

REVIEW

Sex ratios, intersexuality and sex change in copepods

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*Female-biased adult sex ratios are common in copepod species, and have been observed both in wild populations and in cultures. Biased sex ratios are most commonly explained by sex- or stage-specific differences in longevity or mortality. However, neither differential longevity nor differential mortality fully explains skewed sex ratios in planktonic copepod populations. We propose that sex change is an important mechanism determining the adult sex ratio. Though sex change has been proposed for only a few copepod species, intersexuality is widespread. We review the occurrence and causation of intersexuality in planktonic copepods, which is a manifestation of late sex change during development. By way of example, we demonstrate that skewed sex ratios of *Acrocalanus gracilis*, a common tropical paracalanid copepod, could be explained by sex change alone. Our observations suggest that sex determination in copepods is under strong environmental control, and we argue that the food limitation is an important determinant of sex change.*

INTRODUCTION

Studies of copepod community composition frequently use counting protocols that include discrimination of the sexes of adult copepods, and sometimes even the sexes of late copepodite stages. Most of these studies report the occurrence of sex ratios that depart from the expected 1:1 ratio. Moreover, in conducting experimental studies involving cultures of copepods, female copepods often predominate in mature cultures. In both of these cases, the reasons for these observations have attracted surprisingly little attention. In fact, one of the least understood features of copepod development is how an individual's sex is determined (Mauchline, 1998). This weakness in copepod biology needs to be addressed because the mode of sex determination is directly related to the population size and sex ratio

(Voordouw and Anholt, 2002a), and therefore has important implications for the reproductive success of natural populations and for productivity studies.

In this work, we address this issue by focusing on one of the most elusive characteristics of copepod biology: intersexuality. By reviewing the major factors relating to the occurrence of intersexes in copepods, we seek new insights about the factors determining sex change, focusing particularly on the calanoids. We illustrate the potential effects of sex change on the sex ratios of copepods using an experiment where intersexes and skewed sex ratios were observed for a species of the family Paracalanidae from northern Australian waters. We argue that the reason for the observed skewed sex ratios is that there is a strong environmental control of sex determination in copepods, and that food is at least one

component of this. A common manifestation of this process can be seen in the presence of intersexes. We believe our results are generally applicable, and that our observations have broad implications for plankton studies.

SEX DETERMINATION PROCESS AND INTERSEXUALITY

Several sex determining mechanisms can be found in nature (Charnov and Bull, 1977). In dioecious species, two main modes can be observed: genotypic and environmental sex determination (GSD and ESD, respectively) (Bull, 1983). In GSD, the determination of the sex of an individual is mainly by its genotype, whereas in ESD the sex is not necessarily determined at conception, but in response to environmental factors (Bull, 1983; Korpelainen, 1990). However, these two mechanisms are not exclusive: environmental factors can affect GSD, and ESD is ultimately under genetic control (Bull, 1983; Korpelainen, 1990). Charnov and Bull (Charnov and Bull, 1977) proposed that selection for ESD is expected under the following conditions: (i) the offspring enters a patchy environment which has a large effect on its fitness; (ii) the influence of environmental conditions on male and female fitness is different; (iii) the offspring and parents have little control over which environment the offspring will experience; and (iv) mating occurs among individuals from all patches. Under these conditions, sex determination late in the life cycle is expected (Charnov and Bull, 1977; Bull, 1983), and an important consequence is the increased risk of production of intersexes (Bull, 1985). Both ESD and intersexuality have been observed in several taxa (Korpelainen, 1990).

An essential property of the sex determining mechanism is the population sex ratio (Bull, 1985). In species with sexual reproduction, each sex contributes with half the genes to the offspring, and provided that the cost of producing male and female offspring is the same, an equal number of each are produced (the 1:1 “Fisherian sex ratio”). If a deviation happens in the sex ratio, frequency-dependent selection should work to restore it to equality (Fisher, 1930). Nevertheless, skewed sex ratios are common in nature (for instance, Kiørboe, 2006). These biased sex ratios happen because of the violation of any of the several assumptions necessary for the Fisherian sex ratios to hold (Bull and Charnov, 1988). One of the situations in which Fisherian sex ratios do not hold is when fitness varies due to an environmental factor (Bull and Charnov, 1988). This situation can be observed in species showing ESD, and

consequently sex ratios are expected to be biased in these species (Charnov, 1982). In addition, biased sex ratios have also been linked to the process of sex change (Bull and Charnov, 1988), which is an adaptation that allows organisms to optimize their reproductive output (Charnov, 1982). Many species undergo sex change (named “sequential hermaphrodites”) (Policansky, 1982) or ESD in nature (Korpelainen, 1990), and therefore these factors can have important implications at the population level.

BIASED SEX RATIOS OF COPEPODS IN THE FIELD

Many species of copepods have female-biased adult sex ratios (Kiørboe, 2006). For instance, adult paracalanids in nature have a mean sex ratio (males/females) of ~ 0.2 , with values ranging from 0.1 to 0.45 (Kiørboe, 2006; based on data from Hirst and Kiørboe, 2002; mostly for *Paracalanus*). Sex ratios reported for paracalanids around Australia are in most cases strongly skewed toward females (Table I). What mechanisms account for such radical deviations from unity?

In the field, typical female skewed (and highly variable) sex ratios are often explained by differences in sex and/or stage-specific longevity and mortality, and variations in population growth (see Kiørboe, 2006). However, differential longevity and mortality are difficult to estimate, and they do not always explain differences in sex ratios in the population. In an extensive review, Korpelainen (Korpelainen, 1990) observed that the environmental sex determination can be even more important than differential mortality of the sexes on the determination of animal sex ratios. There is evidence suggesting that the variation of sex ratios in copepods may not be linked to mortality. Irigoien *et al.* (Irigoien *et al.*, 2000), for example, raised wild caught *Calanus helgolandicus* copepodites to adults in the lab under different food concentrations, and observed final sex ratios that could not be explained by mortality. Lee *et al.* (Lee *et al.*, 2003) suggested that the change in the sex ratio of food satiated *Pseudocalanus newmani* with temperature could be due to sex change, as there was no evidence of sex-specific differences in mortality rates. As is the case in the field, female-skewed sex ratios are commonly observed in copepod cultures (see Peterson, 1986; Irigoien *et al.*, 2000), and these are also often explained as resulting from the higher mortality of juvenile males despite the fact that sex-specific mortality and the occurrence of intersexes are rarely investigated in these experiments.

Table I: Sex ratios (males/females counts) and percentage of males of paracalanids from tropical Australia

Species	Location					
	Great Barrier Reef ^a		Darwin Harbour ^b		North West Cape ^c	
	♂/♀	%♂	♂/♀	%♂	♂/♀	%♂
<i>Acrocalanus</i> sp.	0/40	0*** ^(F)	3/376	0.8**** ^(F)	18/47	27.7 ^{ns}
<i>Paracalanus indicus</i>	20/167	10.7**	45/64	41.3 ^{ns}	112/835	11.8***
<i>Paracalanus aculeatus</i>	3/33	8.3* ^(F)			34/487	6.5****
<i>Paracalanus nanus</i>					11/77	12.5*
<i>Parvocalanus</i> sp.	81/673	10.7***	439/2770	13.7**	91/718	11.2***
<i>Delius</i> sp.	0/5	0			8/282	2.8****
<i>Bestiolina similis</i>	0/19	0	39/385	9.2***	33/244	11.9**

The data are previously unpublished from the studies indicated. Intersexual individuals were not distinguished from normal females, and only adults were considered in the calculation. The hypothesis of equality of sex ratios to *A. gracilis* in the Timor Sea (19♂/53♀ = 26.4%♂) was assessed with a χ^2 test or Fisher's exact test (indicated with ^(F)). Levels of significance are: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, not significant. References: ^aMcKinnon *et al.* (2005); ^bDuggan *et al.* (2008); ^cMcKinnon and Duggan (2001).

We do not dispute that differential longevity and mortality can affect adult sex ratios. However, we do suggest that sex change during development is possibly an important mechanism in determining the sex ratio in copepods. It seems plausible that the frequently observed female-skewed sex ratios both in incubations and in the field are the result of a proportion of males changing sex during development. In fact, biased sex ratios are theoretically expected and frequently observed in animals showing ESD (Charnov, 1982), and ESD has been considered a potential factor in the determination of sex ratios in copepods (Kiørboe, 2006). Therefore, intersexes have the potential to have a strong effect on the sex ratios of copepods, and accounting for them in the calculation of sex ratios can help to identify the frequency of animals changing sex in the population. Svendsen and Tande (Svendsen and Tande, 1999), for instance, observed a dramatic change in the sex ratio of *Calanus finmarchicus* when quadritek (intersex) females were summed to males instead of the normal females, changing the sex ratio from strongly female-skewed to a value close to unity.

Sex determination and sex change in copepods

Little is known about the sex determination process in copepods, but the presence of intersexes in several species suggests that ESD may be widespread in this group. ESD, chromosomal and polygenic modes of sex determination have been suggested (see reviews by Fleminger, 1985; Legrand *et al.*, 1987; Miller *et al.*, 2005). ESD has been observed in several marine and freshwater copepods (reviewed by Korpelainen, 1990; Mauchline, 1998) and the process is well described in harpacticoid (Voordouw and Anholt, 2002a, b;

Voordouw *et al.*, 2005a, b) and parasitic copepods (Michaud *et al.*, 1999; Michaud *et al.*, 2004). Charnov and Bull's (Charnov and Bull, 1977) conditions for ESD have been validated for one parasitic species (Michaud *et al.*, 2004), and most copepods seem to fulfil at least some of these conditions (Irigoien *et al.*, 2000).

A variety of factors have been suggested to influence the sex determination in copepods: temperature (Katona, 1970); food (Irigoien *et al.*, 2000); population density (Heinle, 1969); parasitism (Sewell, 1951); pH (Egami, 1951) and pressure (Vacquier and Belser, 1965) amongst others (see reviews by Fleminger, 1985; Korpelainen, 1990; Mauchline, 1998). In planktonic calanoid copepods, there is also evidence that sex may also be under environmental control (Mauchline, 1998). Fleminger (Fleminger, 1985) reviewed the occurrence of female dimorphism of several species belonging to the family Calanidae and showed that intersexuality is common. Fleminger hypothesized that these intersex individuals ("quadritek" females) originate from genotypic copepodite (hereafter C) 5 males which have changed sex. This hypothesis has not yet been proven, but some studies have shown that the sex ratio of *Calanus* spp. populations changes seasonally, and that food quantity and quality have a significant effect on the population sex ratio, supporting the existence of a strong environmental influence on sex determination (Svendsen and Tande, 1999; Irigoien *et al.*, 2000; Miller *et al.*, 2005).

The production of intersexes is linked to the endocrine-regulated sex differentiation process in crustaceans (see Sagi and Khalaila, 2001), and it has been hypothesized that this process in copepods is likely to be similar to the malacostracans. The endocrine regulation of the appearance of secondary sexual structures in copepods was first suggested by Sewell (Sewell, 1951), and Fleminger (Fleminger, 1985) later suggested that the

morphogenesis of secondary sexual characteristics may be controlled by the production of androgenic hormones. Despite the endocrine system in copepods not being formally described, processes such as moulting are very similar to those of other crustaceans, suggesting that copepods have an analogous endocrine regulation (Fleminger, 1985). More evidence comes from studies that have shown that the copepod development can be affected by endocrine disrupters (Kusk and Wollenberger, 2007). For example, the naupliar development of *Eurytemora affinis* can be inhibited by the endocrine-disrupting chemicals benzo(a)pyrene, 4-nonylphenol and di(ethyl-hexyl)phthalate (Forget-Leray *et al.*, 2005), and estrogens can accelerate the sexual maturation of females of *Acartia tonsa* (Andersen *et al.*, 1999).

Intersexes are likely to occur in animals undergoing ESD when sex is determined late in development and in response to environmental cues (Bull, 1985; Korpelainen, 1990). Therefore, because intersexes have been observed in many copepod species, it is likely that ESD and change of sex late in the life cycle is common in this group. Probably the first to suggest late sex determination in copepods, Sewell (Sewell, 1929) proposed that the influence of male and female “growth-factors” after C3 were responsible for the occurrence of high dimorphism/intersexuality in copepods. Later, he suggested that intersexuality was linked to parasitism and hormonal regulation in copepods (Sewell, 1951). However, the first in-depth discussion of the link of intersexuality and late sex change in copepods was that of Fleminger (Fleminger, 1985), which is still today one of the most detailed discussions on this issue. More recently, some studies of Calanidae have supported Fleminger’s hypothesis. Miller *et al.* (Miller *et al.*, 2005) found evidence that the final sex determination decision in *Calanus* can occur late in development. Irigoien *et al.* (Irigoien *et al.*, 2000) observed that sex ratios of *Calanus* in mesocosms were similar to the field when animals were raised from C5 to adults, but were different, composed mainly of females, when animals were raised from C2–C4 to adults, suggesting that the sex can still change at C4 and not be completely determined until C5.

We speculate that the late sex determination in the life cycle in copepods might be an adaptation to the determinate model of development in this group. Most sex changing invertebrates (including crustaceans) present sequential hermaphroditism, first maturing as one sex and then switching sex later in the life cycle (Charnov, 1982; Policansky, 1982; Allsop and West, 2004). However, the number of moults in the life cycle of copepods is defined, and once maturity is reached, animals cannot moult further. This peculiar developmental characteristic makes copepods a special case of sex-

switching animals, differing from most of the other sex changing animals in two ways: (i) the sex-switching point occurs while copepods are still immature; and consequently, (ii) mature copepods (sex switched or not) can only reproduce as one sex during their lifetime. Under these circumstances, the “decision” to change sex late in development allows an individual to “choose” the sex that would increase its reproductive output based on the most probable environmental conditions it will encounter once it becomes an adult. If this “decision” occurs too early in development, the environmental conditions can change with time, and the risk of the chosen sex not being the best in adulthood increases.

SEX CHANGE AND SKEWED SEX RATIOS IN EXPERIMENTS: AN EXAMPLE WITH *ACROCALANUS GRACILIS*

As is the case in the field, female-skewed sex ratios are commonly observed in copepod cultures (see Peterson, 1986; Irigoien *et al.*, 2000), and these are also often explained as resulting from the higher mortality of males, despite the fact that sex-specific mortality and the occurrence of intersexes are rarely investigated in these experiments. Consequently, we refer to one of our own experiments with the paracalanid copepod *Acrocalanus gracilis*. The purpose of this example is to show that the careful identification of intersexes in incubation experiments is necessary in order to understand resulting sex ratios.

A detailed description of this experiment can be found elsewhere (Gusmão and McKinnon, in press). Briefly, *Acrocalanus gracilis* females were collected at a mid-shelf station in the Timor Sea and left to produce eggs in deck incubators with environment temperature seawater flowing through for 24 h. After this period, we collected the eggs and nauplii, transferred them to containers in the same deck incubators, and followed the development of the cohort for 12 days until adulthood. The water in the containers was renewed and the cohort was sampled every 1–2 days. From each sample, animals were staged, sexed, the body and the length of the fifth leg measured. The appearance of intersexes and their effect on the stage- and age-specific sex ratio of the population was followed during the development of the cohort, and the sex ratios of the incubated population compared with those in the field.

Our results describe an experimental outcome that is often encountered, but seldom reported—the distortion of sex ratios under experimental conditions from those encountered in the field to a sex ratio that is greatly biased toward females. The proximate cause of this shift

is most likely to be the less favourable food environment animals were exposed to in the incubation experiment in comparison to the field (see Gusmão and McKinnon, in press). One of the most interesting observations in these experiments was the occurrence of intersexes in the late copepodite states. Intersexual individuals originating both from the field and the experiment could be clearly distinguished from males by the presence and size of the fifth leg in C5 and adults (Fig. 1), and from normal females, in which the fifth leg is reduced to only the basipodite. All adult intersex individuals had a genital double somite similar to that of a normal female. Sex could only be determined from C4 to adults, and intersexes were observed in C5 and adults. No parasitism was observed in any individual from the field or from the experiment.

Due to the presence of intersexes, two sex ratios were calculated; either adding intersex individuals to normal females or to males. The sex ratios generated different patterns for C4, C5 and adults, as well as within each of these stages, both in the field and in the experiment (Table II). When comparing sex ratios within the same stage, the sex ratios generated for each stage by these two approaches were not different for C5 or adults in the field, but were significantly different in the experiment (Table II, diagonal grey cells). When comparing sex ratios among stages in the field, only the sex ratios of C5 and adults were significantly different, irrespective of whether intersexes were classified as males or as females. In the experiment, however, sex ratios of C4, C5 and adults were only significantly different when intersexes were classified as females.

The two sex ratio calculations also generated different results when comparing the experimental cohort to the field population (Table III). Adult males were completely absent from the experiment, but contributed 26% of the total adults in the field. Intersex individuals were frequent in the experiment, but comprised only 2 and 1% of the total of C5 and adults in the field, respectively. The sex ratio of C4, and the sex ratios considering intersexes as males in C5 and adults showed no significant difference between the populations in the field and in the experiment. On the other hand, the sex ratios from the experiment were significantly different from the field when intersexes were added to females in C5 and adults. Sex ratios were not significantly different from 1 only in C5 from the field ($\chi^2 = 0.0164$, $P = 0.9$), and in C5 from the experiment when intersexes were summed to males ($\chi^2 = 2.7931$, $P = 0.095$).

Sex ratios in the experimental cohort changed with time and with the method of calculation (Fig. 2). Males were always less abundant than females, which always comprised at least 50% of the total proportion of sexes

in C4, C5 and adults during the whole experiment. Despite C4 males being present during the whole experiment, the sex ratio was always skewed towards females, especially at the end of the experiment. Intersexes were always less frequent than females in adults and in C5. Males in C5 were only recorded up to day 9, but intersexes were present from day 7 until the end of the experiment. Because of this, the C5 sex ratio considering intersexes as females increased with time, but the sex ratio adding intersexes to males showed little variation. Sex ratios calculated by adding intersexes to females were skewed towards females in adults. The low abundance and non-constant occurrence of intersexes in adults contributed to the variability in the sex ratio considering intersexes as males over time.

Sex change, intersexuality and sex ratios in *Acrocalanus gracilis*

In the Paracalanidae, intersexuality has been observed in *Parvocalanus crassirostris* and *Paracalanus aculeatus* (Sewell, 1929), *P. parvus* (Sewell, 1929, 1951, and references therein; Ianora *et al.*, 1987; Ianora *et al.*, 1990), *Paracalanus sp.* (probably *P. quasimodo*, Liang and Uye, 1996), *P. indicus* (Kimmerer and McKinnon, 1990), *Acrocalanus inermis* (Sewell, 1912) and *A. gracilis* (present work). Intersexual adults of *A. gracilis* from the Timor Sea were similar to other intersexual paracalanids described in the literature: all adult intersexes morphologically similar to normal females, but with the presence of a modified pair of fifth legs resembling those of a normal C4 male. The frequency of intersexual individuals in the *A. gracilis* population in the field was very low ($\sim 1-2\%$), as is the case in most crustaceans (LeBlanc, 2007).

In our experiment, and in the field population, none of the intersex individuals of *A. gracilis* showed any sign of parasitization. It is unlikely that an early infection occurred along the development of *A. gracilis* because no copepodite stage was found to be infected, and animals showed no tumour-like protuberances or “protruding guts”, usually associated with the release of parasites in copepods (see Skovgaard, 2004). Despite most of the reports of paracalanid intersexes in the literature being linked to parasitization (e.g. Sewell, 1951), the occurrence of non-parasitized intersex individuals in this family is also common (Ianora *et al.*, 1987; Liang and Uye, 1996). Therefore, we exclude parasitization as the cause of intersexuality observed in *A. gracilis*, both in the field and in the development experiment.

Our data suggest that *A. gracilis* is able to change sex during development. Copepods used in our incubation

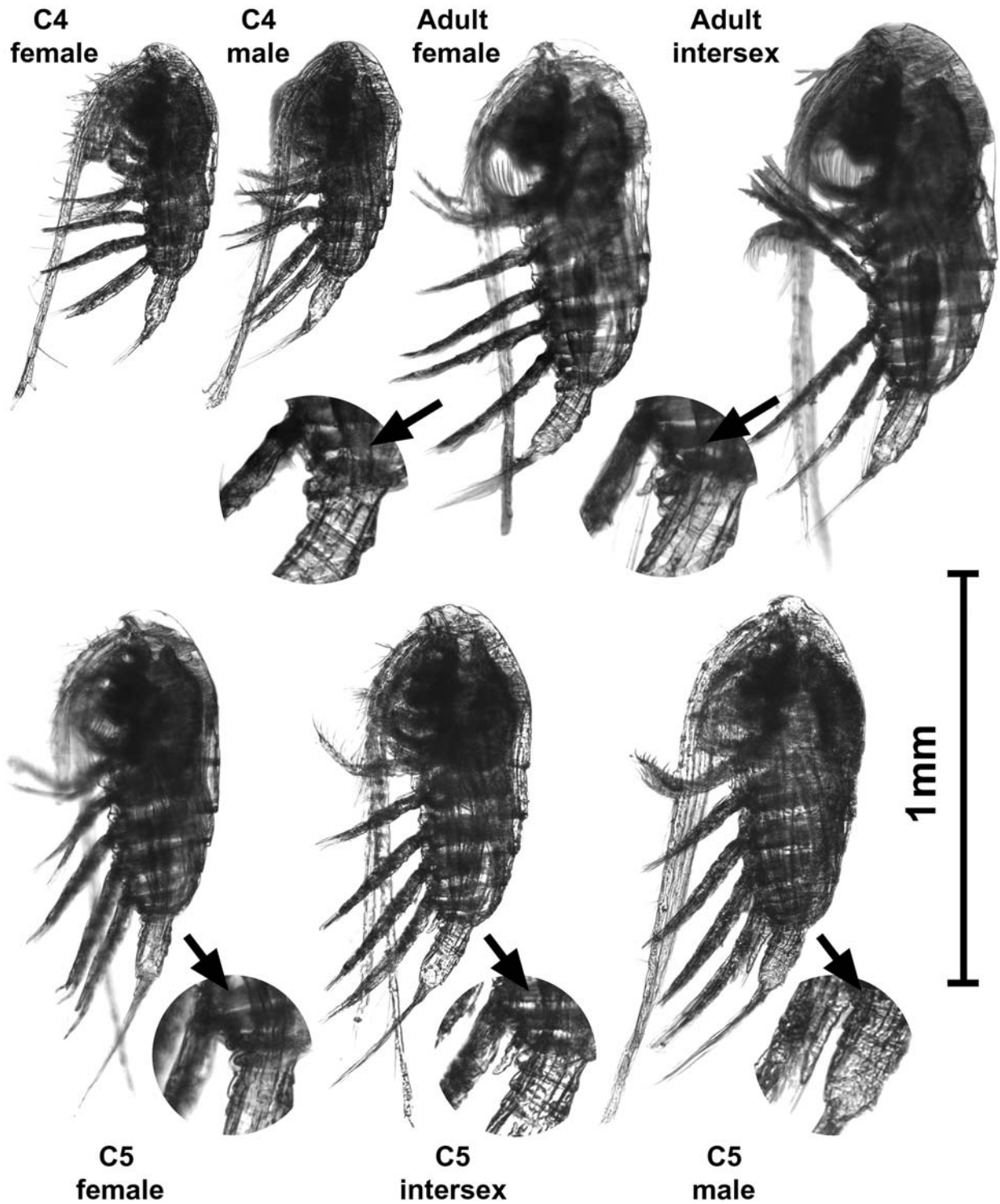


Fig. 1. Examples of different sexes in the last three stages of development of *Acrocalanus gracilis* raised in incubators in the Timor Sea. Normal C4, C5 and adult females do not have a fifth leg, and only the basipodite can be seen. The fifth leg of C4 males is almost half the length of the urosome, and in C5 males the fifth leg is as long as the urosome. In C5 and adult intersex, the fifth leg is as long as in the C4 males.

Table II: Comparison of sex ratios between stages of *A. gracilis* from the field (left) and from the experiment (right, pooled data from all tanks) considering intersexes as males or as females

Field				Experiment			
Stages	C4 (6/9)	C5 (31/30)	Adult (20/52)	Stages	C4 (37/94)	C5 (46/67)	Adult (5/13)
C4 (6/9)	—	ns	ns	As males	C4 (37/94)	—	ns
C5 (30/31)	ns	ns	*	As males	C5 (12/101)	**	****
Adult (19/53)	ns	*	ns	As females	Adult (0/18)	** ^(F)	*** ^(F)
As females				As females			* ^(F)

The sex ratios are inside parenthesis (males/females). The comparison of the sex ratios considering intersexes as males are above the grey cells, and those considering intersexes as females are below the grey cells. The grey cells are the comparison of the two sex ratios (considering intersexes as males and as females) for the same stage either in the field or in the experiment. The Chi-square (χ^2) goodness-of-fit statistic adjusted for continuity with the Yates correction was used to test for differences between the sex ratios either from the experiment (pooled data from all tanks) or from the field. When samples were small, and any of the recorded frequencies was five or less, the Fisher exact test (indicated with ^(F)) was used instead. Significance levels as for Table I.

Table III: Comparison of sex ratios of *A. gracilis* from the field with those from the experiment considering intersexes as males or as females

Allocation of intersexes	Stage								
	C4		C5		Adult				
	Field σ/φ	Experiment σ/φ	Field σ/φ	Experiment σ/φ	Field σ/φ	Experiment σ/φ			
Intersexes as Females	6/9	ns	37/94	30/31	****	12/101	19/53	* ^(F)	0/18
Intersexes as Males				31/30	ns	46/67	20/52	ns	5/13

The Chi-square (χ^2) goodness-of-fit statistic adjusted for continuity with the Yates correction was used to test for differences between the sex ratios from the experiment (pooled data from all tanks) and from the field. When samples were small, and any of the recorded frequencies was five or less, the Fisher exact test (indicated with ^(F)) was used instead. Significance levels as for Table I.

experiments were first generation descendants from the field population, and would be expected to have a similar sex ratio to that in the field. If intersexes were classified as female, and summed to the normal females, the sex ratios in the incubation were significantly different from the field. On the other hand, if we consider intersexes as genetic males (Fleminger, 1985), and added to males, the sex ratios in the incubation were not significantly different from the field. Therefore, the proportion of intersex individuals observed in the incubation accounted for the difference in the proportion of males observed in the incubation in comparison to the original wild population, supporting Fleminger’s (Fleminger, 1985) hypothesis that intersex copepods are actually genetic males that have switched sex during development.

Since intersexes were more common in the experiment than in the field, it is clear that the environmental cue causing the appearance of intersexes was amplified within the experimental containers. Because temperature in the incubators was the same as in the field, the most likely cause of the higher levels of intersexuality in

the incubation was the low food quality/quantity in the incubation water (see Gusmão and McKinnon, in press). This observation supports the hypothesis that food environment is linked to sex determination and the occurrence of intersexuality in copepods (Fleminger, 1985; Irigoien *et al.*, 2000; Miller *et al.*, 2005). If this is also applicable to *A. gracilis*, males would require better trophic conditions in order to promote the appearance of secondary sexual characteristics, otherwise a proportion of genetic males would become females.

Our observations for *A. gracilis* showed that the sex ratios of a copepod population can be affected by the way they are calculated when intersexes are present. In our study, skewed sex ratios towards females were observed only if intersexes were counted as females, as was observed for *Calanus finmarchicus* by Svensen and Tande (Svensen and Tande, 1999). We have not directly estimated mortality in the present experiment, but there was no evidence of differential mortality between sexes, because the sex ratios were not different if intersexes were considered as males.

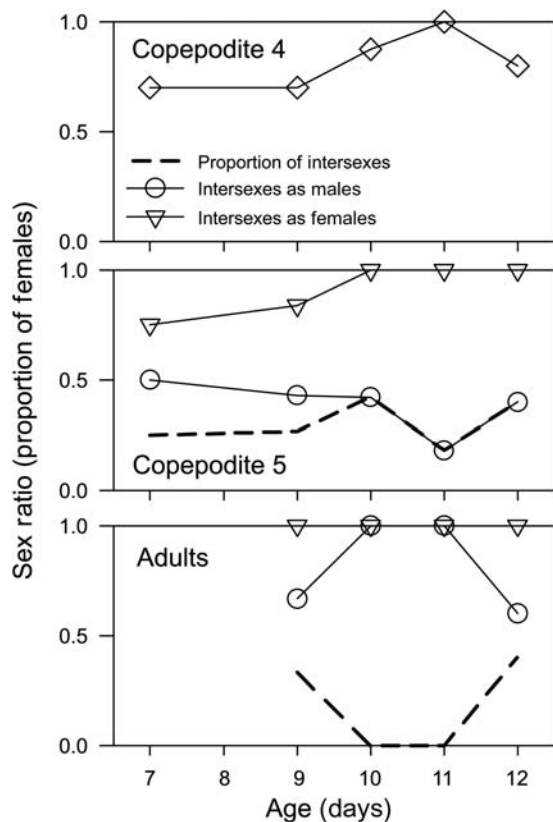


Fig. 2. Sex ratio considering intersexes as males or females and proportion of intersexes for the stages of C4 to adult in relation to the age of the animals in the experiment. Each point represents the pooled data from all replicate containers.

The relationship of intersexuality and sex ratios with age in *Acrocalanus gracilis*

The sex ratios and the proportion of intersexes were not constant in time within a developmental stage in *A. gracilis*. The higher proportion of females towards the end of the experiment, especially in C4 and C5 after the ninth day, may be an indication that males were developing faster than females, as was the case in *Paracalanus parvus* (Landry, 1983). Uye (Uye, 1991) also observed that the occurrence of males in laboratory cohorts of *Paracalanus* was apparently faster than females, and suggested that the stage duration of C5 males was shorter than that of females. This observation also agrees with Irigoien *et al.*'s (Irigoien *et al.*, 2000) hypothesis that the rapid development of individuals through certain key stages will tend to create males, whereas those developing slowly would become females.

The variation of the proportion of C5 intersexes in our experiment suggests that the cue to change sex increased with age. After the ninth day, there were no

C5 males and the continuous presence of C5 intersexes towards the end of the experiment suggests that older C4 males were changing sex more frequently than younger individuals. This observation supports the hypothesis that food quality/quantity may have been the cue to change sex in this experiment: animals growing slower would experience the poor food environment for longer than animals growing faster, thus promoting more animals to change sex.

Sex ratios in the Timor Sea

The sex ratios for paracalanid copepodites in the field are rarely reported and no data for *Acrocalanus* are available. The only other report on the sex ratio of copepodites of a paracalanid species in the field is that of Liang and Uye (Liang and Uye, 1996) who observed mean sex ratios close to unity in C4 and C5 *Paracalanus sp.* In our study, C4 *A. gracilis* sex ratio was slightly skewed towards females, and the sex ratio of C5 was not significantly different from unity only when intersexes were classified as males. In both species, adult sex ratios were different from those of copepodites, and were strongly skewed toward females, which Liang and Uye (Liang and Uye, 1996) attributed to the shorter lifespan of males. Sex-specific longevity could also explain the different sex ratio between adults and copepodites of *A. gracilis* in the field, because intersexes were not abundant. However, the presence of intersexes may produce highly skewed sex ratios towards females if they are summed to females (see Table III). In these cases, it would be extremely hard to discern the effects of intersexuality from differential longevity in the adult sex ratios, stressing the importance of the correct identification of intersexes.

Female skewed sex ratios are frequent in paracalanids (see reviews in Hirst and Kiørboe, 2002; Kiørboe, 2006) similarly to the sex ratio we observed for *A. gracilis* adults. However, the sex ratio of *A. gracilis* in the Timor Sea was less skewed towards females than most sex ratios reported for paracalanids around Australia (Table I), but was similar to *Acrocalanus spp.* from the neighbouring region of Australia's North West Cape. If a rich trophic environment contributes to a higher proportion of individuals maturing as males (Irigoien *et al.*, 2000), we can expect skewed sex ratios towards females in poor trophic environments. In fact, copepods are typically food-limited in tropical Australia (McKinnon and Thorrold, 1993; McKinnon, 1996; McKinnon and Duggan, 2001), which may contribute to the female-skewed sex ratios of most paracalanids in the region. However, it has been suggested that *Acrocalanus spp.* may feed on microzooplankton (see McKinnon, 1996), an

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often abundant resource in the North West Cape region (Moritz *et al.*, 2006) that could contribute to a better nutrition of males and less skewed sex ratios.

When does sex switch in *Acrocalanus gracilis*?

Our observations indicate that the sex change of *A. gracilis* genetic males is most likely to occur in the moult from C4 to C5 (Fig. 3) based on two lines of evidence.

First, the length of the fifth leg in adult intersexes was not significantly different from C5 intersexes and C4

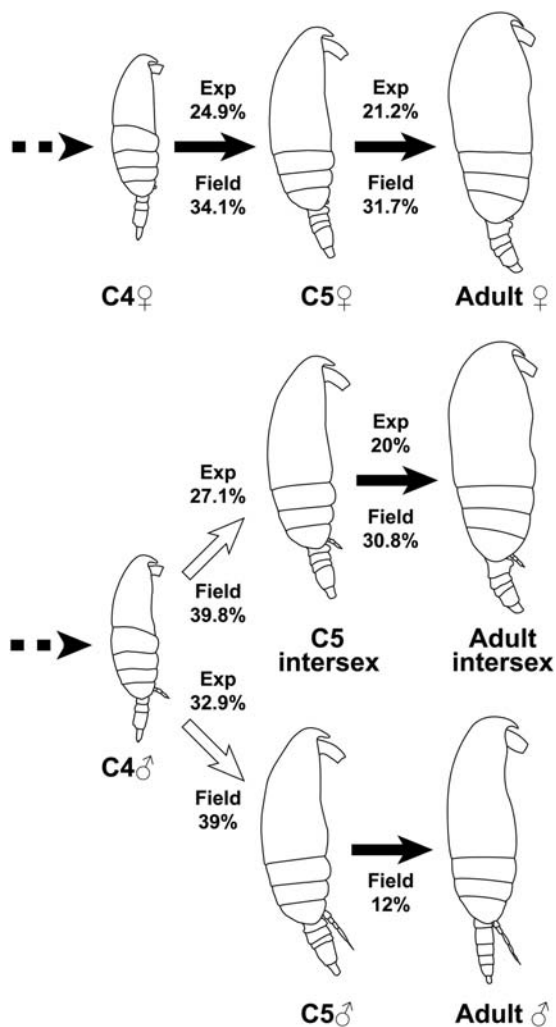


Fig. 3. Conceptual model of development and sex differentiation in *Acrocalanus gracilis*. Only males can change sex along the development, and the moult from C4 to C5 would be the point in which sex can change (white arrows). The percentages of increment in the mean prosome length of consecutive stages in the incubators and in the field are shown for each moult. Dashed arrows indicate previous development and black arrows indicate a normal moult (no sex change).

males (see Gusmão and McKinnon, in press), an indication that this leg showed little or no development in intersexes after C4. It seems that the fifth leg has been “carried” by intersexes since it first appeared in C4. Because normal *Acrocalanus* females do not have a fifth leg at all, it is reasonable to believe that once males switch to females, development of the fifth leg will cease or reduce. Based on the fifth leg length, we have no evidence that the sex could change in the moult from C5 males to adults in *Acrocalanus*. If this were the case, the length of the fifth leg of intersex adults would be similar to that of C5 males. However, the fifth leg of intersex adults was significantly shorter than C5 males, suggesting that C5 males did not change sex.

Secondly, because there was no morphological evidence to distinguish intersexes in C4, and C4 males and females showed no significant differences in size, it is likely that the sex expressed in C4 is the primary sex. Because secondary sexual characteristics were not yet well developed in C4, it is likely that both sexes are physiologically more similar in C4 than in later stages. This hypothesis is supported by the similarity of C4 sex ratios in the experiment and the field. In addition, the sex-specific mean increment in the prosome length between stages is also an indication that major changes in morphometry happened only after C4. The mean prosome increments from C4 to adult females and from C4 males to adult intersexes were similar, and both were different from the mean prosome increments of C4 to adult males (Fig. 3). If the prosome increments are interpreted as a proxy of physiological investment in growth between two stages, the C5 and adult intersexes seem to be physiologically more similar to normal C5 females and adult females than to normal C5 males and adult males, an indication that that sex may have switched before C5.

The size advantage model predicts that sex change occurs at the size where the reproductive success is the same for both sexes (Ghiselin, 1969; Charnov, 1982). This theory was developed for the case of sequential hermaphrodites, in which sex can change after reproduction has begun. Therefore, it cannot be directly applied to copepods because they still cannot reproduce at C4 (the possible point of sex change). However, we propose a reinterpretation of this model in the following way: sex change could occur in copepods at the last point in development in which the physiological condition of both sexes is the same (or similar). Secondary sexual characteristics and gonad differentiation in most calanoid copepods begins to occur in C4 (Mauchline, 1998). After C4, the differences in sizes of males and females could be interpreted as the result of the different physiological investment of each sex towards

adulthood. Therefore, C4 could be considered the last point in development in which both sexes are physiologically similar, and therefore a candidate point for sex change.

The hypothesis of a late sex change in the development of *A. gracilis* is similar to *Calanus* (Svensen and Tande, 1999; Irigoien *et al.*, 2000; Miller *et al.*, 2005). However, our conceptual model is slightly different from the other observations for *Calanus* which suggest that the sex switch occurs from C5 to adults (Svensen and Tande, 1999; Miller *et al.*, 2005). More data are available for *Calanus* than *Acrocalanus*, and though we found no evidence that sex can still change in C5 for *Acrocalanus*, this possibility cannot be ruled out. Nonetheless, both *Calanus* and *Acrocalanus* have intersexes, suggesting that the final sex determination occurs late in development.

INTERSEXUALITY IN COPEPODS

Intersexuality has been observed in several species of copepods (Sewell, 1912, 1929, 1951, and references therein; Fleminger, 1985; Ianora *et al.*, 1987; Ianora *et al.*, 1990; Kimmerer and McKinnon, 1990; Liang and Uye, 1996; Svensen and Tande, 1999; Irigoien *et al.*, 2000; Miller *et al.*, 2005; Bayly and Shiel, 2008; Table IV), but it is still unclear why it occurs, what controls it and what are the consequences for copepod populations. Intersexuality in copepods is usually manifested by the presence of morphological abnormalities in females that resemble structures found in the male. For example, the occurrence of “quadritek” females in *Calanus spp.*, which differ from normal females in the number and organization of aesthetascs on the antennules, resembles that of males (Fleminger, 1985; Svensen and Tande, 1999; Irigoien *et al.*, 2000; Miller *et al.*, 2005). In females of some freshwater calanoids, male-like geniculate antennules sometimes occur (Markevitch, 1982; Sillett and Stemberger, 1998; Dharani and Altaff, 2002). However, the most ubiquitous morphological abnormality observed in intersexual copepods is the presence of a modified fifth pair of legs in females similar to those of males (see Sewell, 1929).

Intersexuality is common in crustaceans (e.g. Legrand *et al.*, 1987; Ford and Fernandes, 2005), especially Malacostraca (Charniaux-Cotton, 1975; LeBlanc, 2007). The basis of sex determination in Crustacea is genetic, but usually shows the influence of various environmental and cytoplasmic factors (Korpelainen, 1990). In several crustaceans, intersexuality results from the disruption of endocrine signalling pathways involving sex determination and/or sex differentiation (LeBlanc,

2007). Sexual differentiation, reproduction and moulting processes have all been shown to be under endocrine regulation (see reviews by Fingerman, 1997; Kusk and Wollenberger, 2007; LeBlanc, 2007) which is intrinsically linked to the environment, perhaps explaining why this group is so successful in nature (LeBlanc, 2007). This link is made through neuroendocrine pathways, which transduce detected environmental cues to regulate several aspects of an individual’s survival, guaranteeing the population’s sustainability (LeBlanc, 2007).

These neuroendocrine pathways, and the whole endocrine system in general, are very well understood in malacostracans (Charniaux-Cotton, 1975; LeBlanc, 2007). Both sexual differentiation and gonadal activity are under hormonal control (Highnam and Hill, 1977), and the androgenic gland hormone (AGH) plays a major role in male sex differentiation and development, regulating the expression of primary and secondary sexual characteristics (Sagi and Khalaila, 2001). Sex is genetically determined, and in males the gonad stimulating hormone (GSH) promotes the formation of the androgenic gland, which releases AGH, which in turn induces the development of testes and secondary sexual characteristics. In females, where AGH is not produced due to the absence of the androgenic gland, ovaries will develop spontaneously and the female secondary sexual characteristics will be stimulated by the ovarian hormone (see review by Kusk and Wollenberger, 2007; LeBlanc, 2007). Sex reversal into functional individuals of the opposite sex was achieved by ablation of the androgenic gland in genetic males of the prawn *Macrobrachium rosenbergii*, and by the implantation of this gland in females (see Sagi and Khalaila, 2001). In addition, intersexuality has been associated with an irregular production of AGH in the amphipod *Echinogammarus marinus* (Ford *et al.*, 2005), and intersexes could be produced by the manipulation of the androgenic gland in the crayfish *Cherax quadricarinatus* (Sagi and Khalaila, 2001).

How widespread is intersexuality in copepods?

The frequency of intersexual individuals in natural populations of most crustaceans is relatively low, usually representing less than 1% of individuals (LeBlanc, 2007). However, higher percentages of intersexes are reported in the literature for copepods (see Sewell, 1951; Fleminger, 1985), suggesting that it may be relatively common. Liang and Uye (Liang and Uye, 1996) found that 2.5–23% of adult females of *Paracalanus sp.* in Fukuyama Harbour in Japan had intersexual characteristics, and Svensen and Tande (Svensen and Tande,

Table IV: Occurrence of fifth leg abnormality and geniculate antennule in adult females, ESD and intersexuality in copepods

Species	Order	Environment	Location	ESD	Parasitized	Intersex	5th Leg	Antenn.	Reference
<i>Clausocalanus farrani</i>	Calanoida	Marine	Red Sea	No	Yes	Yes	Yes	–	Cornils <i>et al.</i> (2007)
<i>Clausocalanus furcatus</i>	Calanoida	Marine	Red Sea	No	Yes	Yes	Yes	–	Cornils <i>et al.</i> (2007)
<i>Ctenocalanus vanus</i>	Calanoida	Marine	Red Sea	No	Yes	Yes	Yes	–	Cornils <i>et al.</i> (2007)
<i>Paracalanus parvus</i>	Calanoida	Marine	Mediterranean Sea	No	Yes	Yes	Yes	–	Ianora <i>et al.</i> (1987)
<i>Paracalanus indicus</i>	Calanoida	Marine	South Australia	No	Yes	Yes	Yes	–	Kimmerer and McKinnon (1990)
<i>Paracalanus sp.</i>	Calanoida	Marine	Sea of Japan	No	–	Yes	Yes	–	Liang and Uye (1996)
<i>Acartia tonsa</i>	Calanoida	Marine	Southern Brazil; Baltic Sea	No	No	No	Yes	–	Montú and Gloeden (1982); Behrends <i>et al.</i> (1997)
<i>Acartia bifilosa</i>	Calanoida	Marine	North Atlantic; Baltic Sea	No	No	No	Yes	–	Brylinski (1984); Behrends <i>et al.</i> (1997)
<i>Acartia longiremis</i>	Calanoida	Marine	Baltic Sea	No	No	No	Yes	–	Behrends <i>et al.</i> (1997)
<i>Acartia discaudata</i>	Calanoida	Marine	Baltic Sea	No	No	No	Yes	–	Behrends <i>et al.</i> (1997)
<i>Acartia clausi</i>	Calanoida	Marine	North Atlantic	No	No	No	Yes	–	Brylinski (1984)
<i>Temora stylifera</i>	Calanoida	Marine	Southern Brazil	No	No	Yes	No	Yes	Martinelli-Filho <i>et al.</i> (in press)
<i>Eudiaptomus gracilis</i>	Calanoida	Freshwater	Russia	–	–	Yes	Yes	Yes	Markevitch (1982)
<i>Leptodiptomus minutus</i>	Calanoida	Freshwater	Northeastern USA	No	No	Yes	Yes	Yes	Sillett and Stemberger (1998)
<i>Boeckella triarticulata</i>	Calanoida	Freshwater	Western Australia	No	No	Yes	Yes	Yes	Bayly and Shiel (2008)
<i>Boeckella poppei</i>	Calanoida	Freshwater	Southern Argentina	No	No	No	Yes	Yes	Menu-Marque (2003)
<i>Sinodiptomus indicus</i>	Calanoida	Freshwater	Southeastern India	Yes	No	No	Yes	Yes	Dharani and Altaff (2002)
<i>Paramphiascella hyperborea</i>	Harpacticoida	Marine	North Atlantic	No	No	Yes	Yes*	Yes*	Moore and Stevenson (1991, 1994)
<i>Stenhelia gibba</i>	Harpacticoida	Marine	North Atlantic	No	No	Yes	Yes*	No*	Moore and Stevenson (1991, 1994)
<i>Halectinosoma similidistinctum</i>	Harpacticoida	Marine	North Atlantic	No	No	Yes	Yes*	No*	Moore and Stevenson (1991, 1994)
<i>Tigriopus californicus</i>	Harpacticoida	Marine	Western Canada	Yes	No	No	–	–	Voordouw and Anholt (2002a, b); Voordouw <i>et al.</i> (2005a, b)
<i>Pachypygus gibber</i>	Cyclopoida	Marine	Southern France	Yes	No	No	–	–	Becheikh <i>et al.</i> (1998); Michaud <i>et al.</i> (1999, 2004)

This is a non-exhaustive review, complementary to Fleming (1985), Korpelainen (1990) and Mauchline (1998). The literature older than 1950 was reviewed by Sewell (1951). ESD, authors explicitly suggested the occurrence of ESD and/or sex change for the species. Parasitized, parasitization was observed amongst intersexual individuals (note that this does not imply that all reported intersexual individuals were parasitized). Intersex, authors referred to abnormal individuals as intersexes. 5th Leg, authors reported the occurrence of an abnormal fifth leg in adult females. Antenn., authors reported the occurrence of a male-like geniculate antennule in adult females. –, no information present. *Most harpacticoid intersexes reported by Moore and Stevenson (1994) have female secondary sexual characters, usually with an abnormal fifth pair of legs, and a variable occurrence of male-like sixth legs, urosome segmentation and prehensile antennules.

1999) reported up to 41% of the females of *Calanus finmarchicus* in Norway to be quadritek (intersexes).

Reports of abnormal fifth legs in phenotypic females are relatively common, and are found in many species (Table IV). Despite this, reports on intersex copepods are not as frequent in the literature as for other crustaceans. We believe that this is simply because they have been widely overlooked. Fifth leg abnormalities are commonly found only in copepod females, and always show a tendency to resemble the male's fifth leg (for instance, abnormal fifth legs in female calanoids often show an asymmetry on the same side as those of males). Researchers might consider these abnormalities a natural variation in the morphology of the species leaving them unreported despite being characteristic of intersexuality. Morphological abnormalities in intersexes vary in their degree of expression, and they are not always obvious, resulting in an unnoticed classification of intersexes as normal females. For instance, the modified fifth leg of intersexes usually varies in size and structure, and sometimes is very similar to a normal female's fifth leg (e.g. Sewell, 1951; Ianora *et al.*, 1987). As a consequence, intersexes may be ignored in field samples, or even worse, misidentified as other species (see *Corycaeus* examples in Sewell, 1951), or misidentified as new species (e.g. Menu-Marque, 2003; Bayly and Shiel, 2008). In fact, Sewell (Sewell, 1951) noted that most of the authors to that date to observe intersexes in copepods considered these individuals to be abnormal females, including in his own earlier work.

Food, parasitization and intersexuality

Of the various factors suggested to affect sex determination and the occurrence of intersexes in copepods, two predominate in the literature: parasitization and resource availability. Here we suggest that parasitization might be linked to food limitation, and that nutritional status is the most important factor controlling sex determination in copepods.

Food quantity and quality are known to influence several aspects of the life of copepods, such as reproduction and development (see review in Mauchline, 1998). For other crustaceans, food quality is also known to directly promote sex change. For example, some species of diatoms are able to promote sex reversal in the protandric shrimp *Hippolyte inermis* (Zupo and Messina, 2007). Sex ratio studies suggest that food quality and quantity can be related to the sex determination process in copepods as well. Irigoien *et al.* (Irigoien *et al.*, 2000) observed higher proportions of *Calanus helgolandicus* males when animals were raised from eggs or nauplii to adults under higher food concentrations. In addition,

they found that animals raised on different algae diets had different adult sex ratios. Bonnet and Carlotti (Bonnet and Carlotti, 2001) observed a higher proportion of males when cultures of *Centropages typicus* were raised fed *Thalassiosira weissflogii*, and sex ratios close to 1 when fed other algae.

More evidence comes from field studies of copepod sex ratio. The sex ratio of copepods is known to vary seasonally (see reviews by Mauchline, 1998; Kiørboe, 2006), and it has been suggested that it may be related to food quantity and quality (see review in Irigoien *et al.*, 2000). For instance, Irigoien *et al.* (Irigoien *et al.*, 2000) observed higher percentages of *Calanus* males in the field coinciding with the spring phytoplankton bloom in the north Atlantic. Despite sex ratio being widely investigated in the field (reviewed by Mauchline, 1998; Kiørboe, 2006), few studies report intersexes. Fleminger (Fleminger, 1985) was the first to analyse the seasonal variation of sex ratios accounting for intersexes, and to suggest a link between food supply with sex determination and intersexuality. He analysed the seasonal variation in the occurrence of intersexes and observed that these individuals were more frequent during the winter, while normal males were more frequent in the spring, when food conditions were better. A similar seasonal variation of intersexes in *Calanus finmarchicus* was also found by Miller *et al.* (Miller *et al.*, 2005). Liang and Uye (Liang and Uye, 1996) observed high proportions of intersexual adults (up to 23%) in *Paracalanus sp.* in a sampling period that also included low Chla levels ($\sim 2\text{--}2.4 \mu\text{g L}^{-1}$).

Can *Blastodinium* parasitization influence the sex change process in copepods?

Copepods are hosts to a diversity of parasites which affect their hosts in a variety of ways (see Chatton, 1920; Sewell, 1951). Among these effects, parasitization has been widely linked to intersexuality in copepods, especially within the Paracalanidae (Sewell, 1929, 1951; Ianora *et al.*, 1987). In Paracalanidae, infections by the dinoflagellates *Syndinium* in *P. parvus* (Ianora *et al.*, 1987; Ianora *et al.*, 1990) and *Ateodinium* in *P. indicus* (Kimmerer and McKinnon, 1987), both in juveniles and adults, were linked to the appearance of intersexual characteristics, but also promoted other morphological and behavioural changes (Ianora *et al.*, 1987), severe gonadal dysgenesis and sexual castration (Ianora *et al.*, 1990), and high mortality rates (Kimmerer and McKinnon, 1987). However, not all intersexuality inducing parasites cause such devastating effects in their copepod hosts. Intersexuality in parasitized copepods has been particularly linked to gut parasitizing

dinoflagellates of the genus *Blastodinium*, which show no evidence of causing major harm to the host (Chatton, 1920; Sewell, 1951; Ianora *et al.*, 1987; Ianora *et al.*, 1990). For instance, Ianora *et al.* (Ianora *et al.*, 1990) found that *P. parvus* parasitized by *Blastodinium* showed no major morphological changes from normal individuals, no alterations of the midgut and gonads, and no apparent sexual castration.

However, the relationship between parasitization (especially by *Blastodinium*) and intersexuality is somewhat unclear. Two facts suggest that parasitization cannot be considered the direct cause of intersexuality in infected populations. First, not all parasitized individuals in these populations are intersexes (see examples in Sewell, 1951; Ianora *et al.*, 1987). Second, intersexuality is also found in non-parasitized animals. For instance, Ianora *et al.* (Ianora *et al.*, 1987) observed a population of *P. parvus* in which most of the intersex individuals were not parasitized. Similarly, intersexuality could not be attributed to parasitization in *P. indicus* (Kimmerer and McKinnon, 1990), *Paracalanus* *sp.* (Liang and Uye, 1996) and in “quadritek” females in Calanidae (Fleminger, 1985). Sewell (Sewell, 1951) suggested that these non-parasitized intersexes may be early infected individuals which later recovered. In fact, zoospores of *Blastodinium* *spp.* may be released through the host’s anus (Chatton, 1920; Skovgaard, 2004), but it is still unknown if copepods can fully recover (Skovgaard, 2005). However, Sewell (Sewell, 1951) also recognized that intersexes may appear in the life history of copepods irrespective of the presence of parasites, as he observed that several early authors referred to intersexes but did not mention parasites.

If parasitization is not the direct cause of intersexuality, why are intersexes more frequent in parasitized populations of copepods (see examples in Sewell, 1951)? We suggest that parasitization “boosts” the intersexuality inducing trigger that is naturally found in non-infected populations. Intersex individuals are particularly common in populations infected by *Blastodinium*, and we suggest that this may be caused by the occupation of most of gut of the host by the parasite, resulting in the host becoming undernourished. Infected individuals are smaller than non-parasitized animals in several species (Sewell, 1951), suggesting undernourishment of infected animals. Therefore, we suggest that one of the most likely triggers of sex change and the appearance of intersexes is food limitation, both in parasitized and non-parasitized populations.

Some other observations of the link between parasitization and intersexuality in copepods could help us to elucidate how the sex change process occurs in this group. These are: (i) juvenile copepodites seem to be

more prone to infection than adults (Sewell, 1951); (ii) parasitized adult males are extremely rare or non-existent (Chatton, 1920; Sewell, 1951; Ianora *et al.*, 1987); and (iii) only females show the occurrence of intersex characteristics (Sewell, 1951; Ianora *et al.*, 1987). The acquisition of the parasite early in life is important because it renders these early infected individuals exposed for longer to the sex change trigger than late infected animals, increasing the likelihood of sex change. Experimental observations support the hypothesis that sex can still change in C3–C4, but not in C5 in *Calanus* (Irigoién *et al.*, 2000). Observations 2 and 3 above indicate that only males can change sex, and this is supported by studies of parasitized and non-parasitized populations of copepods (Sewell, 1951; Fleminger, 1985). This is somewhat similar to protandric hermaphrodites (where only males change sex to females), often found in sex changing invertebrates (Allsop and West, 2004). If a young genetic female is infected by *Blastodinium*, it would continue to develop until adulthood and would not show any morphological difference from a normal female other than being smaller than a non-parasitized female due to undernourishment, as is the case in normal adult females parasitized in the field (Sewell, 1951; Ianora *et al.*, 1987). On the other hand, when a young genetic male is infected by *Blastodinium*, it will also experience an undernourished development, but in this case it would work as a trigger to change sex. At some point during its development, the sex change would occur, and it would reach adulthood as an intersex still carrying the parasite in its gut. These would be the parasitized intersexes reported in the literature (see examples in Sewell, 1951; Ianora *et al.*, 1987).

If the hypothesis that the major trigger to sex change of food limitation is correct, there is still the question of how this could physiologically promote sex switching. Since intersexuality is a direct result of the sex differentiation mechanism, it is possible that the food limitation (through parasitization or not) could affect this mechanism. If copepods indeed possess androgenic hormones (Fleminger, 1985), and intersexuality can result from the disruption of endocrine signaling (LeBlanc, 2007), it is possible that the food limitation could promote sex change in a process similar to the effect of *Cocconeis* *sp.* diatoms in the diet of the decapod shrimp *Hippolyte inermis*, through the promotion of apoptosis in the male gonad and in the androgenic glands (Zupo and Messina, 2007). Unfortunately, there is no conclusive research identifying the sex differentiation mechanism in copepods, and any hypothesis based on the existence of an endocrine mechanism is still purely speculative.

THE EVOLUTIONARY ADVANTAGE OF SEX CHANGE

Whatever the control of sex change during development, this process may have clear evolutionary advantages. The main advantage, discussed in detail in Fleminger (Fleminger, 1985), Svensen and Tande (Svensen and Tande, 1999) and Miller *et al.* (Miller *et al.*, 2005), would be higher reproductive output, guaranteeing the maintenance of the population in the highly patchy planktonic environment. In Calanidae exhibiting diapause and under heavy seasonal influence, Fleminger (Fleminger, 1985) suggested that if sex changed early in the reproductive season, when most animals emerging from diapause are males, there would be an increase in the reproductive output of sex-changed individuals.

We believe that sex change could also promote an increased reproductive output of genetic males in highly variable environments, as was hypothesized by Fleminger (Fleminger, 1985) for *Calanus*. We use *Acrocalanus* in tropical Australia as an alternative model species to *Calanus* as its life cycle is much shorter than *Calanus*, seasonality is clearly less marked in tropical regions, and there is no evidence of diapause in paracalanids. We suggest that since there is high variability in trophic resources in tropical Australia (see Moritz *et al.*, 2006), a higher proportion of males would mature during rich food conditions, and conversely, when the trophic conditions become limiting, that males would switch sex and mature as phenotypic females and have access to the males matured during the previous favourable period. If we consider that females mature later and have a longer longevity than males, the individuals that changed sex would be present in the environment longer than males, hence increasing their chances of mating. Moreover, Sillett and Stemberger (Sillett and Stemberger, 1998) observed that intersexes of *Leptodiaptomus minutus* tended to live longer than normal females. If this is the case in *A. gracilis*, it would be a tremendous advantage to C4 males to mature as intersexes. A consequence of this hypothesis is that for long periods of food limitation, the sex ratio would skew towards females, an observation that accords with reported sex ratio of paracalanids in tropical Australia (Table I), a typically oligotrophic region.

Obviously, the hypothesis above is valid only if sex-changed animals are able to reproduce successfully. The fertility of intersexes is unknown, but there is some evidence in the literature to suggest that crustaceans which changed sex are indeed able to reproduce. For example, *Macrobrachium rosenbergii* that had undergone complete sex reversal were capable of mating with normal

individuals and producing progeny (Sagi and Cohen, 1990). In marine calanoids, because the identification of intersexes is often done *post mortem*, there are only few comparisons of their reproductive output with that of normal females. Cattley (Cattley, 1948) observed spermatophores attached to intersex individuals of *Pseudocalanus elongatus*, and concluded that males find these intersex individuals “as attractive as normal females and effect copulation”. Miller *et al.* (Miller *et al.*, 2005) observed that intersex *Calanus finmarchicus* had similar egg production and hatching to normal females. Reports of intersexes of freshwater calanoids carrying egg sacs (e.g. Sillett and Stemberger, 1998; Dharami and Altaff, 2002) also suggest the successful fertilization of intersexes in the field. Sillett and Stemberger (Sillett and Stemberger, 1998) observed that intersexes mated with males in the laboratory. They also observed that intersexes produced more eggs and almost twice the number of clutches as normal females, but the number of eggs that hatched was similar. This apparently increased reproductive output of intersexes was also observed in females of *Euterpina acutifrons* that mated with large males (intersexes) and had greater clutches than those that mated with small (normal) males (Stacnyk and Moreira, 1988).

Fleminger (Fleminger, 1985) proposed another more general hypothesis on sex change in copepods: sex switching would be most favoured in larger genetic males. This assumption is based on the Ghiselin (Ghiselin, 1969) size-advantage model for sex change and the Charnov and Bull (Charnov and Bull, 1977) model for ESD, in which sex change would be expected when an animal can reproduce more effectively as a member of the opposite sex. In copepods, there is a positive relationship between female body size and reproductive output or success, but apparently not in males, so large males that switch sex would be able to become productive females, while small males would take advantage of remaining as males, as they could mate with larger females (Fleminger, 1985). This hypothesis may be true for *A. gracilis*, as we observed that C5 intersexes were significantly larger than normal C5 females in the incubation. However, Miller *et al.* (Miller *et al.*, 2005) raised an important question: if larger body sizes imply better nutrition, and good nutrition has been positively linked to the maturation of males (Irigoién *et al.*, 2000), a reduction in the proportion of intersex individuals would be coupled to larger sizes. Because good nutrition would generate larger genetic males, which would not switch sex, Fleminger’s (Fleminger, 1985) hypothesis would be best confirmed when trophic conditions are poor. Irigoien *et al.* (Irigoién *et al.*, 2000) hypothesized that *Calanus*

individuals under growth-rate limitation will tend to mature as small females, whereas those individuals developing at maximum growth rates will become normal males and large females. Our results support this idea, as *A. gracilis* in the incubation were significantly smaller in size and the frequency of intersexes was much higher than in the field, implying a better nutrition in the field than in the incubation.

CONCLUDING REMARKS

It is plausible that the frequently observed female-skewed sex ratios both in incubations and in the field are the result of a proportion of males changing sex during development. In fact, skewed sex ratios are expected in animals showing ESD (Charnov, 1982), which has been considered a potential factor in the determination of sex ratios in copepods (Kiørboe, 2006). Therefore, intersexes have the potential to have a strong effect on the sex ratios of copepods, and accounting for them in the calculation of sex ratios can help identify the frequency of animals changing sex in the population. Svensen and Tande (Svensen and Tande, 1999), for instance, observed a dramatic change in the sex ratio of *Calanus finmarchicus* when quadratek (intersex) females were summed to males instead of the normal females, changing the sex ratio from strongly female-skewed to a value close to unity.

Our observations for *Acrocalanus* support Irigoien *et al.*'s (Irigoien *et al.*, 2000) idea that copepods fulfil some of the conditions described by Charnov and Bull (Charnov and Bull, 1977) in which ESD is expected. In the planktonic environment, probably the most evident of these conditions is that parents and offspring do not control, or have little predictive ability over, their future environment. Under this condition, sex determination late in the lifecycle is expected (Charnov and Bull, 1977; Bull, 1983), and because sex is determined late, it increases the risk of production of intersexes (Bull, 1985).

Resolution of the mechanism underlying sex change and intersexuality in copepods remains a formidable challenge. There is increasing evidence of the widespread occurrence of intersexuality in copepods (e.g. Sewell, 1951; Fleminger, 1985; Table IV), and the process of sex change seems to be complex and variable among copepod groups. For instance, the large male morphotype in *Euterpina acutifrons* is believed to originate from females that switched sex during development (Stancyk and Moreira, 1988). Correct identification and quantification of intersexes in the field is necessary, but the misidentification of these individuals may be

relatively common, especially in routine analysis of zooplankton samples (Bayly and Shiel, 2008). Intersexes can have a significant impact on the sex ratio of the population (e.g. Svensen and Tande, 1999; present work), and we suggest that skewed sex ratios may be related to sex change in paracalanids in the field. In addition, there is evidence that skewed sex ratios influence the reproductive output of a population. For instance, McKinnon (McKinnon, 1996) observed in *A. gibber* that animals from experimental populations with a skewed sex ratio towards females failed to produce eggs. While the current evidence in the literature helps elucidate important parts of this complex puzzle, very important questions such as how sex is determined and differentiated, including the existence of an endocrine system in copepods, are yet to be answered.

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