocera branchialis* (L.) (Pennellidae) and *Lepeophtheirus pectoralis* (Müller) (Caligidae), parasitic on flounder. *J. Crustac. Biol.*, **12**, 31–40.
- Atalay, D. and Schausberger, P. (2018) Balancing in- and out-breeding by the predatory mite *Phytoseiulus persimilis*. *Exp. Appl. Acarol.*, **74**, 159–169.
- Barreto, F. S. and Burton, R. S. (2013) Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. *Proc. R. Soc. B. Biol. Sci.*, **280**, 7.
- Barreto, F. S., Watson, E. T., Lima, T. G., Willett, C. S., Edmands, S., Li, W. and Burton, R. S. (2018) Genomic signatures of mitonuclear coevolution across populations of *Tigriopus californicus*. *Nat. Ecol. Evol.*, **2**, 1250–1257.
- Blades-Eckelbrager, P. I. (1991) Functional morphology of spermatophores and sperm transfer in calanoid copepods. *Crustac. Biol.*, **246–270**.
- Bozic, B. (1960) Le Genre *Tigriopus* Norman (Copépodes Harpacticoides) et ses formes européennes: recherches morphologiques et expérimentales.
- Burris, Z. P. and Dam, H. G. (2014) Female mating status affects mating and male mate-choice in the copepod genus *Acartia*. *J. Plankton Res.*, **37**, 183–196.
- Burton R.S., Ellison C.K., and Harrison J.S. (2006) The sorry state of F2 hybrids: consequences of rapid mitochondrial DNA evolution in allopatric populations. *Am. Nat.*, **168**, S14.
- Burton, R. S. (1985) Mating system of the intertidal copepod *Tigriopus californicus*. *Mar. Biol.*, **86**, 247–252.
- Burton, R. S., Pereira, R. J. and Barreto, F. S. (2013) Cytonuclear genomic interactions and hybrid breakdown. *Annu. Rev. Ecol. Evol. Syst.*, **44**, 281–302.
- Buskey, E. J. (1998) Components of mating behavior in planktonic copepods. *J. Mar. Syst.*, **15**, 13–21.
- Ceballos, S. and Kjørboe, T. (2010) First evidences of sexual selection by mate choice in marine zooplankton. *Oecol.*, **164**, 627–635.
- Ceballos, S. and Kjørboe, T. (2011) Senescence and sexual selection in a pelagic copepod. *PLoS One*, **6**, e18870.
- Chang, W. (2014) *extrafont: Tools for using fonts*.
- Detsky, A. S., Naylor, C. D., O'Rourke, K., McGeer, A. J. and L'Abbé, K. A. (1992) Incorporating variations in the quality of individual randomized trials into meta-analysis. *J. Clin. Epidemiol.*, **45**, 255–265.
- Dougherty, L. R. and Shuker, D. M. (2015) The effect of experimental design on the measurement of mate choice: a meta-analysis. *Behav. Ecol.*, **26**, 311–319.
- Dur, G., Souissi, S., Schmitt, F. G., Beyrend-Dur, D. and Hwang, J.-S. (2011) Mating and mate choice in *Pseudodiaptomus annandalei* (Copepoda: Calanoida). *J. Exp. Mar. Bio. Ecol.*, **402**, 1–11.
- Dur, G., Souissi, S., Schmitt, F. G., Cheng, S. and Hwang, J.-S. (2012) Sex ratio and mating behavior in the calanoid copepod. *Zool. Stud.*, **51**, 589–597.
- Edmands, S. (2001) Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. *Mol. Ecol.*, **10**, 1743–1750.
- Eysenck, H. J. (1994) Systematic reviews: meta-analysis and its problems. *BMJ*, **309**, 789–792.
- Gabry, J. and Mahr, T. (2018) *bayesplot: Plotting for Bayesian models*.
- Garlitska, L., Neretina, T., Schepetov, D., Mugev, N., De Troch, M., Baguley, J. G. and Azovsky, A. (2012) Cryptic diversity of the “cosmopolitan” harpacticoid copepod *Nannopus palustris*: genetic and morphological evidence. *Mol. Ecol.*, **21**, 5336–5347.
- Goetze, E. (2003) Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. *Proc. R. Soc. B. Biol. Sci.*, **270**, 2321–2331.
- Goetze, E. (2008) Heterospecific mating and partial prezygotic reproductive isolation in the planktonic marine copepods *Centropages typicus* and *Centropages hamatus*. *Limnol. Oceanogr.*, **53**, 433–445.
- Goetze, E. and Kjørboe, T. (2008) Heterospecific mating and species recognition in the planktonic marine copepods *Temora stylifera* and *T. longicornis*. *Mar. Ecol. Prog. Ser.*, **370**, 185–198.
- Hadfield, J. D. and Hadfield, J. D. (2010) MCMC methods for multi-response generalized linear mixed models: the {MCMCglmm} {R} package. *J. Stat. Softw.*, **33**, 1–22.
- Hill, G. (2019) *Mitochondrial Ecology*, Oxford University Press, Oxford.
- Hillebrand, H. and Cardinale, B. J. (2010) A critique for meta-analyses and the productivity–diversity relationship. *Ecol.*, **91**, 2545–2549.
- Höglund, J. and Alatalo, R. (2014) *Leks*, Princeton University Press, New Jersey.
- Hui, J. H. L. (2018) Copepod incompatibilities. *Nat. Ecol. Evol.*, **2**, 1203–1204.
- Hull, M. Q., Pike, A. W., Mordue, A. J. and Rae, G. H. (1998) Patterns of pair formation and mating in an ectoparasitic caligid copepod *Lepeophtheirus salmonis* (Kroyer 1837): implications for its sensory and mating biology. *Philos. Trans. R. Soc. B. Biol. Sci.*, **353**, 753–764.
- Ianora, A., Scotto di Carlo, B. and Mascellaro, P. (1989) Reproductive biology of the planktonic copepod *Temora stylifera*. *Mar. Biol.*, **101**, 187–194.
- Johnstone, R. A. (1996) Multiple displays in animal communication: “backup signals” and multiple messages. *Philos. Trans. R. Soc. B.*, **351**, 329–338.
- Katona, S. K. (1973) Evidence for sex pheromones in Planktonic copepods. *Limnol. Ocean.*, **18**, 574–583.
- Kelly, L. S. and Snell, T. W. (1998) Role of surface glycoproteins in mate-guarding of the marine harpacticoid *Tigriopus japonicus*. *Mar. Biol.*, **130**, 605–612.
- Kelly, L. S., Snell, T. W. and Lonsdale, D. J. (1998) Chemical communication during mating of the harpacticoid *Tigriopus japonicus*. *Philos. Trans. R. Soc. London. Ser. B. Biol. Sci.*, **353**, 737–744.
- Khodami, S., McArthur, J. V., Blanco-Bercial, L. and Martinez Arbizu, P. (2017) Molecular phylogeny and revision of copepod orders (Crustacea: Copepoda). *Sci. Rep.*, **7**, 1–11.
- Kjørboe, T. (2006) Sex, sex-ratios, and the dynamics of pelagic copepod populations. *Oecol.*, **148**, 40–50.
- Kjørboe, T. (2007) Mate finding, mating, and population dynamics in a planktonic copepod *Oithona davisae*: there are too few males. *Limnol. Oceanogr.*, **52**, 1511–1522.

- Kjørboe, T., Bagoien, E. and Thygesen, U. H. (2005) Blind dating - mate finding in planktonic copepods. II. The pheromone cloud of *Pseudocalanus elongatus*. *Mar. Ecol. Prog. Ser.*, **300**, 129–133.
- Kokko, H. and Johnstone, R. A. (2002) Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philos. Trans. R. Soc. London. Ser. B. Biol. Sci.*, **357**, 319–330.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, **35**, 1547–1549.
- Lazzaretto, I., Franco, F. and Battaglia, B. (1994) Reproductive behaviour in the harpacticoid copepod *Tigriopus fulvus*. In *Ecology and Morphology of Copepods*, Springer Netherlands, Dordrecht, pp. 229–234.
- Lee, C. E. (2000) Global Phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate “populations”. *Evol.*, **54**, 2014–2027.
- Lonsdale, D. J., Snell, T. W. and Frey, M. A. (1996) Lectin binding to surface glycoproteins on *Coullana* spp. (Copepoda: Harpacticoida) can inhibit mate guarding. *Mar. Freshw. Behav. Physiol.*, **27**, 153–162.
- Loyau, A., Jalme, M. S., Cagniant, C. and Sorci, G. (2005) Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*). *Behav. Ecol. Sociobiol.*, **58**, 552–557.
- Lynch, M. (1991) The genetic interpretation of inbreeding depression and outbreeding depression. *Evol.*, **45**, 622–629.
- Mauchline, J. (1998) The biology of calanoid copepods. *Adv. Mar. Biol.*, **33**, 710.
- Ohtsuka, S. and Huys, R. (2001) Sexual dimorphism in calanoid copepods: morphology and function. *Hydrobiol.*, **453/454**, 441–466.
- Palmer, C. A. and Edmands, S. (2000) Mate choice in the face of both inbreeding and outbreeding depression in the intertidal copepod *Tigriopus californicus*. *Mar. Biol.*, **136**, 693–698.
- Raisuddin, S., Kwok, K. W. H., Leung, K. M. Y., Schlenk, D. and Lee, J. S. (2007) The copepod *Tigriopus*: a promising marine model organism for ecotoxicology and environmental genomics. *Aquat., Toxicol.*, **83**, 161–173.
- Von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. and Wittzell, H. (1999) Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. London. Ser. B. Biol. Sci.*, **266**, 1–12.
- Seuront, L. (2013) Chemical and hydromechanical components of mate-seeking behaviour in the calanoid copepod *Eurytemora affinis*. *J. Plankton Res.*, **35**, 724–743.
- Sichlau, M. H., Nielsen, E. E., Thygesen, U. H. and Kjørboe, T. (2015) Mating success and sexual selection in a pelagic copepod, *Temora longicornis*: evidence from paternity analyses. *Limnol. Oceanogr.*, **60**, 600–610.
- Smith, J. and Harper, D. (2003) *Animal Signals*, Oxford University Press, Oxford.
- Snell, T. (2010) Contact chemoreception and its role in zooplankton mate recognition. In *Chemical Communication in Crustaceans*, Springer, New York, pp. 451–466.
- Subramoniam, T. (1993) Spermatophores and sperm transfer in marine crustaceans. In *Advances in Marine Biology*, Academic Press, Massachusetts, pp. 129–214.
- The R Core Team (2019) R: A language and environment for statistical computing.
- Ting, J. H., Kelly, L. S. and Snell, T. W. (2000) Identification of sex, age and species-specific proteins on the surface of the harpacticoid copepod *Tigriopus japonicus*. *Mar. Biol.*, **137**, 31–37.
- Ting, J. H. and Snell, T. W. (2003) Purification and sequencing of a mate-recognition protein from the copepod *Tigriopus japonicus*. *Mar. Biol.*, **143**, 1–8.
- Titelman, J., Varpe, Ø., Eliassen, S. and Fiksen, Ø. (2007) Copepod mating: chance or choice? *J. Plankton Res.*, **29**, 1023–1030.
- Tsuboko-Ishii, S. and Burton, R. S. (2017) Sex-specific rejection in mate-guarding pair formation in the intertidal copepod, *Tigriopus californicus*. *PLoS One*, **12**, 1–16.
- Tsuda, A. and Miller, C. B. (1998) Mate-finding behaviour in *Calanus marshallae* frost. *Philos. Trans. R. Soc. B. Biol. Sci.*, **353**, 713–720.
- Viechtbauer, W. (2010) Conducting meta-analyses in R with the metafor package. *J. Stat. Softw.*, **36**, 1–48.
- Waser, N. M. and Price, M. V. (1989) Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. *Evol.*, **43**, 1097–1109.
- Weaver, R. J., Koch, R. E. and Hill, G. E. (2017) What maintains signal honesty in animal colour displays used in mate choice? *Philos. Trans. R. Soc. B. Biol. Sci.*, **372**, 20160343.
- Wickham, H. (2016) ggplot2: elegant graphics for data analysis.
- Yen, J. and Lasley, R. (2010) Chemical communication between copepods: finding the mate in a fluid environment. In *Chemical Communication in Crustaceans*, New York, Springer New York, pp. 177–197.