



REVIEW

# No evidence of predation causing female-biased sex ratios in marine pelagic copepods

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**ABSTRACT:** Although sex ratios close to unity are expected in dioecious species, biased sex ratios are common in nature. It is essential to understand causes of skewed sex ratios *in situ*, as they can lead to mate limitation and have implications for the success of natural populations. Female-skewed sex ratios are commonly observed in copepods *in situ*. Here we discuss the challenges of copepod sex ratio research and provide a critical review of factors determining copepod sex ratios, focusing on 2 main objectives. The first is a critique of the male predation theory, which is currently the main process thought to be responsible for female-skewed sex ratios. It assumes that males have higher mortality because of increased vulnerability to predation during their search for mates. We show that there is little support for the male predation theory, that sex ratios skewed toward females occur in the absence of predation, that sex ratios are not related to predation pressure, and that where sex-skewed predation does occur, it is biased toward females. Our second objective is to suggest alternative hypotheses regarding the determination of sex ratios. We demonstrate that environmental factors, environmental sex determination and sex change have strong effects on copepod sex ratios, and suggest that differential physiological longevity of males and females may be more important in determining sex ratios than previously thought. We suggest that copepod sex ratios are the result of a mixture of factors.

**KEY WORDS:** Sex-specific predation · Sex change · Environmental sex determination · Intersexuality · Longevity · Prey size · Seasonality · Sex ratio · Zooplankton

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## INTRODUCTION

Sex ratios are a fundamental trait of sexually reproducing populations. Consequently, sex ratio is one of the most studied traits of natural populations, and the theory of sex allocation is one of the most successful in evolutionary ecology (Charnov 1982, Hardy 2002, West 2009). In dioecious species, sex ratios are pre-

dicted to be close to unity if the investment in the production of each sex is the same (Charnov 1982). However, skewed sex ratios are often observed in populations with separate sexes (Charnov 1982, Kjørboe 2006, Gusmão & McKinnon 2009, Hirst et al. 2010). Skewed sex ratios can be the result of various factors, such as the presence of sex allocation-distorting elements (the occurrence of selfish genetic elements and

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endosymbionts such as *Wolbachia*, e.g. Bouchon et al. 1998), environmental sex determination and sex change (Stouthamer et al. 2002, West 2009), differential physiological longevity, and mortality of the sexes linked to predation (Vollrath & Parker 1992, Acharya 1995). Understanding the causes of skewed sex ratios is essential, as they have important implications for the success of populations, such as in the case of the low frequency of males limiting mate finding and reproduction in copepods (Kiørboe 2007).

Planktonic copepod populations are often characterised by adult sex ratios strongly skewed toward females, with some copepod families, such as Oithonidae and Paracalanidae, often showing extremely female-skewed sex ratios *in situ* (Kiørboe 2006, Gusmão & McKinnon 2009, Hirst et al. 2010). Uneven sex ratios of adult copepods have been noticed since the early days of zooplankton research (e.g. Sewell 1912), and in fact many species are known only from the female. It is easy to discriminate the sex of adult copepods, and the fourth and fifth copepodite stages (C4 and C5) also show sexual differences in morphology, though the ease of observation of these features differs among families. Unfortunately, it is not possible to discriminate the sex of nauplius or early copepodite stages. As a consequence, the origin of the skewed sex ratios observed in adults has largely escaped the attention of researchers.

Gusmão & McKinnon (2009) reviewed factors determining the sex of copepods. These fall into 2 main categories: genotypic determination and environmental sex determination. In the case of the latter, sex determination late in the life cycle is expected, and intersex individuals may occur. Deviation from a 1:1 'Fisherian' sex ratio occurs when fitness varies because of an environmental factor, or because organisms change sex during development (Charnov 1982, Hardy 2002). The strongly skewed sex ratios observed in natural populations of planktonic copepods are often explained by differences in stage-specific mortality, sex or stage-specific differences in longevity or mortality, and variations in population growth (Kiørboe 2006).

Kiørboe (2006) reviewed the relationship between copepod sex ratio, mortality, and the reproductive structures in some copepods. Diaptoimoidea (Centropagoidea in that publication) require repeated mating because of the absence of a seminal receptacle and are characterised by sex ratios close to unity. Strongly skewed sex ratios are only found in copepods belonging to other superfamilies ('non-Diaptoimoidea'). Kiørboe (2006) concluded that these differences in sex ratio are due to differential mortality as

a result of the increased vulnerability to predation of the males from non-diaptomoid families during their search for mates. Subsequently, Hirst et al. (2010) asked 'Does predation control adult sex ratios and longevities in pelagic copepods?' and concluded that 'adult sex ratio skew in pelagic copepods is primarily due to differential mortality of the sexes [greater predation on males] in the adult stage and not juveniles'. We refer to this as the male skewed predation (MSP) theory.

Here, we provide a critical review of factors determining copepod sex ratios, focusing on 2 main objectives: a critique of the MSP theory and suggestion of alternative hypotheses regarding the determination of copepod sex ratios. This review is organised around 3 major themes. The first sets the scene by discussing the challenges of studying copepod sex ratios, an essential pre-requisite for improving our knowledge in this area and interpreting results. The second major theme is reviewing the evidence of male-biased copepod predation. Here, we show that there is little support for the theory of MSP. By using data available in the literature, and reanalysis of data from Hirst et al. (2010), we highlight that skewed sex ratios toward females occur in the absence of predation, that sex ratios are not related to invertebrate or vertebrate predation pressure, and that where sex-skewed predation does occur, it is biased toward females, not males. Our third theme discusses alternative hypotheses for explaining the widespread skewed sex ratios towards females in copepods. We propose that copepod sex ratios are not controlled mainly by predation, but are the result of a mixture of factors including the differential physiological longevity of males and females (shorter male lifespans), environmental factors such as temperature and food, and environmental sex determination and sex change.

Unless stated otherwise, all references to copepods are to holoplanktonic copepods, predators are defined as pelagic predators that have the ability to capture individual copepods, and sex ratios are reported as the proportion of females.

## OBSTACLES IN COPEPOD SEX RATIO RESEARCH

Sex ratio is probably the most understudied population trait in copepod ecology. A major reason is that copepod ecologists face many challenges when studying sex ratios, most notably the scarcity of *in situ* sex ratio data, the effect of vertical migration (in pelagic species) and emergence from the benthos (in

demersal species) on sex ratios, and, probably most importantly, the difficulty of identifying sexes in immature copepodites.

### Scarcity of *in situ* sex ratio data

Most studies of zooplankton community structure report the species of copepods present, but rarely report sex ratios. In many cases, these data exist in raw form (i.e. in the original count data), but where sex ratio is reported at all, it is only that skewness is present or at best an average sex ratio value is reported. Further, what data do exist is not always appropriate for sex ratio analysis because of poor count statistics or inadequate sampling strategies. The lack of data is a serious obstacle to the study of copepod sex ratios, as it limits our ability to identify patterns and test hypotheses.

### Effects of vertical migration and emergence on sex ratios

Sex ratio estimation in pelagic copepod populations assumes that the sampling strategy efficiently samples the whole population non-selectively. If there are differences in the distribution of sexes and/or the sampling strategy does not adequately sample the whole population, then biased sex ratios observed *in situ* may be due to sampling artefacts. Both vertical migration (in pelagic species) and emergence behaviour (in demersal species, mostly in shallow water) can be responsible for uneven spatiotemporal distribution of sexes and stages. To illustrate, we refer to studies of 3 copepod genera used by Hirst et al. (2010) to support the MSP theory but that are likely to suffer from inadequately sampling: *Pleuromamma*, *Pseudodiaptomus*, and *Pseudocalanus*.

*Pleuromamma* species are strong vertical migrators. Since males and females differ in their vertical migration behaviour (Ferrari & Hayek 1990), estimation of sex ratios of species of this genus is limited by the ability to properly sample the entire population. Ferrari & Hayek (1990) acknowledged that their data for the abundance of males of *Pleuromamma xiphias* may have been compromised for this reason.

The same argument applies to *Pseudodiaptomus* species, which are coastal copepods that show demersal behaviour. Adults of this genus live close to the bottom during the day, and males and females may show different emergence patterns at night (e.g. Jacoby & Greenwood 1989, Kouassi et al. 2001).

Therefore, sex ratios of *Pseudodiaptomus* populations determined *in situ* are likely to be highly variable as a result of the time of the day and the technique used for sampling the population. To demonstrate this point, we calculated sex ratios from a study of *P. colefaxi* in a coastal environment that used various sampling techniques (Jacoby & Greenwood 1989). *P. colefaxi* sex ratios derived from plankton nets were all female biased or not significantly different from unity, while sex ratios determined from emergence traps were closer to unity or male biased (Jacoby & Greenwood 1989; Table 1). Since most studies report the abundance of *Pseudodiaptomus* species from plankton net samples collected during the day (including those of *P. marinus* in Fig. 2 in Hirst et al. 2010), sex ratios from these studies, which are often female skewed, are not likely to be representative of the population.

Finally, the vertical distribution of sexes of pelagic copepod species may also vary seasonally, resulting in similar obstacles to the determination of sex ratio as do vertical migration or emergence. In the Mediterranean Sea, Kouwenberg (1993) observed different sex ratios with depth and that changed seasonally for several copepod species. Populations of *Pseudocalanus* species were observed to have female skewed adult sex ratios *in situ* (e.g. Hirst et al. 2010), but this may originate from the different vertical distribution of males and females in different seasons. For instance, Pertsova & Kosobokova (1996) observed strong variation in sex ratios of *P. minutus* in the water column in the White Sea (Table 2). While *P. minutus* sex ratios were female-biased in summer in the whole water column, sex ratios closer to unity were observed in various strata during winter and spring.

Table 1. *Pseudodiaptomus colefaxi*. Effect of different sampling strategies on *P. colefaxi* sex ratios (proportion of females) estimated using 4 collection techniques in 2 seasons in Moreton Bay (Australia). Data are sex ratios calculated from Table 1 from Jacoby & Greenwood (1989). The 'total' value is the sex ratio calculated summing all individuals of each sex captured by all collection techniques. The difference from unity was tested with a Chi-square test based on counts. Significance levels: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns: not significant

	Summer	Winter
Re-entry trap	0.33***	0.60*
Net emergence trap	0.21***	0.45 <sup>ns</sup>
Rotary emergence trap	0.56 <sup>ns</sup>	0.61**
Plankton net tow (night)	0.73*	0.67 <sup>ns</sup>
Plankton net tow (day)	0.63 <sup>ns</sup>	1.00
Total	0.37***	0.58**

Table 2. *Pseudocalanus minutus*. Seasonal variation in vertical distribution of sex ratios (proportion of females) in the White Sea. *Pseudocalanus* adult sex ratios show significant seasonal and vertical variation. Depth: depth strata from which sex ratios were determined (for the 0–100 stratum, the sex ratio was calculated by integrating all strata). Recalculated from data from Pertsova & Kosobokova (1996)

Depth (m)	Winter (Feb 1977)	Spring (Jun 1992)	Summer (Aug 1976)
0–10	0.56	1.00	1.00
10–25	0.91	0.25	1.00
25–50	0.71	0.50	0.94
50–100	0.71	0.77	0.97
0–100	0.67	0.67	0.97

#### Point in development when sex can be identified

Copepods have determinate development, in which there are a defined number of developmental stages. This is observed in all pelagic copepod species. Determinate development has 2 important consequences for copepods: (1) the last developmental stage is the only stage that is sexually mature and able to reproduce, and (2) when individuals reach adulthood they do not moult. Since moulting is necessary for crustaceans to grow and to change structurally, when copepods become adult their sexual structures cannot change.

Another peculiarity of determinate development in copepods is that sexes can only be morphologically distinguished late in the development. Eggs and nauplii do not show morphological characteristics that allow the identification of sex, and the sex of copepodite stages younger than C3 or C4 cannot be inferred in most species from morphological characteristics. There are currently no alternative techniques to morphology (such as molecular or cytological techniques) that allow the identification of sex in copepod eggs and juveniles. The sex of late copepodite stages (especially C5) can be inferred in some species from morphological similarities to adults.

The point in development when sex can be identified is a crucial factor in sex ratio studies. The earlier in the development the sex is identified the better, as it will allow the researcher to identify the factors affecting the population sex ratios from that point of development on (Kraak & Pen 2002). In copepods, we cannot determine the sex ratio at the point of zygote formation or at fertilization (the primary sex ratio), nor the sex ratio at birth (the secondary sex ratio). We can only determine with certainty the sex ratio at maturity (the tertiary sex ratio). Consequently, any factor that affects the primary or secondary sex ratios

and promotes the occurrence of skewed sex ratios cannot be detected early in the development in copepods. Moreover, the adult (tertiary) sex ratio is the result of processes from fertilization to adulthood. Since we can only determine with confidence the tertiary sex ratios in copepods, we cannot identify with certainty what point in the development the skew in the sex ratio occurs. This problem is largely because sex identification in copepods is made on morphological grounds. Only the development or application of alternative techniques for the identification of sex that do not rely on morphological features (such as molecular techniques) will allow us to determine with certainty at what point in development skews in the sex ratio occur. Such techniques have been applied with success in other animals showing biased sex ratios. For instance, Komdeur et al. (1997) used a molecular technique to identify the sex of Seychelles warbler nestlings, and demonstrated that the skewed production of sexes, not differential mortality of embryos, was the main cause of biased hatching sex ratios in this species. The possibility of applying similar techniques in copepods remains unknown.

#### LITTLE EVIDENCE OF MALE-BIASED PREDATION

According to the MSP theory, copepods show female-biased sex ratios because planktonic predators consume more adult male copepods than females. The theory is based on the assumption that mate searching and courtship behaviour of male copepods increases their predation risk. Since these sex-specific behavioural differences apparently only occur in adult copepods, sex-specific predation in juvenile copepods is extremely unlikely. Here, we show that evidence, both from the predator and prey point of view, does not support the MSP theory. We reanalysed published sex ratio data related to sex-specific predation in copepods, and demonstrate that adult male copepods are not the main prey of plankton predators, that the relationship between *in situ* copepod sex ratios and predation pressure is not consistent with the MSP theory, and that copepods have skewed sex ratios in the absence of predation.

#### Pelagic predators are size-selective, opportunistic, and generalist feeders

For MSP to cause the widespread and extremely female-skewed sex ratios observed in copepod spe-

cies *in situ*, strong sex-skewed predation must be common in planktonic predators. If only a few planktonic predators consume more males than females of some copepod species, then, in theory, the predation pressure upon male copepods would be insufficient to create the heavily female-skewed adult sex ratios observed in so many copepod species. We discuss 2 common behavioural adaptations of planktonic predators—generalist and opportunistic feeding, and size selectivity of prey—that demonstrate planktonic predators show feeding behaviour that do not favour strong sex-skewed predation *in situ*.

#### Generalist and opportunistic feeding

Generalist (or polyphagous) predators consume a variety of prey species, and opportunistic feeders adjust their diet to whatever food item is available in the environment. Since these feeding behaviours are observed in most pelagic predators, the validity of the MSP theory is challenged simply because the more generalist and opportunistic the predators are, the less 'selective' the overall predation pressure will be. It is true that most generalist species in nature can show a degree of selectivity in the form of preferences when choosing what to eat when alternative food items are available (Begon et al. 2005). However, 'choosing' prey items is probably not the case for most planktonic predators. In the patchy planktonic environment, planktonic predators are likely to consume anything of suitable size they encounter (size selectivity is discussed below), increasing their chances of survival. Consequently, if there is male-skewed predation upon some copepod species in the field, this is likely to represent only a fraction of the diet of these predators. Even when these predators are abundant enough in the environment to cause an impact in the prey population abundance, this skewed predation might still be too low to cause a shift in the sex ratio of the prey copepod population. Unfortunately, there is no experimental evidence of this process. However, there are many studies confirming the generalist and opportunistic feeding behaviour of planktonic predators.

Most planktonic predators such as fish larvae, chaetognaths and jellyfish show generalist and opportunistic feeding behaviour in the wild. For instance, though larvae of many fish species feed on copepods, they also feed on phytoplankton, microzooplankton, meroplanktonic larvae, appendicularia, and other fish larvae (Hunter 1981). Chaetognaths are opportunistic predators and their diet reflects the composition of the prey in the field (Feigenbaum & Maris 1984). For ex-

ample, Álvarez-Cadena (1993) observed that the most abundant copepod species in plankton were also recorded in the guts of the chaetognath *Sagitta elegans* in the Irish Sea. The preferred prey of *S. elegans* varies from inshore to offshore populations (Terazaki 2004), indicating that *S. elegans* may adapt its diet to the available prey *in situ*. Reviewing the predation of gelatinous predators on zooplankton, Mills (1995) reported that scyphomedusae and ctenophores are more generalist predators than hydromedusae and siphonophores. Such generalist feeding behaviour has been reported, for instance, for the hydrozoan *Aglantha digitale* in the White Sea (Berger et al. 2003). However, Mills (1995) also noted that hydromedusae and siphonophores 'usually occur in multi-species assemblages that may together have a more general effect' (Mills 1995, p. 579). In the recent review of Sullivan & Kremer (2011) on the feeding ecology of gelatinous zooplankton in coastal environments, copepods are ubiquitous in the gut contents of most ctenophores and cnidarians, but the diet of these 2 phyla are by no means limited to copepods, and encompasses several other crustacean groups, meroplanktonic larvae, gelatinous organisms as well as microzooplankton and phytoplankton (see their Table 5). Interestingly, Sullivan & Kremer (2011) also noted that the same species of gelatinous zooplankton can prey on different prey species in different regions, confirming the opportunistic behaviour of this group.

#### Size selectivity

Another important aspect of planktonic predator-prey relationships is that prey size is the main factor controlling prey selectivity in planktonic predators, even when other factors such as prey behaviour are taken into account (Kjørboe 2008). Considering only copepods as prey, this aspect has 2 implications for the validity of the MSP theory. First, theoretically, even if there are behavioural differences in the swimming patterns of male and female copepods, it is the copepod body size (not its sex) that will be the main factor determining whether an individual is captured or not by a predator. Second, planktonic predators will feed on a range of sizes of copepods *in situ*, encompassing several copepod stages, including adults. Consequently, it is reasonable to expect that adult copepods are only a fraction of the copepod stages consumed by planktonic predators *in situ*. Therefore, the predation pressure on adult male copepods is likely not enough to contribute to the skew in adult copepod sex ratios observed in nature.

There are no studies comparing the effects of prey size, behaviour, and sex on prey selectivity of planktonic predators, but evidence from the literature suggests that adult copepods are frequently only a fraction of the copepod stages consumed by planktonic predators *in situ*.

Evidence from the field and experiments support both the existence of strong size selectivity in nature and that adult copepods may not be the main copepod prey. For instance, size-specific selection of copepod prey has been observed in herring (Munk 1992) and cod (Munk 1997) larvae, which feed on different size ranges of developmental stages of various copepod species depending on the size of the larva itself. Larvae of walleye pollock feed preferentially on copepod nauplii and *Pseudocalanus* copepodites (Kendall et al. 1987). Pearre (1980) showed that there is a strong relationship between prey size and the head width of several chaetognath species, and Saito & Kiørboe (2001) observed that 99% of the copepods found in the gut of the chaetognath *Sagitta elegans* were copepodites. There is also evidence of ontogenetic changes in size selectivity of Hydrozoa, such as in the scyphomedusa *Aurelia aurita*, which shows a shift in prey size selectivity, prey type, and diversity with increasing bell sizes (Sullivan et al. 1994, Graham & Kroutil 2001).

Therefore, size-specific predation, rather than sex-specific predation, is prevalent in planktonic systems, and adult copepods might only be a fraction of the diet of planktonic predators. If adult copepods are not the most common prey, and prey sex is not a determinant in planktonic predation, then MSP is likely to be negligible in most cases. Nevertheless, adult copepods are a ubiquitous part of the diet of most planktonic predators, and to understand any possible effect of predation on copepod sex ratios, we focus our discussion in the following sections on the specific interaction of planktonic predators with adult copepods.

### **Fish larvae consume more female copepod prey**

Fish larvae are the most common vertebrate predators of pelagic copepods. Fish larvae are cruise feeders that swim actively to encounter prey (Kiørboe 2011). Fish larvae detect their prey visually, and the visibility of prey is determined by size, colour, movement, and bioluminescence. Since adult female copepods are almost always larger than the males of the same species, fish larvae (especially large larvae) should theoretically consume more female than male

copepods. To test this, we reviewed the studies reporting sex-selection of adult copepod prey by fish and invertebrate predators (Table 3, some of these studies derived from literature cited in Hirst et al. 2010). Most fish data in Table 3 are derived from gut contents of wild-caught fish larvae, and only a few are derived from laboratory feeding experiments. From the 31 predator–prey relationships found in the literature, 21 showed preference for female copepods, 7 showed no preference for either sex, and only in 3 cases did fish larvae prefer male copepods (all from Ohman 1986). Considering only female or male selectivity (excluding cases in which fish showed no sex preference), female copepod predation is significantly more likely than male copepod predation by fish larvae (binomial test,  $N = 25$ ,  $p < 0.0001$ , Table 4).

Interestingly, for copepod species that carry egg sacs, the preference is often towards egg-carrying females, probably because they are more visible. For instance, Mahjoub et al. (2011) investigated the predator–prey interaction of the sea bass *Dicentrarchus labrax* and the copepod *Eurytemora*, and found that fish consumed ovigerous females over non-ovigerous females, but that there was no difference in predation rate between non-ovigerous females and males. Data from Table 3 also suggests that the sex selectivity of fish larvae is species-specific, so the preference of a predator for females or for either sex of the prey depends on both the predator and the prey species. For example, *Gadus morhua* shows strong preference towards females of *Pseudocalanus* and *Oithona*, but shows no preference for either sex of *Temora* and *Calanus* (Lough et al. 2005, Robert et al. 2011).

The evidence from feeding studies of larval fish does not support the MSP theory. The female skewed sex ratios of field copepod populations cannot be a result of sex-specific predation by fish larvae because, apparently, fish larvae prefer females. In addition, the prey sex selectivity of fish larvae appear to be species-specific, so each predator species will have a different effect on the population of different prey species. Therefore, the overall effect of sex-skewed predation by fish on adult copepod populations would be the removal of more female copepods.

### **Weak evidence for male copepod skewed predation by invertebrate predators**

The most common invertebrate predators of marine planktonic copepods (chaetognaths, ctenophores, and medusae) are ambush predators (Kiørboe 2011). Chaetognaths are active ambush predators that are

Table 3. Sex-specific predation of adult copepods by fish, gelatinous zooplankton, and chaetognaths. Predation on female copepods is significantly more likely than on males (binomial test,  $p < 0.0001$ ). Rows are ordered by predator type. Gut: data from gut content analysis; Exp: experimental data; ?: unconfirmed, unclear, or disputed evidence; M: male; F: female

Predator	Data	Prey	Predation skew			Source
			M	None	F	
<b>Fishes</b>						
<i>Gasterosteus aculeatus</i>	Gut	<i>Acartia hudsonica</i>			X	Landry (1978)
<i>Cymatogaster aggregata</i>	Gut	<i>Acartia hudsonica</i>			X	Landry (1978)
<i>Hypomesus pretiosus</i>	Gut	<i>Acartia hudsonica</i>		X		Landry (1978)
<i>Clupea harengus</i>	Gut	<i>Acartia hudsonica</i>			X	Landry (1978)
	Gut	<i>Eurytemora affinis</i>			X	Viitasalo et al. (2001)
	Gut	<i>Acartia</i> sp.			X	Viitasalo et al. (2001)
	Gut	<i>Temora longicornis</i>			X	Viitasalo et al. (2001)
	Gut	<i>P. elongatus</i>		X		Viitasalo et al. (2001)
	Gut	<i>Eurytemora</i> spp.			X	Sandström (1980)
	Gut	<i>Limnocalanus grimaldi</i> <sup>a</sup>		X		Sandström (1980)
<i>Morone saxatilis</i>	Exp	<i>Eurytemora</i>		X		Meng & Orsi (1991)
<i>Dicentrarchus labrax</i>	Exp	<i>Eurytemora affinis</i>			X	Mahjoub et al. (2011) <sup>a</sup>
<i>Engraulis mordax mordax</i>	?	<i>Acartia californiensis</i>			X	Johnson (1981) <sup>b</sup>
	?	<i>Acartia clausi</i>			X	Johnson (1981) <sup>b</sup>
<i>Hypomesus pretiosus pretiosus</i>	?	<i>Acartia californiensis</i>			X	Johnson (1981) <sup>b</sup>
	?	<i>Acartia clausi</i>			X	Johnson (1981) <sup>b</sup>
<i>Atherinops affinis affinis</i>	?	<i>Acartia californiensis</i>			X	Johnson (1981) <sup>b</sup>
	?	<i>Acartia clausi</i>			X	Johnson (1981) <sup>b</sup>
<i>Gadus morhua</i>	Gut	<i>Pseudocalanus</i> spp.			X	Lough et al. (2005) <sup>a</sup>
<i>Gadus morhua</i> (9–20 mm)	Exp	<i>Pseudocalanus</i> spp.			X	Robert et al. (2011) <sup>a</sup>
	Exp	<i>Oithona similis</i>			X	Robert et al. (2011) <sup>a</sup>
	Exp	<i>Temora longicornis</i>		X		Robert et al. (2011) <sup>a</sup>
	Exp	<i>Calanus finmarchicus</i>		X		Robert et al. (2011) <sup>a</sup>
<i>Gasterosteus aculeatus</i>	Exp	<i>Eurytemora affinis</i>		? <sup>d</sup>	X	Vuorinen et al. (1983)
	Gut	<i>Paracalanus parvus</i>			X	Ohman (1986) <sup>a</sup>
	Gut	<i>Pseudocalanus</i> sp.			X	Ohman (1986) <sup>a</sup>
	Gut	<i>Metridia lucens</i>			X <sup>f</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Calanus</i> spp.	X			Ohman (1986) <sup>a</sup>
	Gut	<i>Euchaeta elongata</i>	X <sup>e</sup>			Ohman (1986) <sup>a</sup>
<i>Oncorhynchus keta</i>	Gut	<i>Metridia lucens</i>			X	Ohman (1986) <sup>a</sup>
	Gut	<i>Calanus</i> spp.			X <sup>f</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Euchaeta elongata</i>	X <sup>e,f</sup>			Ohman (1986) <sup>a</sup>
<b>Coelenterate</b>						
<i>Chrysaora quinquecirrha</i>	Exp	<i>Acartia tonsa</i>		X		Suchman & Sullivan (1998)
<b>Chaetognath</b>						
<i>Sagitta elegans</i>	Gut	<i>Pseudocalanus</i> spp.			X	Ohman (1986) <sup>a</sup>
	Gut	<i>Corycaeus anglicus</i>			X	Ohman (1986) <sup>a</sup>
	Gut	<i>Acartia longiremis</i>			X <sup>e,f</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Paracalanus parvus</i>			X <sup>e</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Oithona similis</i>			X <sup>e</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Oithona spinirostris</i>			X <sup>e</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Metridia lucens</i>			X <sup>e</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Calanus</i> spp.			X <sup>e</sup>	Ohman (1986) <sup>a</sup>
	Gut	<i>Tortanus discaudatus</i>	X <sup>f,g</sup>			Ohman (1986) <sup>a</sup>
	Gut	<i>Acartia clausi</i>		? <sup>g</sup>		Álvarez-Cadena (1993)
	Gut	<i>Paracalanus parvus</i> <sup>c</sup>		? <sup>g</sup>		Saito & Kiørboe (2001)

<sup>a</sup>Information not included in Hirst et al. (2010). <sup>b</sup>Information as reported in Hirst et al. (2010): the citation was Johnson (1990), not in the reference list; the correct reference is probably Johnson (1981) (a thesis to which we did not have access). <sup>c</sup>Wrongly reported as *Acartia clausi* in Hirst et al. (2010). <sup>d</sup>Authors report no preference between males and non-ovigerous females, but a strong preference for ovigerous females. <sup>e</sup>Based on text information and data from Tables III and IV from Ohman (1986). <sup>f</sup>The values reported are either low or occurred only once; the reported *Oncorhynchus keta* selectivity is low and appears to vary with predator size. <sup>g</sup>The evidence for male-skewed predation of *Sagitta elegans* is unclear, see text for details

Table 4. Copepod sex ratios (proportion of females) in the absence of predation. Data are from laboratory cultures and aquaculture of copepods. Food type denotes the main food type used in the experiment and those in parenthesis indicate mixed food treatments containing either a subset or all the food types indicated. Sex ratios are the range reported for each species

Copepod species	Food type	Sex ratio	Source
<i>Acartia tonsa</i>	( <i>Isochrysis galbana</i> , <i>Rhinomonas reticulata</i> )	0.56–0.67	Medina & Barata (2004)
	<i>Rhodomonas salina</i>	0.75	Jepsen et al. (2007)
	<i>Rhodomonas salina</i>	0.54–0.69	Drillet et al. (2008)
<i>Acartia sinjiensis</i>	( <i>Tetraselmis</i> , <i>Isochrysis</i> )	0.65–0.74	Camus & Zeng (2009)
<i>Pseudocalanus newmani</i>	( <i>Pavlova</i> , <i>Heterocapsa</i> , <i>Chaetoceros</i> )	0.42–0.72	Lee et al. (2003) <sup>e</sup>
<i>Bestiolina similis</i>	<i>Isochrysis</i> sp.	0.79	Camus & Zeng (2010)
	<i>Pavlova salina</i>	0.74	Camus & Zeng (2010)
	( <i>Isochrysis</i> , <i>Pavlova</i> , <i>Tetraselmis</i> , <i>Chaetoceros</i> )	0.77–0.86	Camus & Zeng (2010)
<i>Calanus helgolandicus</i>	<i>Chaetoceros curvisetus</i>	1	Paffenhöfer (1970)
	<i>Skeletonerna costatum</i>	0.98	Paffenhöfer (1970)
	<i>Lauderia borealis</i>	0.76–0.95	Paffenhöfer (1970)
	<i>Gyrrnodinium splendens</i>	0.76–0.77	Paffenhöfer (1970)
	( <i>Chaetoceros curvisetus</i> , <i>Gyrrnodinium splendens</i> )	0.98	Paffenhöfer (1970)
	<i>Thalassiosira weissflogii</i>	0.87	Irigoien et al. (2000) <sup>d</sup>
	<i>Isochrysis galbana</i>	0.97	Irigoien et al. (2000) <sup>d</sup>
	<i>Prorocentrum micans</i>	0.96	Irigoien et al. (2000) <sup>d</sup>
	<i>Gladioferens pectinatus</i>	<i>Isochrysis</i>	0.40–0.58
<i>Oithona similis</i>	( <i>Rhodomonas</i> , <i>Heterocapsa</i> , <i>Oxyrrhis</i> )	0.91–0.97	Sabatini & Kiørboe (1994)
<i>Pseudodiaptomus pelagicus</i>	<i>Isochrysis</i>	0.46–0.53	Rhyne et al. (2009) <sup>a</sup>
<i>Pseudodiaptomus marinus</i>	<i>Phaeodactylum</i> , <i>Dicrateria</i>	0.52–0.55	Huang et al. (2006) <sup>a,b</sup>
<i>Centropages typicus</i>	<i>Hymenomonas elongata</i>	0.49	Bonnet & Carlotti (2001)
	<i>Thalassiosira weissflogii</i>	0.35	Bonnet & Carlotti (2001)
	<i>Strombidium sulcatum</i>	0.46	Bonnet & Carlotti (2001)
	( <i>Hymenomonas elongata</i> , <i>Strombidium sulcatum</i> )	0.53	Bonnet & Carlotti (2001)
	( <i>Thalassiosira weissflogii</i> , <i>Strombidium sulcatum</i> )	0.51	Bonnet & Carlotti (2001)
<i>Eurytemora affinis</i>	<i>Rhodomonas</i>	0.25–0.44	Souissi et al. (2010) <sup>c</sup>
	( <i>Isochrysis</i> , <i>Cyclotella</i> , <i>Platymonas</i> , <i>Skeletonema</i> )	0.49–0.79	Katona (1970) <sup>e</sup>
<i>Eurytemora herdmani</i>	( <i>Isochrysis</i> , <i>Cyclotella</i> , <i>Platymonas</i> , <i>Skeletonema</i> )	0.33–0.48	Katona (1970) <sup>e</sup>
	( <i>Skeletonema</i> , <i>Monochrysis</i> , <i>Tetraselmis</i> , <i>Rhodomonas</i> )	0.20–0.55	George (1985)
<i>Temora stylifera</i>	<i>Prorocentrum minimum</i>	0.44–0.46	Carotenuto et al. (2011)
	<i>Thalassiosira rotula</i>	1	Carotenuto et al. (2011) <sup>f</sup>
	( <i>Prorocentrum minimum</i> , <i>Skeletonema marinoi</i> )	1	Carotenuto et al. (2011)
	( <i>Prorocentrum minimum</i> , <i>Thalassiosira rotula</i> )	0.22	Carotenuto et al. (2011)
<i>Calanoides carinatus</i>	( <i>Phaeodactylum</i> , <i>Isochrysis</i> , <i>Prorocentrum</i> , <i>Scrippsiella</i> , <i>Skeletonema</i> , and/or <i>Gyrrnodinium</i> )	1	Hirche (1980)

<sup>a</sup>Authors report sex ratios are not significantly different from equality; <sup>b</sup>sex ratios from experimental controls; <sup>c</sup>intersexes were reported; <sup>d</sup>sex ratios of animals raised from eggs; <sup>e</sup>temperature affected sex ratios; <sup>f</sup>calculated from few individuals

stationary until sensing prey remotely, at which point they attack. Ctenophores and medusae are passive ambush predators in which motile prey collide with the predator. In either case, sex-selective predation in ambush predators depends on the presumption that one sex of the prey organism is more mobile (hence encountering passive ambush predators more often) or more detectable from hydrodynamic disturbances than the other, as has been suggested for the males of non-Diaptomoidea (Kiørboe 2006). Unfortunately, most studies of invertebrate predation on copepods do not report the sex of the prey, only prey species/ group and/or size. To the best of our knowledge, the only

study investigating copepod sex-specific predation in gelatinous zooplankton is the study of Suchman & Sullivan (1998). They showed that *Chrysaora quinquecirrha* consumed both sexes of the copepod *Acartia tonsa* equally. Note that *C. quinquecirrha* is a cruising predator, but its lengthy tentacles can also be used to ambush prey (Costello et al. 1998).

#### Sex-selective predation by chaetognaths

The case for MSP rests on 3 studies of one chaetognath species, *Sagitta elegans* (Ohman 1986, Álvarez-

Cadena 1993, Saito & Kiørboe 2001). We believe that the evidence from these is weak. In these studies, the copepod species for which MSP was apparent were not the main prey items. Ohman (1986) reported only one occurrence of male *Tortanus* in the guts of *S. elegans*. For the other 8 copepod species consumed, including the most common prey species (*Pseudocalanus* spp. and *Oithona similis*), he found female-skewed predation. The study by Álvarez-Cadena (1993) reported skewed predation upon male *Acartia clausi* from an unpublished experiment for which neither the experimental design nor the data are available. In addition, *A. clausi* was not the main prey of *S. elegans*, and there was no reference to sex-specific predation in the other more frequently eaten copepod species. Again, in the study of Saito & Kiørboe (2001), *Paracalanus parvus* (misreported as *A. clausi* in Hirst et al. 2010) was not the most common prey item of *S. elegans*.

Since chaetognaths are opportunistic, active ambush predators, it is necessary to understand the ambient prey field to evaluate the impact of predation upon copepod sex ratios. Saito & Kiørboe (2001) neither report the abundance of each sex nor the sex ratio of *Paracalanus parvus*. Common prey items in the guts of chaetognaths may not be abundant in the associated plankton samples (acknowledged by Saito & Kiørboe 2001), and the differential availability of prey due to vertical migration of both copepod prey and chaetognath predator may also be an important factor. This is a crucial point because patchiness that leads to higher availability of a certain sex would contribute to a local tendency to feed upon that sex.

Results from Saito & Kiørboe (2001) indicate that *Sagitta elegans* consumed both sexes of *Paracalanus parvus*. Of the 16 samples where the gut content of chaetognaths was analysed, females and C5 were found in 15 samples, while males were observed in 11. In 5 of these samples (31% of the total), the clearance of males was not reported but the clearance of females was, leading to the conclusion that the sex-specific predation of *S. elegans* was toward females, not males. It seems that if sex-specific predation really occurs in *S. elegans*, it is variable, and the predation upon one sex or another may shift in time. This observation is in accord with the generally opportunistic feeding behaviour observed in chaetognaths (Feigenbaum & Maris 1984) and also with the possible variability in availability of *P. parvus* males and females in the environment. More importantly, the fact that both males and females were consumed by *S. elegans*, and that only 8.7% of the items in *S. elegans* guts were *P. parvus* (including males, females,

and copepodites), indicates that the overall effect of any MSP by *S. elegans* on the *P. parvus* population sex ratio may be negligible.

Finally, although none of these studies report sex ratios of the copepod prey *in situ*, all of them indicate that *Oithona* was an important prey item of *Sagitta elegans*, in accordance with the conclusions of a worldwide study of this species (Terazaki 2004). *Oithona* species typically show strongly female-skewed sex ratios (Kiørboe 2006) and are, thus, good model species for the detection of MSP by chaetognaths. Despite this, there is no evidence of MSP on *Oithona*, and the only available evidence suggests female *Oithona* are the most common prey. Data from Ohman (1986) suggests that female copepod predation is significantly more likely than male copepod predation by *S. elegans* (binomial test,  $N = 9$ ,  $p < 0.05$ ). We conclude that, despite the elegant theoretical framework (Kiørboe 2011), there is insufficient evidence to support the statement of Hirst et al. (2010) that 'feeding by mechanoreception in *Sagitta* is strongly biased toward male consumption' (Hirst et al. 2010, p. 2203).

#### **Copepod sex ratios are not correlated with predation pressure**

In theory, if the primary sex ratio is 1:1, the higher the sex-specific predation pressure upon this population, the more skewed its sex ratio will be towards the least ingested sex. Since predation pressure increases with predator abundance, assuming that the overall degree of sex selectivity is constant for the predator population, it is reasonable to expect that if predation is skewed towards adult males, the extent of female-biased sex ratios in the prey population ought to be proportional to the abundance of predators.

To investigate the relationship between adult sex ratios and predator abundance, we used the longest and most complete dataset we could find in the literature. This was from the White Sea (Russia), which included counts of both the sex of 8 adult copepods and the abundance of potential plankton predators (*Sagitta elegans* and *Aglantha digitale*) over several seasonal cycles (Berger et al. 2003). While the White Sea dataset contains a vast amount of data for the sex of several copepod species, it has adequate data for only 2 planktonic predators, *S. elegans* and *A. digitale*. Only data from 1965 to 1970 were used in our analysis as the sampling effort in this period was higher and more samples were collected under ice,

giving a better characterization of the zooplankton during winter. The mean adult copepod sex ratios and abundance of predators were calculated for each date using the respective abundances in the 3 depth strata in the dataset. The sex ratio difference from equality was tested using one-sample *t*-tests, and the linear correlation between predator abundance and copepod sex ratio was calculated for all potential predator–prey pairs. To improve homogeneity of variance and normality, copepod sex ratios were arcsin–square root transformed and predator abundance was  $\log(x + 1)$  transformed. After transformation, residuals were improved but still heteroscedastic, so a conservative value of alpha was used ( $p = 0.01$ ) to reduce Type I error (Underwood 1997).

Both *Sagitta elegans* and *Aglantha digitale* showed high abundances during the summer in the White Sea, up to 521 ind.  $m^{-3}$  and 350 ind.  $m^{-3}$ , respectively, and average abundances were 17.3 ind.  $m^{-3}$  and 13.4 ind.  $m^{-3}$ . We believe the overall predator abundance in the White Sea, especially during the summer/spring period, has the potential to impact the copepod standing stock in the region. The average abundance of *S. elegans* in the White Sea was much higher than the average abundance of the same species reported by Saito & Kiørboe (2001) (4 ind.  $m^{-3}$ ) or Álvarez-Cadena (1993) (10 ind.  $m^{-3}$ , calculated from his Table 1). While *S. elegans* is known to impact the standing stock of copepods, reports of *A. digitale* are contradictory. Some studies suggest that *A. digitale* in high abundances can significantly impact copepod standing stock (e.g. Nicholas & Frid 1999, off the northeast coast of England), but not when present at low abundance (e.g. Pagès et al. 1996, in Hardangerfjord, Norway). Maximum abundances of *A. digitale* recorded in the White Sea (350 ind.  $m^{-3}$ ) were much higher than those reported in Hardangerfjord (57.4 ind.  $m^{-3}$ , Pagès et al. 1996) and northeast coast of England (13.9 ind.  $m^{-3}$ , Nicholas & Frid 1999), suggesting that *A. digitale* predation pressure during the summer in the White Sea might have been significant.

Of the 8 copepod species in the White Sea dataset, 4 showed significant female-skewed sex ratios (*Oncaea borealis*, *Acartia longiremis*, *Pseudocalanus minutus*, and *Oithona similis*), 3 showed sex ratios not significantly different from unity (*Temora longicornis*, *Metridia longa*, and *Centropages hamatus*), and only 1 species (*Calanus glacialis*) showed a significant male-skewed sex ratio (Fig. 1). These sex ratio patterns accord with most literature reports for these species with the exception of *Calanus*, which often shows female-skewed sex ratios (Kiørboe

2006). For copepod species with sex ratios not different from equality or skewed towards males (*T. longicornis*, *M. longa*, *C. hamatus*, and *C. glacialis*), the MSP theory is not applicable, as the predation pressure was either towards females or the effect of the sex-specific predation on the population sex ratio was negligible and/or undetectable.

Most of the correlations of the sex ratios of the 8 copepod species with the abundance of *Sagitta* and/or *Aglantha* were not significant and the direction of each predator–prey relationship varied for each predator–prey pair (Fig. 1). There was no significant difference in the sex ratio of adult copepods with the increase of the abundance of *Sagitta* (meta-

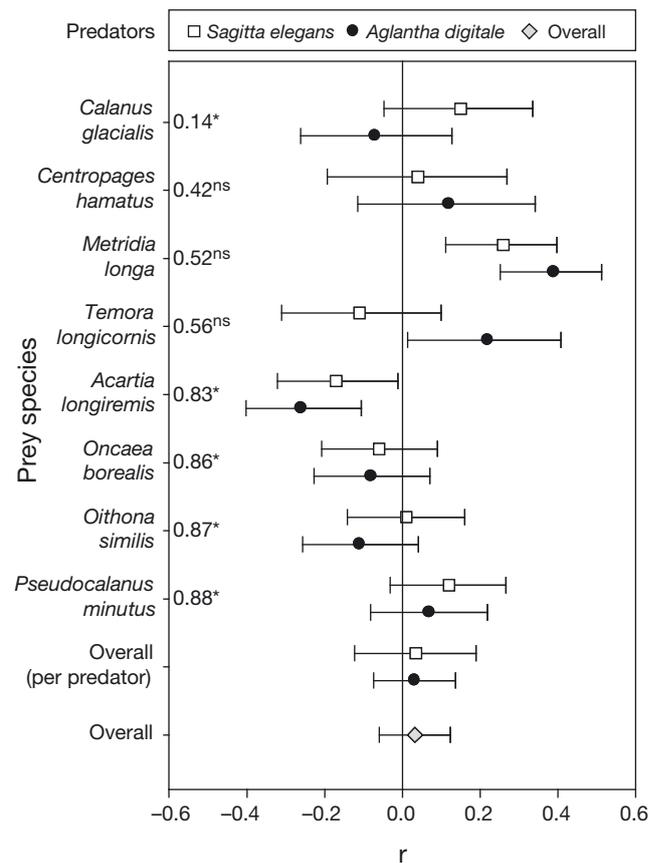


Fig. 1. Linear correlation of adult sex ratio (proportion of females) of copepods and the abundance of 2 potential invertebrate predators in the White Sea from 1965 to 1970. Sex ratios of adult copepods showing female bias are not correlated with the abundance of predators as expected if male-skewed predation (MSP) occurs. Points are correlation values, and whiskers are the confidence interval. Mean adult sex ratios are indicated at the right of each copepod species. \* denotes sex ratios significantly different from equality (*t*-test); ns not significant; r: linear correlation. Data are from Berger et al. (2003), freely available at [www.nodc.noaa.gov/OC5/WH\\_SEA/WWW/HTML/atlas.html](http://www.nodc.noaa.gov/OC5/WH_SEA/WWW/HTML/atlas.html)

analytic  $r = 0.04$ ,  $Z = 0.434$ ,  $p = 0.664$ ), *Aglantha* (meta-analytic  $r = 0.03$ ,  $Z = 0.597$ ,  $p = 0.55$ ), or both invertebrate predators (meta-analytic  $r = 0.03$ ,  $Z = 0.701$ ,  $p = 0.483$ ). If the MSP theory is correct, the relationship between predator abundance and sex ratio (the proportion of females) would be positive and significant. The only significant correlations between sex ratio and predator abundance were observed for *Acartia longiremis* and *Metridia longa*. *A. longiremis* showed a significant negative correlation with *Sagitta elegans* abundance, suggesting female-skewed predation. Correlations between *M. longa* sex ratio and both predators were positive, but sex ratios of *Temora longicornis* were not significantly different from unity.

Of the 4 species showing female-skewed sex ratios in the White Sea, *Oithona*, *Pseudocalanus*, and *Acartia* species are common prey items of *Sagitta elegans*. *Oncaea* spp. are not commonly reported in gut contents of *S. elegans* (Saito & Kiørboe 2001, Terazaki 2004), and when *Oncaea* is observed in the guts of *S. elegans*, it is a minor component of the diet, with no clear skew in prey sex consumption (e.g. Ohman 1986). The fact that the sex ratios of these 4 species were not correlated, or were negatively correlated (in *Acartia*), with *S. elegans* abundance, suggests that sex-specific predation by this chaetognath was not responsible for the female-skewed sex ratios of these species observed *in situ*.

To further assess whether predators cause skewing of the sex ratios, we reanalysed the sex ratio data presented in Fig. 3 of Hirst et al. (2010) together with the abundance of 5 potential plankton predator groups (from the L4 station data repository, [www.westernchannelobservatory.org.uk](http://www.westernchannelobservatory.org.uk)). The analysis was restricted to the same period in Hirst et al. (2010), because they directly estimated sex ratios from additional zooplankton counts. Since both predator and prey abundances vary seasonally, predation pressure should also vary seasonally, so the most robust estimates of sex-specific predation will be across whole seasonal cycles. The White Sea dataset covers several complete seasonal cycles with periods of high and low abundance of predators and prey, whereas the L4 data subset is limited to <1 yr of data and should thus be interpreted with caution.

Relationships between the copepod adult sex ratio and predator abundance from station L4 showed similar features to the White Sea (Fig. 2). Adult sex ratios of *Paracalanus parvus*, *P. elongatus*, and *Acartia clausi* were skewed towards females *in situ*. However, copepod sex ratios were not correlated

with potential predator abundances, and the direction of the sex ratio–predator abundance relationship varied with each predator–prey pair. There was no significant difference in the sex ratio of adult copepods with the increase of the abundance of chaetognaths (meta-analytic  $r = 0.30$ ,  $Z = 1.45$ ,  $p = 0.15$ ), cnidaria ( $r = 0.07$ ,  $Z = 0.33$ ,  $p = 0.74$ ), ctenophora ( $r = 0.02$ ,  $Z = 0.1$ ,  $p = 0.92$ ), euphausiids ( $r = -0.05$ ,  $Z = -0.24$ ,  $p = 0.81$ ), fish larvae ( $r = 0.30$ ,  $Z = 1.45$ ,  $p = 0.15$ ), or all predators together ( $r = 0.06$ ,  $Z = 0.66$ ,  $p = 0.51$ ).

Based on results from both the White Sea and L4 datasets, we conclude that MSP is unlikely to be responsible for female-skewed sex ratios of common copepods in these environments.

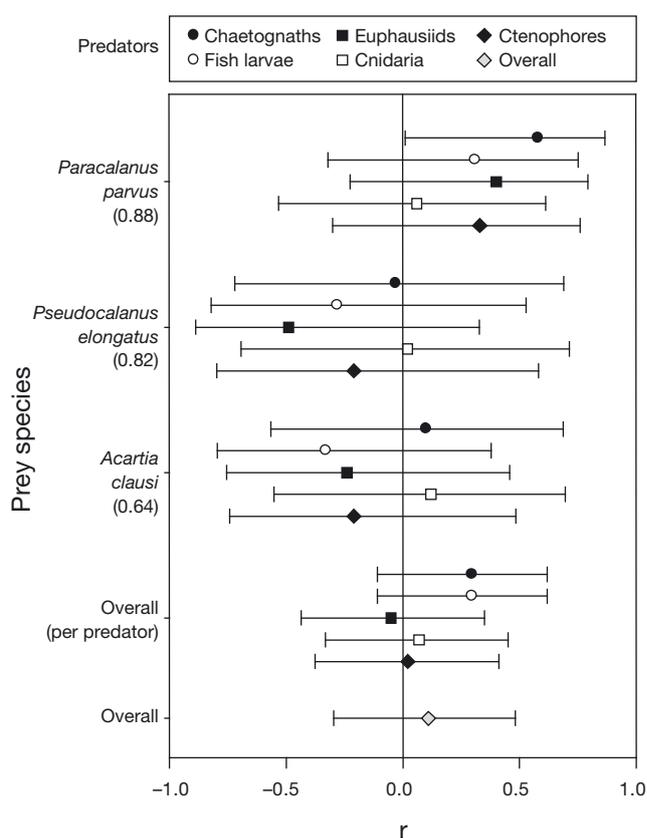


Fig. 2. Linear correlation of the adult sex ratio (proportion of females) of 3 copepod species and the abundance of potential predators *in situ* from the L4 Station (western English Channel). Sex ratios of adult copepods showing female bias are not correlated with the abundance of predators as expected if MSP occurs. Points are correlation values, and whiskers are the confidence interval. Mean adult sex ratios are indicated underneath each copepod species name.  $r$ : linear correlation value. Adult sex ratio data are from Fig. 3 in Hirst et al. (2010), and predator abundance data are from the 2008 L4 dataset, freely available at [www.westernchannelobservatory.org.uk](http://www.westernchannelobservatory.org.uk)

### Skewed sex ratios occur in the absence of predation

We have shown in the preceding section that field observations do not support the MSP theory. However, the 'coup de grâce' to the MSP theory comes from aquaculture and laboratory cultures of copepods, where predation is absent. If the MSP theory is correct, then adult sex ratios of populations kept isolated from predation should be less skewed than those *in situ* and, assuming 1:1 sex ratios at birth, close to unity.

On the contrary, available data indicate that female-skewed sex ratios are common in controlled copepod cultures in the absence of predation (Table 4). Sex ratios of copepod cultures can also be as skewed as those observed for populations *in situ*. Of the 6 copepod families presented in Figs. 2 & 3 of Hirst et al. (2010), species from 4 families (Acartiidae, Paracalanidae, Calanidae, and Pseudocalanidae) showed skewed sex ratios in culture (Table 4). Skewed sex ratios also occur in cultures of species from the families Oithonidae, Centropagidae, and Temoridae (Table 4). Assuming that the population is female-dominated if the average sex ratio (the mean of the range limits) in a study is  $>0.5$ , then copepod sex ratios across all experiments are significantly skewed towards females in the absence of predation (binomial test,  $p = 0.012$ ). If copepod sex ratios become skewed in the absence of predation, then predation must not be the main control of adult copepod sex ratios.

Evidence from field experiments also demonstrates skewed sex ratios in adult copepods can occur in the absence of predators. For instance, Richardson (1998) showed that skewed sex ratios in 2 copepod species in the Southern Benguela cannot be a consequence of differential adult male mortality, either by predation or longevity. Based on data from 24 h moulting ratio experiments on field-collected *Calanus agulhensis* (108 experiments using 3466 C5s) and *Calanoides carinatus* (15 experiments using 465 C5s), he showed sex ratios were already highly skewed toward females as soon as C5s moulted to adulthood. The overall sex ratio (percentage of males) of *Calanus agulhensis* from moulting ratio experiments was 33.0% and that for *Calanoides carinatus* was 27.0%, suggesting that adult male-specific predation is not a key determinant of skewed sex ratios in these species.

Though sex-specific predation is apparently widespread in nature (Boukal et al. 2008), the available evidence indicates that where sex-specific predation pressure is present it is mostly toward adult females,

not males (Table 3). Since female-skewed predation appears to be prevalent, copepod sex ratios should theoretically be skewed towards males *in situ*, which is not the case for most pelagic copepods. Moreover, for a given predator species, sex-specific predation appears to vary according to the prey species. Consequently, the same predator species may have a different effect on the population of different prey species. Since female-skewed sex ratios are observed in many copepod species *in situ*, for MSP to be responsible it must be consistent among predator and prey species. The only reasonable conclusions we can reach based on the evidence shown above are that (1) MSP is unlikely or extremely rare in plankton predators, and that (2) the extent of MSP is variable within and between predator-prey pairs. We do not dispute that MSP could still be a factor contributing to the skew in sex ratios of some copepods, but it is likely to occur only in special situations.

### MULTIPLE FACTORS ARE LIKELY TO DETERMINE COPEPOD SEX RATIOS

If predation is not the main control of copepod sex ratios, what else could be? We believe skewed sex ratios in adult copepods are not the result of one major factor, but a complex interaction of various factors. Skewed sex ratios can be a result of a multitude of factors in nature (Stouthamer et al. 2002, West 2009). Here, we discuss some of the factors for which there is enough data in copepods: the differential stage duration of juveniles, differential adult physiological longevity, the response of sex ratios to environmental effects, and environmental sex determination and sex change processes.

#### Differential stage duration of juveniles and differential adult longevity

The simplest explanation for biased sex ratios is that they originate from the differential stage duration of juveniles and the differential physiological longevity of adult males and females. For instance, adult *Pseudocalanus* males have a natural lifespan one-fourth that of females (Pertsova & Kosobokova 1996) and adult *Oithona davisae* male longevity is half of that of the female (Ceballos & Kiørboe 2011). These findings could explain, at least in part, the commonly observed female-skewed sex ratios of species of these genera *in situ*. Effects of stage duration and longevity on sex ratios have been discussed in detail elsewhere (Hirst et

al. 2010, Ceballos & Kiørboe 2011). Hirst et al. (2010) applied several corrections to the observed sex ratios to account for effects of differential stage duration and differential adult longevity. However, any correction of sex ratios for differential longevity is compromised by the paucity of physiological longevity data (which is especially true for male copepods, e.g. Fig. 5 in Hirst et al. 2010), the variability of available estimates, and the highly complex processes that influence longevity (e.g. Ceballos & Kiørboe 2011). This might have contributed to the conclusion by Hirst et al. (2010) that predation is the main factor controlling adult copepod sex ratios.

Little is known about the physiological longevity of copepods, but a recent study demonstrated that the controls of sex-specific adult longevities can be complex. Ceballos & Kiørboe (2011) demonstrated that the physiological longevity of virgin adult *Oithona davisae* is significantly longer than in mated individuals, and that this effect is much more pronounced in males than females. If Ceballos & Kiørboe's (2011) observations for *Oithona* are applicable to other copepods in varying degrees, then the intertwined relationship of physiological longevity and mating might be a major factor promoting female-skewed sex ratios in pelagic copepods. Moreover, as is the case for other physiological processes, sex differences in adult physiological longevity in copepods are likely to vary with environmental factors such as temperature and trophic conditions. If this is true, longevity differences between males and females will also vary seasonally in the field, making it difficult to evaluate the true extent of the effect of differential physiological longevity on copepod population sex ratios. In conclusion, the effect of physiological longevity on copepod sex ratios may be underestimated due to the scarcity of data available. This problem can only be resolved with direct estimations of physiological longevity of both males and females of various copepod genera in the laboratory.

### Environmental effects on copepod sex ratios

Copepod sex ratios are affected by several environmental factors, independent of the effects of predation. Two factors appear to prevail: temperature and food (Gusmão & McKinnon 2009).

Experimental evidence indicates that temperature affects sex ratios of copepods in the absence of differential mortality. Lee et al. (2003) demonstrated in laboratory experiments that the sex ratio of food-satiated and predator-free *Pseudocalanus newmani*

populations shifts towards females at warmer temperatures. Since all animals had the same parentage, skewed sex ratios could only arise as a result of (1) temperature influencing the production of males and females at birth, or (2) temperature affecting the final sex determination during development. Either way, *P. newmani* skewed sex ratios were not affected by predation on adult stages or differential mortality of the sexes. There is also evidence that the effect of temperature on copepod sex ratios might be species specific. Katona (1970) observed a different effect of temperature on the sex ratio of *Eurytemora affinis* and *E. herdmani* raised from eggs in the laboratory (Fig. 3). Both species showed sex ratios close to unity at warm temperatures, but at cooler temperatures *E. affinis* sex ratios were female-skewed, while *E. herdmani* sex ratios were more male-skewed. Considering the different thermal tolerances of the species, Katona (1970) suggested that more females are produced under thermal stress, and more males are produced during favourable conditions to maximize reproduction.

Food quantity and quality can also affect copepod sex ratios. Paffenhöfer (1970) and Irigoien et al. (2000) observed more males of *Calanus helgolandicus* in high-food than in low-food concentration treatments. Similarly, Gusmão & McKinnon (2009) observed only females and intersexes in a cohort of

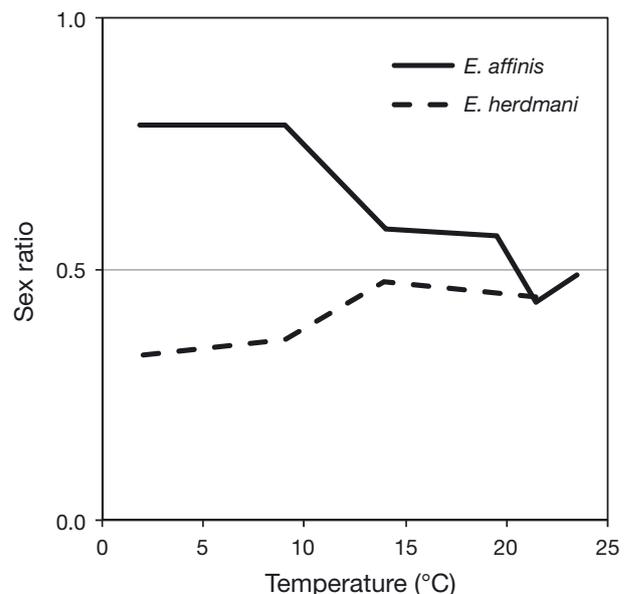


Fig. 3. *Eurytemora affinis* and *Eurytemora herdmani*. The effect of temperature on the sex ratio (proportion of females) of *E. affinis* and *E. herdmani* reared from eggs under satiating food conditions, at salinity 20 and various temperatures. Sex ratios were calculated based on the sum of all individuals of each sex raised at each temperature. Recalculated and redrawn from Fig. 4 in Katona (1970)

*Acrocalanus gracilis* raised from eggs under limited food availability. *Temora stylifera* (Carotenuto et al. 2011), *Centropages typicus* (Bonnet & Carlotti 2001), and *Calanus helgolandicus* (Paffenhöfer 1970, Irigoien et al. 2000) reared with different microalgae species as food in the laboratory show remarkable variation in sex ratios (Table 4). Also, there is indication that the effect of food on adult sex ratios may also be species-specific. For instance, Bonnet & Carlotti (2001) observed male-skewed sex ratios in *Centropages typicus* reared with the diatom *Thalassiosira weissflogii* as opposed to more equal sex ratios in animals reared with other microalgae, and Katona (1970) observed a more male-biased sex ratio in *Eurytemora herdmani* and a more female-biased sex ratio in *E. affinis* when both species were fed the same microalgae (Table 4).

The evidence above demonstrates that environmental factors can affect copepod sex ratios in experimental conditions, but it is difficult to determine the true extent of similar effects on copepod populations in the field. One of the few field observations showing that ambient food availability can skew sex ratios at the onset of adulthood is by Richardson (1998). Using data from 24 h moulting ratio experiments on *Calanus agulhensis* collected in the field in the Southern Benguela, he found sex ratios were related to ambient chlorophyll *a* (chl *a*) concentration. A significantly lower proportion of individuals moulted to males in areas of poor food (29.9%,  $\leq 2 \text{ mg m}^{-3}$  chl *a*) than under good food (38.3%,  $> 2 \text{ mg m}^{-3}$  chl *a*) conditions. There was no observed effect on sex ratio of temperature or density of males, females, or the population as a whole, suggesting that more females are produced under poor food conditions.

Long-term monitoring data sets can also shed light on the interaction of environmental factors and copepod sex ratios *in situ*. If copepod sex ratios are controlled by temperature and food, then sex ratios will vary seasonally. Evidence from literature supports this conclusion. For instance, Irigoien et al. (2000) analysed zooplankton time series and observed that the highest proportion of males in different populations of *Calanus* is during the spring bloom and before the peak in female abundance. Kouwenberg (1993) observed significant seasonal changes in sex ratios of *Clausocalanus*, *Paracalanus parvus*, *Calanus helgolandicus*, *Acartia clausi*, *Temora stylifera*, *Centropages typicus*, *Pleuromamma gracilis*, *Candacia armata*, and *Euchaeta* spp. in the Mediterranean Sea. Hopkins (1982) observed remarkable variation of sex ratios of C4 and C5 and adults of *Euchaeta norvegica*

in Scotland during one seasonal cycle. Our analysis of copepod sex ratios from the White Sea also demonstrated a remarkable seasonal variation in sex ratios (Fig. 4). More males were observed during the spring bloom in the populations of all copepods that showed skewed sex ratios; the effect is clear in *Oithona similis*, *Pseudocalanus minutus*, *Acartia longiremis*, and *Calanus glacialis*, and less evident in *Oncaea borealis*. The spring bloom in the White Sea begins in late March/early April, peaking around May, beginning to decline in June, and phytoplankton growth terminates in October (Filatov et al. 2005).

Seasonality in copepod sex ratios demonstrates that the controls of copepod sex ratios *in situ* are not constant. In addition, the existence of a relationship between copepod sex ratios and environmental variables compromises the MSP theory as the main determinant of female-skewed copepod sex ratios *in situ*. However, more importantly, this relationship between environmental variables and copepod sex ratios implies the existence of much more significant underlying processes controlling copepod sex ratios than sex-specific predation: environmental sex determination and sex change.

#### **Environmental sex determination and sex change can skew copepod sex ratios**

The sex-determination process in copepods is poorly understood, but there is growing evidence of the importance of environmental factors. Environmental factors can cause some genotypic male copepods to switch sex during their development and become females when adults (Fleminger 1985, Gusmão & McKinnon 2009). Therefore, the adult phenotypic sex of a genotypic male copepod can be a result of the environment experienced during development. The lability of phenotypic sex in genotypic males (but not in genotypic females) is better understood in malacostracans practising sequential hermaphroditism. In malacostracans, sex change occurs in sexually active animals, and is linked with individual size — genotypic males first mature as males and then change sex at a larger size. Whereas body size triggers sex change in male malacostracans, environmental factors such as food and temperature appear to trigger sex change in male copepods (Fleminger 1985, Gusmão & McKinnon 2009).

Environmental sex determination (ESD) and sex change have been observed in copepods, and the process described in some species (reviewed in Gusmão & McKinnon 2009). An expected theoretical out-

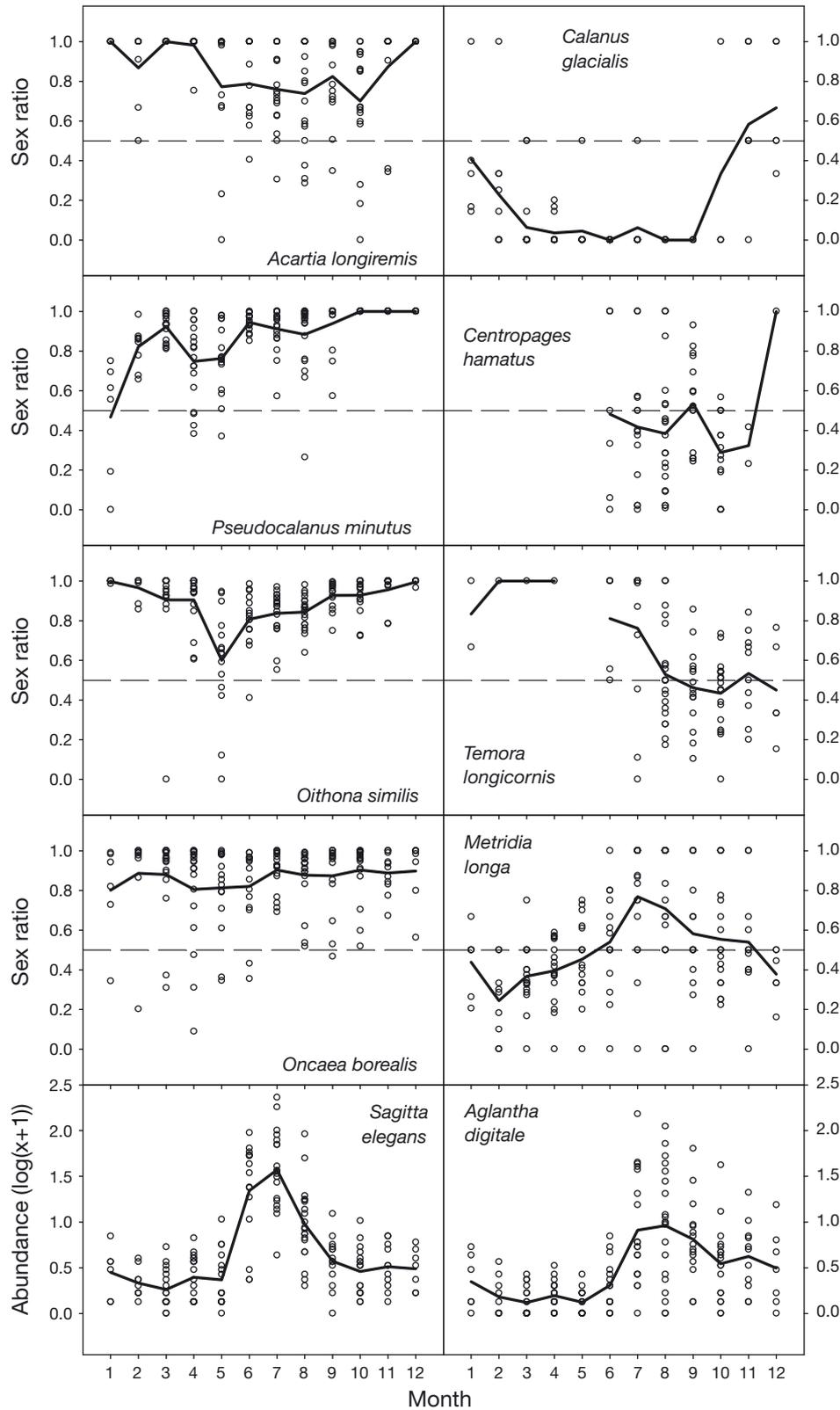


Fig. 4. Seasonal variation of mean sex ratios (proportion of females) of various species of adult copepods and abundance (ind.  $m^{-3}$ ) of 2 potential planktonic predators in the White Sea. The black line connects the mean values in each month; the dashed line is the 1:1 sex ratio. Data are from Berger et al. (2003), freely available at [www.nodc.noaa.gov/OC5/WH\\_SEA/WWW/HTML/atlas.html](http://www.nodc.noaa.gov/OC5/WH_SEA/WWW/HTML/atlas.html)

come of species with ESD and sex change is that they can show very biased sex ratios (Charnov 1982, Charnov & Bull 1989). Therefore, female-biased sex ratios in several copepod species may be a result of the influence the environment has on the sex determination of copepods. If environmental factors can affect the final sex determination of male copepods during development, then individuals hatching and developing under different environmental conditions will have different probabilities of being female when reaching adulthood. This process is more easily visualized if we think in terms of the development of cohorts; as each cohort of individuals experiences a different environment during development, adult sex ratios will be different for each cohort. This process has been observed in laboratory experiments on copepod cohorts, with temperature influencing adult sex ratios of *Pseudocalanus* (Lee et al. 2003), and food quantity influencing adult sex ratios of *Calanus* (Paffenhöfer 1970) and food quality in *Eurytemora affinis* (Souissi et al. 2010).

A consequence of the process of sex change in animals is the risk of the appearance of intersex individuals. Theory predicts that the risk of occurrence of intersexes is higher when sex change occurs late in development (Bull 1985). However, while the occurrence of intersexes is a strong indicator of sex change, the absence of intersexes does not mean that sex change did not occur. Consequently, considering intersexes are rarely reported, the assumption that the frequency of intersex copepods represents the proportion of the population that changed sex will inevitably lead to the incorrect conclusion that sex change is negligible.

Intersexes have been reported *in situ* in most of the genera listed in Fig. 2 of Hirst et al. (2010). While there are several reports of intersexes of *Paracalanus*, *Acartia*, and *Calanus* species (see Gusmão & McKinnon 2009), there are no reports for *Pseudodiaptomus* and *Pleurommama* species *in situ*. However, Huang et al. (2006) observed both skewed sex ratios and intersexes of *Pseudodiaptomus marinus* in laboratory experiments. Further, Ferrari & Hayek (1990) reported the occurrence of one individual of *Pleurommama xiphias* with 'reduced leg 5, partially fused urosome 1 and 2, and a protruding boss-like structure on urosome 2'; these characteristics are consistent with those of an intersex copepod.

Although many groups have intersexes, there are several reasons for the relatively low frequency of these being reported. First, they are generally difficult to identify. While their identification is relatively easy in some genera (for instance, *Acrocalanus*

females do not have fifth legs but C4 and C5 intersexes do), in most species the morphological identification of abnormalities is more difficult. Thus, researchers either unknowingly ignore these individuals, report only that intersexes were present in the samples, or count intersexes in a small fraction of their samples (e.g. Liang & Uye 1996). Although Hirst et al. (2010) argue that intersexes were not observed in their field samples, this might be because the set of diagnostics for developmental stages they used (Conway 2006) is largely based on morphometric characters that are not sufficiently detailed to discriminate intersexes.

Second, intersexes are often reported simply as abnormal individuals, not intersexes, for many copepod genera (see Table 4 in Gusmão & McKinnon 2009). However, the widespread occurrence of these abnormal individuals (intersexes) in several copepods suggests that labile sex determination is frequent in copepods.

Third, there would theoretically be a low probability of occurrence of intersexes if sex change occurs early in development. For example, Miller et al. (2005) studied the occurrence of quadrithek antennules (a male characteristic) in female *Calanus*. They concluded intersex characters in *Calanus* are most likely evidence of switching after first initiation of male development in C5 and that many sex-changed individuals may mature as females and not show any intersex characteristics. Results from mesocosm experiments suggest that *Calanus* C3 to C4 can change sex (Irigoien et al. 2000). In a comprehensive review of sequential hermaphrodites, sex change is expected at ~72% of the individual's maximum size (Allsop & West 2003). If the controls of sex change in copepods are similar to sequential hermaphrodites, then, similarly to *Calanus*, most pelagic calanoid copepods would change sex around C4, which is roughly 72% of the size of an adult. If this is the case, sex change at the C4 stage would generate very few intersex copepods, as secondary sexual features are underdeveloped at this stage (Mauchline 1998). There is also evidence that the final sex determination can occur very early in the development of copepods. For instance, in harpacticoids, intersexes are observed, but often at an even lower frequency than in calanoids. In *Tigriopus japonicus*, a species in which intersexes have been reported but are extremely rare *in situ* (Ito 1970), C1 is a crucial point in the final sex determination (Takeda 1950). Individuals showing intersexual characteristics are also extremely rare, but have been observed in pelagic poecilostome copepods (R. Böttger-Schnack pers. comm.).

The frequency of intersexes in some copepod species might also be a result of both life history and the environment. For instance, in tropical Paracalanidae, chances of observing an intersex may be higher than in other groups because the development time of these individuals is very fast and tropical species are often food-limited. Therefore, in food-poor environments where food patches are ephemeral, changing sex late in the life cycle increases the chances of genetic males choosing the most appropriate sex for the conditions they will experience. It may be for this reason that the majority of reports of intersexes in paracalanids are from tropical waters. By contrast, larger copepod species inhabiting strongly seasonal environments have slower development due to cooler temperatures, and due to their comparatively large body size, juveniles of these animals experience a more prolonged exposure to the environment than tropical species. Consequently, these animals would be able to 'decide' whether they should change sex or not earlier in development. Since sex change may occur earlier in life, intersex morphological characters may not be evident, or even not be formed at all—sex-switched males would be perfect females.

The main consequence of not determining the frequency of sex change in copepod populations and/or ignoring or misidentifying intersexes is the assignment of sex-changed animals to one sex or another, which can drastically change the sex ratios of natural populations (see Gusmão & McKinnon 2009). Since only male copepods can change sex, sex ratios of populations undergoing sex change will tend to be skewed towards females. To demonstrate this, we use studies by Crain & Miller (2000) and Miller et al. (2005) on *Calanus finmarchicus* and Liang & Uye (1996) on *Paracalanus* as examples. Studies of Crain & Miller (2000) and Liang & Uye (1996) were used in Hirst et al. (2010) to support the MSP theory. The analysis by Hirst et al. (2010) of Crain & Miller (2000) data showed that sex ratios of C5 *C. finmarchicus* were not significantly different from unity, but adult sex ratios were significantly biased towards females. Although intersexes are not reported in Crain & Miller (2000), the same authors later published one of the most comprehensive studies of intersexuality on the same population of *C. finmarchicus* (Miller et al. 2005). Miller et al. (2005) demonstrated that sex determination in *C. finmarchicus* was labile, that sex change was an important factor controlling adult sex ratios *in situ*, and that adult sex ratios can be biased even if copepodite sex ratios are close to unity. Further, Miller et al. (2005) found evidence that intersexes may be rare, even if sex change is frequent in

the population. The fact that no intersexes were reported in Crain & Miller (2000) suggests that sex-changed animals were counted as females in that study. Similarly, Hirst et al. (2010) analysed sex ratios from Liang & Uye (1996) study of *Paracalanus*, and showed that while adult sex ratios were biased, copepodite sex ratios were, on average, relatively even. Liang & Uye's (1996) study was the only study explicitly reporting intersexes that was used by Hirst et al. (2010). Liang & Uye (1996) analysed intersexes in only 14 of 80 samples, and in 12 of these 2.5 to 23% of adult females were found to have intersex characteristics, providing strong evidence of frequent sex change in that population. Since intersexes were not quantified in all samples, intersexes were thus reported as females in their study.

Studies of Liang & Uye (1996), Crain & Miller (2000), and Miller et al. (2005) are examples that, when sex change is frequent in the population, adult copepod sex ratios can be biased towards females, even when copepodite sex ratios are not biased. Therefore, the conclusion by Hirst et al. (2010) that assigning sex-changed animals from one sex to another would 'very slightly affect' their results (Hirst et al. 2010, p. 2198) is incorrect. More importantly, these studies, and the other evidence shown in this section, suggest that sex change could also contribute significantly to the female-skewed sex ratios *in situ*.

## CONCLUSION: NO SILVER BULLET

In this review, we have demonstrated that there is little evidence to support the hypothesis that predation on male copepods can explain the widespread female-skewed sex ratios observed in marine pelagic copepod populations. However, we have also shown that controls of sex ratios in copepod populations are far from straightforward, and many factors can contribute to skewing sex ratios in natural populations. Consequently, we are led to the same conclusion that Moore & Sander (1983) reached 30 years ago, that 'it is therefore obvious that single explanations of sex ratio variations [in copepods] are not realistic' (Moore & Sander 1983, p. 120). In a group such as the copepods, where species show extremely diverse life strategies, it is unrealistic to assume that a single process will control sex ratios in all species.

While we have not found evidence of MSP controlling adult copepod sex ratios in marine pelagic copepods, the existence of a relationship between copepod sex ratios and predation may still exist in other environments or in special conditions. Most of the

reports of MSP in copepods *in situ* are for benthic harpacticoids and freshwater copepods. However, even in these cases, the extent of the effect of MSP on copepod sex ratios is unclear. For instance, Boix et al. (2006) observed that the anacostracan *Triops cancriformis* more frequently ingests males of the freshwater cyclopoid copepod *Megacyclops viridis*, but not on the co-occurring cyclopoid *Cyclops* sp. However, when predation on *M. viridis* males was high, sex ratios were even in the copepod population. Another example is the sex-specific predation of *Chaoborus americanus* larvae on freshwater diaptomidae copepods (Blais & Maly 1993). Male predation in this case was species-specific: when offered males and females of *Diaptomus minutus* and *Diaptomus leptopus*, *C. americanus* consumed more males than females of *D. leptopus* and showed no preference for either sex of *D. minutus*. In the marine environment, Hicks & Marshall (1985) reported the occurrence of male-specific predation on deep-sea harpacticoid copepods by 2 species of bivalves, but the evidence in their study is weak, as they reported as many unidentified individuals as males in the guts of the bivalve predators. More studies are necessary to determine the true extent of male-specific predation and its relationship with copepod sex ratios in benthic and freshwater environments.

To make progress in the study of sex ratios beyond our current thinking, fundamental questions about the biology of copepods have to be answered.

- How is sex determined in copepods?
- How variable are the possible modes of sex determination among copepod families?
- Is there endocrine control of secondary sexual characteristics in copepods? If so, can the endocrine system be disrupted by the environment and or pollution?
- How can sex be identified in copepod eggs (the primary sex ratio), nauplii and early juveniles?
- To what extent is sex ratio a heritable trait in copepods (e.g. Voordouw & Anholt 2002)?
- Do sex-allocation-distorting elements such as endosymbionts *Cardinium* and *Wolbachia* (e.g. Bouchon et al. 1998) occur in copepods? For instance, *Cardinium* has recently been observed in *Nitocra spinipes*, which could be linked with biased sex ratios in this copepod (Edlund et al. 2012)
- What are, and how universal are, controls of physiological longevity in copepods (e.g. Ceballos & Kjørboe 2011)?
- How do genetics and physiological processes interact with environmental factors such as food and temperature in determining sex ratios in copepods?

These basic questions will require innovative approaches such as molecular techniques, and renewed interest in the study of copepod sex. While field data will always be important, many of these questions can only be answered through experimental work. Some questions will require the combination of field and experimental work (e.g. Irigoien et al. 2000) for providing an integrated understanding of the mechanisms observed in the lab and their applicability in the field.

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