

# Larval density and the Charnov–Bull model of adaptive environmental sex determination in a copepod

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**Abstract:** Charnov and Bull suggested that environmentally induced variation in adult body size coupled with sex-specific differences in fitness can select for the evolution of adaptive environmental sex determination (ESD). In this study we determine whether larval density affects sex determination in the copepod *Tigriopus californicus* (Baker, 1912), as predicted by Charnov and Bull. Individuals reared at low densities developed faster and were significantly larger than siblings reared at high densities. For these laboratory-reared individuals, sexual selection on male body size was stronger than fecundity selection on female body size, but this sex-specific pattern of selection was reversed in the field. Differences in food availability (for females) and the mode of competition (for males) may account for the conflicting results between laboratory and field. We found a weak effect of larval density on sex determination in a pilot experiment but no effect in a second, more powerful experiment. While larval density did not affect the sex ratio of *T. californicus*, our sex-specific estimates of selection on adult body size will inform future models of adaptive ESD in this species and other copepods.

**Résumé :** Charnov et Bull avancent que la variation de la taille adulte induite par le milieu en combinaison avec les différences sexuelles de fitness peut sélectionner l'évolution de la détermination sexuelle adaptative due au milieu (ESD). Dans notre étude, nous déterminons si la densité larvaire affecte la détermination sexuelle chez le copépode *Tigriopus californicus* (Baker, 1912) comme le prédisent Charnov et Bull. Les individus élevés à densités faibles se développent plus rapidement et sont significativement plus grands que leurs frères et soeurs élevés à forte densité. Chez ces individus élevés en laboratoire, la sélection sexuelle faite sur la taille corporelle des mâles est plus forte que la sélection reliée à la fécondité sur la taille corporelle des femelles; mais ce pattern de sélection relié au sexe est inversé en nature. Des différences de disponibilité de nourriture (chez les femelles) et le mode de compétition (chez les mâles) peuvent peut-être expliquer les résultats contradictoires au laboratoire et en nature. Nous avons observé un faible effet de la densité larvaire sur la détermination sexuelle dans une expérience préliminaire, mais aucun effet dans une seconde expérience plus puissante. Bien que la densité larvaire n'affecte pas le sex-ratio chez *T. californicus*, nos estimations de la sélection spécifique au sexe de la taille corporelle adulte affecteront les modèles futurs de ESD adaptative chez cette espèce et chez les autres copépodes.

## Introduction

In animals with separate sexes, sex-determining mechanisms are often classified as genotypic sex determination (GSD) or environmental sex determination (ESD) (Bull 1983). GSD is determination of an individual's sex phenotype predominantly by its genotype (Bull 1983), whereas ESD is determination of an individual's sex phenotype predominantly by an environmental factor at some point during development (Bull 1983; Adams et al. 1987; Korpelainen

1990). GSD mechanisms can be influenced by environmental factors (Baker and Ridge 1980) and, conversely, ESD mechanisms often exhibit a genetic component (Bull et al. 1982; Conover and Heins 1987b; Janzen 1992; Rhen and Lang 1998); hence, the distinction between these two is not always clear (Bull 1983).

At the population level, the sex-determining mechanism is important because it determines the primary sex ratio (defined here as the proportion of males at the time of sex determination), which in turn influences population genetics

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and demography. For GSD systems where the sex ratio genes are under autosomal control, Fisher (1930) predicted a 50:50 primary sex ratio at equilibrium. In GSD systems with sex chromosomes, Mendelian segregation at meiosis also ensures a balanced primary sex ratio (Williams 1979; Bull and Charnov 1988). In contrast, under ESD the primary sex ratio of the population is often biased towards one sex (Bull 1981; Charnov and Bull 1989) and can fluctuate over space and time as the environment changes (Janzen 1994; Michaud et al. 2004).

The adaptive significance of ESD was elucidated by Charnov and Bull (1977), who suggested that differences in the strength of selection on adult body size between males and females can select for ESD. Their logic is as follows: (i) the environment is patchy; (ii) individuals developing in high-quality patches reach a larger body size at sexual maturity than individuals developing in low-quality patches; (iii) adult body size has greater fitness consequences for one sex than the other; hence, (iv) individuals that find themselves in high-quality patches should differentiate into the sex that benefits the most from being large (Charnov and Bull 1977). In summary, under adaptive ESD we expect the sex-specific pattern of selection on adult body size to reflect the spatiotemporal variation in the primary sex ratio (Bull 1983).

A classic example of adaptive ESD is photoperiod-dependent sex determination in the amphipod *Gammarus duebeni* Lilljeborg, 1851 (Bulnheim 1967). In annual populations of *G. duebeni*, the environment is patchy with respect to the duration of the growing season, and individuals that are born early in the season have more time to grow large than do individuals that are born late (Naylor et al. 1988a; Watt and Adams 1994). The precopulatory mate-guarding and assortative mating habits of *G. duebeni* exert strong selection on male body size because large males are capable of mating with females of all sizes, whereas small males are unable to mate with larger females (McCabe and Dunn 1997). While females also benefit from large body size (owing to increased egg production), females that are too large have difficulty finding a suitable partner (Hatcher and Dunn 1997). Because males benefit more from being large, they are produced early in the season under long daylight hours, whereas females are produced late in the season under short daylight hours. Other classic examples of adaptive ESD include temperature-dependent sex determination in Atlantic silversides (*Menidia* spp.) (Conover 1984; Conover and Heins 1987a; Lagomarsino and Conover 1993; Yamahira and Conover 2003) and density-dependent sex determination in mermithid nematodes (Blackmore and Charnov 1989).

Organisms with ESD rarely exhibit a unified response to the sex-determining environmental factor. Variation in the level of ESD has been reported among populations of *Menidia* spp. (Conover and Heins 1987a; Yamahira and Conover 2003) and *G. duebeni* (Naylor et al. 1988b; Watt and Adams 1994). Even within populations, individuals vary in their sensitivity, and experiments on such populations typically document variation in the primary sex ratio among families for a standardized set of environmental conditions (Conover and Kynard 1981; Rhen and Lang 1998; Voordouw and Anholt 2002a). While among-family variation in the primary sex ratio is not a theoretical corollary of

ESD (Charnov and Bull 1977; Bull 1981), experiments and statistical analyses that incorporate this variation in their design are usually more powerful (Cook 2002; Wilson and Hardy 2002) and of greater interest to the evolutionary biologist (Orzack 2002).

Recent work on calanoid copepods (Irigoien et al. 2000) and the parasitic copepod *Pachypygus gibber* (Thorell, 1859) (Becheikh et al. 1998) has shown that food levels can affect sex determination during larval development. These studies motivated us to investigate the effect of larval-density-induced food limitation on the primary sex ratio of the harpacticoid copepod *Tigriopus californicus* (Baker, 1912). Although Egloff (1966) conducted this experiment almost 40 years ago (and found no effect), we decided to repeat his experiment for a number of reasons. First, it is not clear whether his three larval density treatments actually limited per capita food availability. Second, he measured ESD as a population-level response instead of quantifying among-family variation in the degree of sex-ratio plasticity (Rhen and Lang 1998; Voordouw and Anholt 2002a). Third, the level of ESD varies among populations (Watt and Adams 1994; Yamahira and Conover 2003), and its absence in Oregon does not preclude its presence on Vancouver Island.

In copepods, larval-density-induced variation in per capita food availability can have important consequences for adult body size, as this trait is often fixed at sexual maturity (Gilbert and Williamson 1983). The importance of female body size for egg production in copepods is well established from taxonomic surveys (Kiorboe and Sabatini 1995; Hopcroft and Roff 1998). Likewise, the prevalence of precopulatory mate guarding in this group suggests that body size also plays a role in male reproductive success (Jormalainen 1998). While several workers have compared the strength of selection on adult body size between male and female amphipods (Ward 1988; McCabe and Dunn 1997; Bertin and Cezilly 2003), to our knowledge this has never been done in copepods. Manipulating larval density increases the range in adult body size and therefore the probability of detecting selection (Anholt 1991). Even if larval density has no effect on the primary sex ratio, our sex-specific estimates of selection on adult body size will inform future models of adaptive ESD in this species and other copepods.

This study consists of two experiments. In the first experiment, we reared paired sibships at high and low densities to determine the effect of larval density on the primary sex ratio. We also compared siblings reared at high and low densities with respect to adult body size, female fecundity, and male mating success. Our density manipulation created two unnatural conditions: (1) females were mated several days after reaching sexual maturity, and (2) males competed over virgin adult females. Under natural conditions, males compete over sexually immature female copepodites and guard them until their terminal molt, after which mating immediately occurs (Haderlie et al. 1980; Burton 1985). Hence, in the second experiment we used naturally mated, field-sampled copepods to quantify fecundity and sexual selection on female and male body size, respectively.

In most harpacticoid copepods, females are generally larger than males (Hicks and Coull 1983). According to Gilbert and Williamson (1983), female-biased sexual size di-

Table 1. Larval density treatments in experiments 1A, 1B, and 1C.

Experiment	Source	Volume	Plate	Food	<i>N</i>	Well density	Absolute density
1A	Field (GH)	2 mL	24-well	12 drops, no flakes	23	1 nauplii/well	500 nauplii/L
						4 nauplii/well	2000 nauplii/L
1B	Field (AC)	10 mL	6-well	2 mL, ~0.2 mg flakes	50	2 nauplii/well	200 nauplii/L
						10 nauplii/well	1000 nauplii/L
1C	Lab (AC)	2 mL	24-well	4 × 10 <sup>6</sup> cells, ~0.2 mg flakes	72	1 nauplii/well	500 nauplii/L
						6 nauplii/well	3000 nauplii/L

Note: Shown are the source populations of *Tigriopus californicus* (field versus laboratory; GH, Gordon Head; AC, Arbutus Cove) from which the copepods were sampled, the volume of filtered seawater and the type of tissue culture plate (24-well or 6-well) in which the nauplii were reared, the amount of food that each well received (the volume or abundance of cultured *Isochrysis galbana* and the amount of Tetramin flakes), the sample size (*N*), the density of nauplii in wells, and the absolute density of nauplii for the low- and high-density treatments in all three experiments.

morphism in copepods suggests that selection is stronger on female than on male body size (but see Fairbairn 1997). From Gilbert and Williamson (1983), we predict that low-density environments will produce a female-biased primary sex ratio and that individuals developing in high-density environments will make the best of a bad lot by differentiating into small males. We also predict that individuals in low-density environments will be larger, more fecund (in the case of females), and more competitive (in the case of males) than their siblings in high-density environments and that the slope of the relationship between fitness and adult body size (i.e., the selection coefficient) will be steeper for females than for males.

## Materials and methods

### Experiment 1: Charnov–Bull model of adaptive ESD

#### General overview

In the summer of 2001, 2002, and 2003 we conducted experiments 1A, 1B, and 1C, respectively. The original purpose of experiment 1A was to investigate sexual selection on male body size using male–male competition trials for a single virgin adult female. In experiment 1A, we used differences in larval density to generate variation in male body size between high- and low-density competitors. The purpose of experiments 1B and 1C was to test the Charnov–Bull model of adaptive ESD in *T. californicus* with respect to larval density. Specifically, experiments 1B and 1C were designed to answer the following three questions: (1) Does larval density affect adult body size? (2) How does larval-density-induced variation in adult body size affect female fecundity and male reproductive success? and (3) Does larval density affect the primary sex ratio in a manner consistent with the Charnov–Bull model? Because male mating success in experiments 1B and 1C was measured in the same way as in experiment 1A, we were able to combine data from the three experiments.

#### Larval density treatments

We used low and high larval density treatments to create large and small individuals, respectively, and to test the effect of larval density on the primary sex ratio. In the low-density treatment, larval density ranged between 200 and 500 nauplii/L; in the high-density treatment, larval density ranged between 1000 and 3000 nauplii/L (Table 1). Our density treatments (200–3000 nauplii/L) were similar to the

ones used by Egloff (1966) (200–2000 individuals/L) and fall in the lower part of the range of densities observed in the field (200 to 20 000 individuals/L; Powlik 1998). The choice of this conservative effect size was chiefly governed by our desire to minimize larval mortality.

For experiment 1A, nauplii in the high- and low-density treatments were obtained from different females sampled from Gordon Head (48°29'48" N, 123°18'24" W), Victoria, British Columbia, Canada. For experiments 1B and 1C, we used a paired design in which nauplii from the same full-sib family were reared under both low and high densities. The advantage of this paired design is that it allows one to control for among-family effects on the sex ratio (Cook 2002; Yusa 2004), which have been shown to be important in *T. californicus* (Voordouw and Anholt 2002a, 2002b). In experiment 1B, we removed egg sacs from 50 field-captured females sampled from Arbutus Cove (48°28'36" N, 123°18'00" W), Victoria, British Columbia, Canada, obtained 20 F<sub>1</sub> offspring from each female, and haphazardly assigned the offspring to either the low-density treatment or the high-density treatment (i.e., 10 offspring per combination of family × density treatment). In experiment 1C, we removed egg sacs from 72 laboratory-reared females (originally sampled from Arbutus Cove), obtained 48 F<sub>1</sub> offspring from each female, and assigned the offspring to either density treatment (i.e., 24 offspring per combination of family × density treatment).

In all three experiments the nauplii were initially reared on a diet of cultured *Isochrysis galbana* cells. In experiments 1A and 1B, the density of the *I. galbana* solution was not known. For experiment 1C, we controlled algal density and abundance (2 mL of 2 × 10<sup>6</sup> cells/mL = 4.0 × 10<sup>6</sup> cells). From previous grazing experiments (unpublished data), we calculated daily per capita algal consumption rates for each stage (Appendix A). At 20 °C, development from a stage I nauplius to a sexually mature adult takes ~16 days, during which the average individual consumes 1.9 × 10<sup>6</sup> cells of *I. galbana*. Hence, in experiment 1C, the initial food supply of 4.0 × 10<sup>6</sup> cells was not enough for the six siblings in the high-density treatment to complete development (Table 1), and we calculate that they ran out of algae sometime during the third copepodite stage. To prevent individuals in the high-density treatment from starving to death (in experiments 1B and 1C), we added Tetramin® flakes at the third copepodite stage; for consistency, we also added flakes in the low-density treatment. We are confident that the

Tetramin supplement did not affect sex determination because sex is determined before the third copepodite stage in *T. californicus* (Burton 1985). Plates were stacked in plastic containers and stored in incubators at 20 °C with a 12 h light : 12 h dark cycle. We did not randomize the positions of the plates in the stack; however, we have previously shown that the effect of such microenvironmental variation among plates is small (Voordouw and Anholt 2002b). In experiments 1B and 1C, we sexed all individuals at adulthood to estimate the sex ratio.

#### *Larval density and adult body size*

For experiment 1A, high- and low-density males were visually selected to maximize size asymmetry in the subsequent competition trials. Hence, in experiment 1A, males were not randomly selected from the two treatments and we cannot use their difference in size to estimate the effect of larval density on male body size. For experiment 1B, we randomly selected one brother from each of the low- and high-density treatments for all of the families to determine the effect of larval density on male body size. For experiment 1C, we determined the effect of larval density on both male and female body size by randomly selecting one individual of each sex from each of the low- and high-density treatments for all of the families. For experiments 1B and 1C, these randomly selected individuals were also used in the subsequent fitness comparisons (i.e., female fecundity and male mating success) between the high- and low-density treatments. To prevent our measuring protocol from harming the contestants, we measured adult body size after conducting these fitness assays.

#### *Adult body size measurements*

In experiment 1A, male body size was measured as the width of the cephalosome. For males in experiment 1B and for males and females in experiment 1C, adult body size was defined as the distance between the tip of the rostrum and the last abdominal segment. To obtain adult body size measurements, copepods were mounted on a slide in three drops of water and immobilized with a cover slip. We viewed the slides with a Leica inverted microscope (2.5× magnification) and, after taking a photograph with a Nikon D1 digital camera, placed the slide in a petri dish filled with seawater. We flushed the cover slip off the slide using a pipette and recovered the individual (generally unharmed). This procedure allowed us to mount some individuals at least twice to estimate the repeatability of the mounting and measurement procedure. In a series of pilot experiments, the repeatability of male cephalosome width and female adult body length was found to be 0.62 and 0.52, respectively. This suggests that the variation in these measurements was due to actual size differences between individuals and not due to the mounting procedure.

#### *Larval density, female body size, and female fecundity*

In experiments 1B and 1C, we investigated the effect of larval density on female fitness by comparing fecundity (number of eggs per egg sac) between randomly sampled low- and high-density sisters from the same full-sib family. For experiments 1B and 1C, we obtained fecundity and body

size estimates from low- and high-density sisters for 19 and 42 families, respectively.

#### *Larval density, male body size, and male mating success*

In experiments 1A, 1B, and 1C, we investigated the effect of larval density on male mating success through male–male competition trials. In experiment 1A, competition occurred between males from different families, with one male from the low-density treatment and the other from the high-density treatment to ensure size asymmetry between competitors. This size asymmetry between high- and low-density males allowed us to assign treatment identity to the winner. In experiments 1B and 1C, we controlled for family effects by pairing randomly sampled high- and low-density brothers from the same full-sib family. Because the size asymmetry between the high- and low-density males was less pronounced than in experiment 1A, we randomly dyed one of the males with a drop of red food coloring to facilitate identification. In a pilot experiment, we determined that this dye protocol had no effect on the outcome of the mating trial.

In all of the experiments, we placed the two males in a mating arena (one well in a 6-well tissue culture plate stocked with 10 mL of seawater and *I. galbana*) and allowed them to acclimatize for a few minutes before adding one virgin adult female. Once the trial began, we scanned the mating arena every 5 min. When a male had clasped the female for two successive scans (>5 min), we terminated the trial and measured the body size of each male (as outlined above). For experiments 1A, 1B, and 1C, we conducted 23, 30, and 54 male–male competition trials, respectively. The number of successful trials in which we managed to measure the body size of both competitors was slightly less (23, 23, and 52, respectively).

#### **Experiment 2: Selection on adult body size of females and males**

##### *Fecundity selection on female body size*

On 20 July 2004, we sampled 80 gravid females from a pool in Arbutus Cove (48°28'36" N, 123°18'00" W), Victoria, British Columbia, Canada. We immediately removed all egg sacs and hatched these in glass spot plates to determine female fecundity. We placed each female in her own well in a 24-well tissue culture plate stocked with 2 mL of filtered seawater and abundant *I. galbana* for food. Once the female produced a second egg sac, we removed it as well and then measured the female's body size. Female body size was defined as the distance between the tip of the rostrum and the last abdominal segment and was measured using the protocol from experiment 1. The first and second egg sacs represent egg production in the field and the laboratory environment, respectively.

##### *Sexual selection on male body size*

On 30 July 2004, we obtained a field sample from Arbutus Cove that contained 112 adult males, 85 gravid females, and 163 solitary copepodites. Of the 112 adult males, 94 were in precopula with a female copepodite and 18 were single. To quantify sexual selection on male body size, we measured the body size of all 112 males after separating the 94 precopula pairs with a thin metal needle. Male body size

was defined and measured in the same way as female body size in experiment 2.

In the field sample, the proportion of single males was low (18/112 = 16.1%), so sexual selection on male body size (if any) was relatively weak. To increase the probability of detecting sexual selection, we conducted a laboratory experiment in which the operational sex ratio was more male-biased. We isolated 140 single males from several Arbutus Cove field samples and placed them in a 4.2-L plastic box containing 0.5 L of filtered seawater. After allowing the males to acclimatize for 1 h, we added 50 virgin female copepodites (obtained by separating 50 precopula pairs). Seventeen hours later, we recovered 47 precopula pairs, 93 single males, and 3 single female copepodites. The high proportion of single males in the laboratory sample (93/140 = 66.4%) means that sexual selection was much stronger in the laboratory than in the field sample.

## Statistical procedures

### *Experiment 1: Charnov–Bull model of adaptive ESD*

Individuals from the same full-sib family reared at high and low densities represent a paired experimental design. Hence, for normally distributed data such as adult body size and female fecundity, we used a paired-sample *t* test to determine whether larval density had a significant effect on these variables. For binomial data such as survivorship (dead or alive), male mating success (low-density male wins or loses the mating competition), and sex ratios (male or female), we used a generalized linear model (GLM) with a binomial error distribution using the `glm()` function in S-PLUS 4.5 (Mathsoft, Inc.). By including a “family” term in our models, we incorporate the paired nature of the experimental design and account for overdispersion in the data (Krackow and Tkadlec 2001; Wilson and Hardy 2002). We assessed significance of model terms using likelihood ratio tests that are asymptotically  $\chi^2$  distributed.

### *Experiment 2: Selection on adult body size in females and males*

#### *Fecundity selection on female body size*

We used a paired *t* test to determine whether fecundity changed between the first (field) egg sac and the second (laboratory) egg sac. We measured selection on female body size as the slope of the linear regression of fecundity on female body length. We conducted separate fecundity – body size regressions for the field and laboratory egg sacs. We did not use ANCOVA to compare the slopes and (or) intercepts because the two regressions are not independent (i.e., both regressions contain the same 80 females).

#### *Sexual selection on male body size*

We used an independent two-sample *t* test to compare mean body length between precopula and single males in both the field sample and the laboratory sample. We measured selection on male body size as the slope of the logistic regression of precopula success (precopula male = 1, single male = 0) on male body length for the field and laboratory samples combined.

## Results

### Experiment 1: Charnov–Bull model of adaptive ESD

#### *Survivorship*

Survivorship was defined as the number of sexually mature individuals at the time of the sex-ratio assay relative to the total number of nauplii with which the plate had been stocked (i.e., 10 and 24 nauplii for each combination of family  $\times$  treatment in experiments 1B and 1C, respectively). When plates were assayed for the sex ratio, adult males and females were counted regardless of whether they were dead or alive, whereas dead or living copepodites and missing individuals were assigned a value of zero because their sex could not be determined. In the high-density treatment, more individuals went missing and fewer dead individuals were recovered than in the solitary, low-density treatment. This suggests that the missing individuals in the high-density treatment were dead individuals that had been consumed by their siblings.

In experiment 1B, mean survivorship for the 50  $F_1$  families was  $81.6\% \pm 1.72\%$  and  $82.8\% \pm 2.23\%$  in the low- and high-density treatments, respectively. In experiment 1C, mean survivorship for the 72  $F_2$  families was  $89.4\% \pm 2.28\%$  and  $85.8\% \pm 2.45\%$  at low and high density, respectively. We used a GLM with binomial errors to test whether survivorship influenced the sex ratio in experiments 1B and 1C. The full model includes the terms “experiment”, “larval density treatment”, their interaction, the random factor “family”, and the covariate “survivorship”. The full model was a good fit to the data (residual *df* = 119, residual deviance = 119.735), and survivorship did not account for a significant portion of the deviance (*df* = 1,  $\Delta$ deviance = 0.092, *p* = 0.762). This result suggests that sex-specific differences in survivorship were unlikely to bias the analysis of the sex-ratio data.

#### *Larval density and adult body size*

In experiment 1B, males reared at low density ( $0.89 \pm 0.017$  mm) were significantly larger than their brothers in the high-density treatment ( $0.84 \pm 0.011$  mm; *t* = 2.780, *df* = 34, *p* = 0.009). In experiment 1C, males reared at low density ( $0.77 \pm 0.005$  mm) were the same size as their high-density brothers ( $0.77 \pm 0.005$  mm; *t* = 0.595, *df* = 58, *p* = 0.554), but low-density females ( $0.82 \pm 0.007$  mm) were significantly larger than their high-density sisters ( $0.79 \pm 0.008$  mm; *t* = 3.092, *df* = 53, *p* = 0.003). Taken together, these three results suggest that the density manipulation worked and that low-density adults are generally larger than their high-density siblings. Furthermore, delayed development of high-density individuals (M.J. Voordouw, personal observation) lends additional support to the conclusion that our density manipulation achieved the desired effect.

#### *Larval density and female fecundity*

In experiment 1B, the mean fecundity of low-density females ( $38.3 \pm 3.49$  offspring) was not significantly greater than that of their high-density sisters ( $32.4 \pm 4.36$  offspring; *t* = 1.500, *df* = 18, *p* = 0.151). In experiment 1C, the mean fecundity of the larger, low-density females ( $47.3 \pm 1.52$  offspring) was actually significantly less than that of their smaller, high-density sisters ( $53.3 \pm 2.07$  offspring; *t* =

**Table 2.** Analysis of deviance of mating trials in experiments 1A, 1B, and 1C.

(A) Maximum likelihood estimates.					
Experiment	Model No.	Model	No. of parameters	Deviance	
1A	1	$Y \sim B_0$	1	26.402	
	2	$Y \sim B_0 + B_1X_1$	2	20.594	
1B	3	$Y \sim B_0$	1	24.085	
	4	$Y \sim B_0 + B_1X_2$	2	20.536	
1C	5	$Y \sim B_0$	1	64.193	
	6	$Y \sim B_0 + B_1X_2$	2	64.171	
(B) Likelihood ratio tests.					
Experiment	Effect	Comparison	df	$\Delta$ Deviance	<i>p</i>
1A	$B_1$	1 vs. 2	1	5.808	0.016
1B	$B_1$	3 vs. 4	1	3.549	0.060
1C	$B_1$	5 vs. 6	1	0.022	0.883
(C) Parameter estimates.					
Experiment	Model No.	Estimate	df	<i>t</i>	<i>p</i>
1A	2	$B_0 = 1.49 \pm 0.680$	21	2.197	0.039
		$B_1 = 1.46 \pm 0.734$	21	1.995	0.059
1B	4	$B_0 = 1.71 \pm 0.729$	21	2.342	0.029
		$B_1 = 1.39 \pm 0.920$	21	1.505	0.147
1C	6	$B_0 = 0.81 \pm 0.301$	51	2.699	0.013
		$B_1 = -0.05 \pm 0.305$	51	0.147	0.884

**Note:** For experiments 1A, 1B, and 1C, the number of successful trials for which we managed to measure the body size of both competitors was 23, 23, and 52, respectively. For each trial, the low-density male either wins (outcome = 1) or loses (outcome = 0) the mating competition. (A) Maximum likelihood estimates of the deviance of models incorporating parameters for the difference in cephalothorax width ( $X_1$ ) and body length ( $X_2$ ). For each experiment,  $X_1$  or  $X_2$  was standardized to have a mean = 0 and a standard deviation = 1. Each model is given a numerical ID (Model No.) that is used in the likelihood ratio tests in (B) (Comparison). (B) Likelihood ratio tests of the effects of  $X_1$  and  $X_2$ . (C) Parameter estimates from the full model; the intercept of the model ( $B_0$ ) is the log odds of the low-density male winning the contest; the slope ( $B_1$ ) indicates the strength and direction of sexual selection on male body size.

2.593,  $df = 41$ ,  $p = 0.013$ ). Hence, there was no support for the idea that low larval density increased female fecundity, even though it increased female body size in experiment 1C. This may be because resources were not limiting in the laboratory environment.

#### *Larval density, male body size, and male mating success*

We used a GLM with binomial errors to test what factors influenced the outcome of the male mating trials (low-density male wins = 1, low-density male loses = 0) in experiments 1A, 1B, and 1C. The full model included the difference in body size between the two competitors, the “experiment”, and their interaction. Because male body size was measured differently in the three experiments (i.e., cephalosome width in 1A versus body length in 1B and 1C), we transformed the difference in body size to  $z$ -scores for each experiment. The full model fit the data well (residual  $df = 92$ , residual deviance = 105.302). The interaction term accounted for a significant portion of the deviance ( $df = 2$ ,  $\Delta$ deviance = 6.509,  $p = 0.039$ ). Hence, we analyzed the three experiments separately (Table 2).

The difference in body size accounted for a significant ( $p = 0.016$ ), a marginally nonsignificant ( $p = 0.060$ ), and a nonsignificant ( $p = 0.883$ ) portion of the deviance in experiments 1A, 1B, and 1C, respectively (Table 2). For experiments 1A and 1B, the estimates of the slope of the size difference ( $B_1$ ) were roughly the same (Table 2), indicating

that the intensity of sexual selection on male body size was similar in these two experiments. For these two experiments, the positive slope ( $B_1 > 0$ ) indicates that larger males were more likely to win the contest than smaller males. For all three experiments, the intercept ( $B_0$ ) was significantly greater than zero (Table 2), indicating that, all else being equal, the low-density male was significantly more likely to win the mating competition than the high-density male (71 of 98 trials,  $p < 0.001$ ).

#### *Larval density and the adult sex ratio*

In experiment 1B, across both density treatments and across all families, there were 552 males and 270 females, whereas in experiment 1C, there were 1587 males and 1461 females. The mean proportion of males ( $\pm 95\%$  confidence limit) in experiments 1B ( $0.67 \pm 0.033$ ) and 1C ( $0.52 \pm 0.018$ ) was significantly more male-biased than the expected 50:50 sex ratio (1B:  $\chi^2 = 96.060$ ,  $df = 1$ ,  $p < 0.001$ ; 1C:  $\chi^2 = 5.126$ ,  $df = 1$ ,  $p = 0.024$ ). In addition, the sex ratio in experiment 1B was significantly more male-biased than that in experiment 1C ( $\chi^2 = 58.993$ ,  $df = 1$ ,  $p < 0.001$ ).

We used a GLM with binomial errors to test the effect of larval density on the sex ratio in experiments 1B and 1C. The full model included the terms “experiment” and “larval density treatment”, their interaction, and a random factor for “family”. Because there is only one sex-ratio estimate for each combination of family and larval density treatment

(i.e., Latin square design), it is not possible to include a family  $\times$  treatment interaction term. The full model was a good fit to the data (residual  $df = 120$ , residual deviance = 119.827). The interaction between experiment and the larval density treatment accounted for a significant portion of the deviance ( $df = 1$ ,  $\Delta deviance = 6.549$ ,  $p = 0.010$ ) and so we analyzed the two experiments separately.

In experiment 1B, the mean proportion of adult males in the low-density treatment ( $0.71 \pm 0.027$ ) was more male-biased than that in the high-density treatment ( $0.64 \pm 0.030$ ), and this difference was statistically significant ( $df = 1$ ,  $\Delta deviance = 5.695$ ,  $p = 0.017$ ). In experiment 1C, the mean proportion of adult males in the low-density treatment ( $0.52 \pm 0.024$ ) was slightly less male-biased than that in the high-density treatment ( $0.54 \pm 0.023$ ), but this difference was not statistically significant ( $df = 1$ ,  $\Delta deviance = 0.895$ ,  $p = 0.344$ ). The results of experiment 1B suggest that high larval density feminizes putative males, but there was no effect of larval density on sex determination in experiment 1C.

#### Family effects on the adult sex ratio

The family term accounted for a significant portion of the deviance in the sex ratio in experiments 1B ( $df = 49$ ,  $\Delta deviance = 114.807$ ,  $p < 0.001$ ) and 1C ( $df = 71$ ,  $\Delta deviance = 461.899$ ,  $p < 0.001$ ). This family effect can also be visualized as a strong correlation of the proportion of males between the low- and high-density treatments in experiments 1B ( $r = 0.470$ ,  $n = 50$ ,  $p < 0.001$ ) and 1C ( $r = 0.727$ ,  $n = 72$ ,  $p < 0.001$ ; Fig. 1). These significant differences in the sex ratio among families suggest that there is genetic variation for the primary sex ratio in *T. californicus*.

#### Experiment 2: Selection on adult body size in females and males

##### Fecundity selection on female body size

For females, body length ranged between 0.74 and 0.97 mm (mean =  $0.83 \pm 0.005$  mm,  $N = 70$ ) and average fecundity (for the two consecutive egg sacs) ranged between 26.0 and 80.0 eggs per clutch (mean =  $52.9 \pm 1.25$  eggs per clutch,  $N = 80$ ). The first (field) clutch ( $47.3 \pm 1.40$  eggs per clutch,  $N = 66$ ) was significantly smaller ( $t = 8.265$ ,  $df = 65$ ,  $p < 0.001$ ) than the second (laboratory) clutch ( $61.0 \pm 1.75$  eggs per clutch,  $N = 66$ ), suggesting that egg production in *T. californicus* females is resource-limited in the field.

The regression of the size of the first (field) clutch on body size was statistically significant ( $F_{[1,68]} = 5.414$ ,  $p = 0.023$ , slope =  $66.6 \pm 28.62$  eggs/mm; Fig. 2) but the regression of the size of the second (laboratory) clutch on body size was not ( $F_{[1,60]} = 2.199$ ,  $p = 0.143$ , slope =  $56.9 \pm 38.39$  eggs/mm; Fig. 2). This suggests that fecundity selection on female body size is stronger in the field than in the laboratory, and this is expected if egg production is resource-limited. After averaging the field and laboratory clutches for each female, the equation for the regression of fecundity versus body length is  $Y = 4.1 + 59.7 \times \text{body length}$  ( $F_{[1,68]} = 4.249$ ,  $p = 0.043$ ; intercept SE = 24.03, slope SE = 28.94).

##### Sexual selection on male body size

In the field sample, the mean body length of the single males ( $0.74 \pm 0.013$  mm,  $N = 18$ , range = 0.66–0.89 mm) was slightly larger than that of the precopula males ( $0.72 \pm$

Fig. 1. Correlation of the proportion of males between full sibs (from the same egg sac) exposed to the high- and low-density treatments in experiment 1C. For each *Tigriopus californicus* family (data point), there are 24 offspring in the high-density treatment and 24 offspring in the low-density treatment.

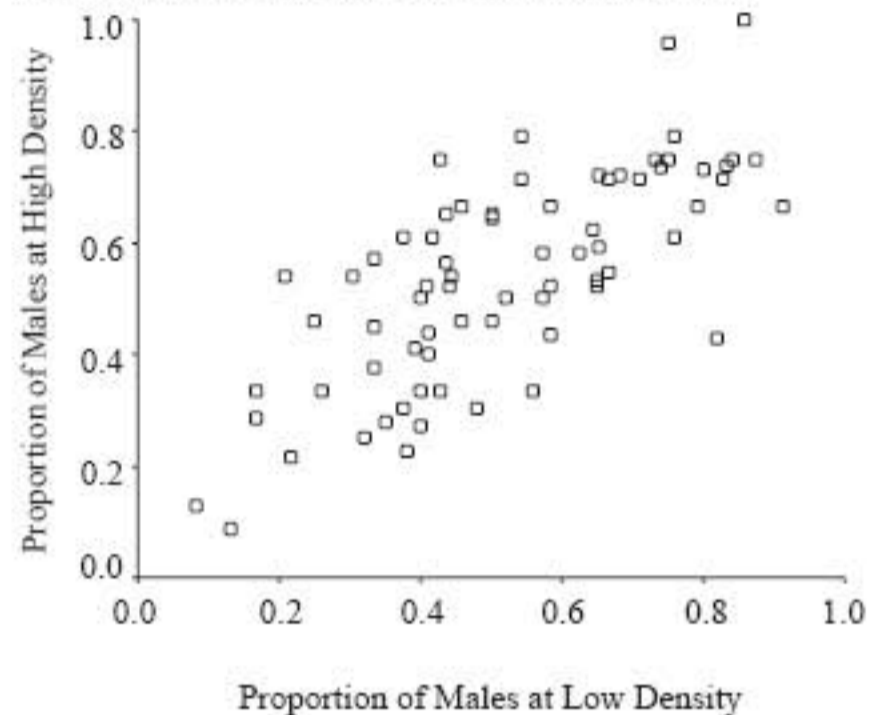
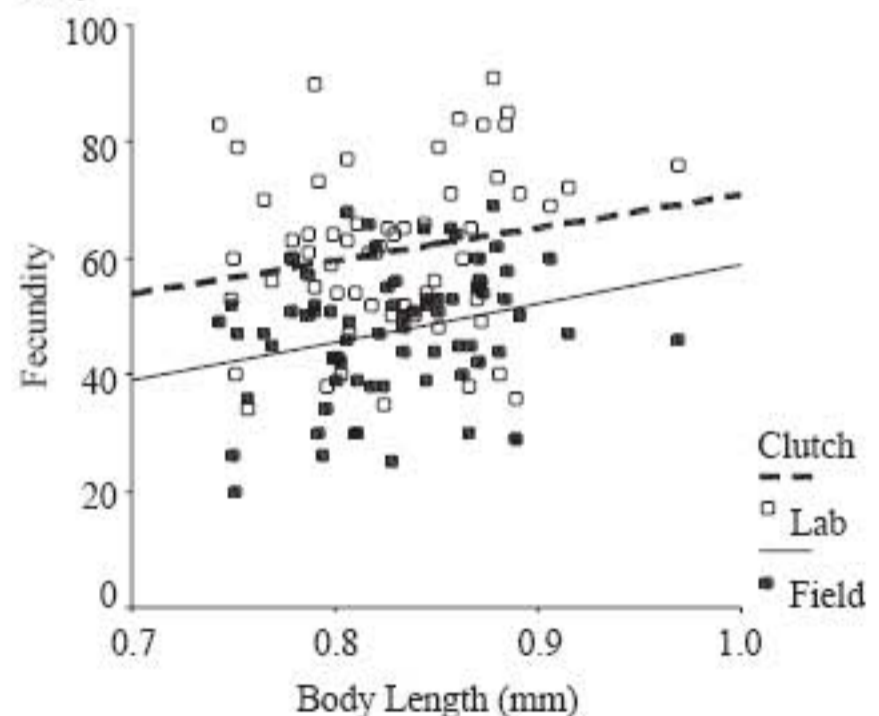


Fig. 2. Relationship between fecundity (number of eggs per clutch) and body length in *T. californicus* females for the first clutch, born in the field, and the second clutch, born in the laboratory.



$0.006$  mm,  $N = 94$ , range = 0.53–0.85 mm), although the difference was not statistically significant ( $t = 1.260$ ,  $df = 110$ ,  $p = 0.210$ ; Fig. 3). Likewise, for the laboratory sample the mean body length of the single males ( $0.74 \pm 0.004$  mm,  $N = 93$ , range = 0.65–0.82 mm) was larger than that of the precopula males ( $0.72 \pm 0.008$  mm,  $N = 47$ , range = 0.62–0.88 mm), and in this case the difference was statistically significant ( $t = 2.383$ ,  $df = 138$ ,  $p = 0.019$ ; Fig. 3).

When field and laboratory samples were combined, the mean body length of single males ( $0.74 \pm 0.004$  mm,  $N = 111$ ) was significantly larger ( $t = 2.435$ ,  $df = 250$ ,  $p = 0.016$ ) than that of the precopula males ( $0.72 \pm 0.005$  mm,  $N =$

141). The logistic regression equation is  $Y = 1/(1 + \exp(-Z))$ , where  $Y$  is the probability of a male being in the precopula state,  $X$  is male body length,  $Z = b_0 + b_1X$ ,  $b_0 = 4.77 \pm 1.906$  (residual  $df = 1$ , residual deviance = 6.262,  $p = 0.012$ ), and  $b_1 = -6.22 \pm 2.607$  (residual  $df = 1$ , residual deviance = 5.688,  $p = 0.017$ ). The negative value of the logistic regression coefficient for male body size ( $b_1$ ) suggests that smaller males are significantly more likely to be found in precopula than larger ones.

#### Differential selection on body size in males and females

To compare the strength of selection on adult body size between the sexes, we used the female fecundity selection (average fecundity =  $4.1 + 59.7 \times$  female body length) and male sexual selection (probability of precopula =  $1/[1 + \exp(-4.77 + 6.22 \times$  male body length)]) equations to generate fitness values (average fecundity for females, probability of precopula for males) for a range of body lengths (0.5–1.0 mm). For each sex, we standardized these fitness values to relative fitness (individual fitness divided by the mean fitness for that sex). As body size increased, relative fitness increased for females but decreased for males (Fig. 4). With respect to larval density and the Charnov–Bull model of adaptive ESD, this data suggests that large, low-density individuals should differentiate as females.

## Discussion

The primary objective of this study was to determine whether larval density affects sex determination in *T. californicus*. Although the high-density treatment produced significantly more adult males in experiment 1B, there was no effect of larval density on the adult sex ratio in experiment 1C. In terms of absolute density, the effect size in experiment 1C (high density = 3000 nauplii/L) was three times that in experiment 1B (high density = 1000 nauplii/L; Table 1) and cannot account for the lack of a density effect in experiment 1C. A post hoc power analysis found that in experiment 1C there was a 60% chance of detecting the level of ESD found in experiment 1B and a 90% chance of detecting a difference in the proportion of males between the high- and low-density treatments  $>0.10$ . Across the two experiments (1B and 1C) and weighted by sample size, the mean difference in the proportion of males between the low- and high-density treatments was  $<0.02$ . This small effect size suggests that larval density has a negligible role in the sex determination of *T. californicus* compared with the differences among families, which can range from  $<20\%$  to  $>90\%$  males (Fig. 1).

Our results are similar to those of Egloff (1966), who also found that larval density did not affect the primary sex ratio in *T. californicus*. Egloff (1966) used a similar range of densities (low, intermediate, and high densities of 200, 1000, and 2000 nauplii/L, respectively) and had high survivorship ( $>96\%$ ); however, he had no data to show that larval-density-induced food limitation actually occurred. Our calculations of the daily per capita algal consumption rates during development (Appendix A) indicate that high-density individuals were food-limited in experiment 1C. Further evidence for density-induced food limitation comes from the observation that high-density individuals took longer to reach sexual maturity and were significantly smaller than their low-density

Fig. 3. Male body length versus precopula status (single or precopula) in the sexual selection assays conducted in experiment 2 with field and laboratory samples of *T. californicus*. The box represents the interquartile range that contains 50% of the values. The line across the box indicates the median. The whiskers represent the highest and lowest values, excluding outliers. Outliers (○) are values that are between 1.5 and 3 box lengths from the upper or lower edge of the box. Extremes (\*) are values that are more than 3 box lengths from the upper or lower edge of the box.

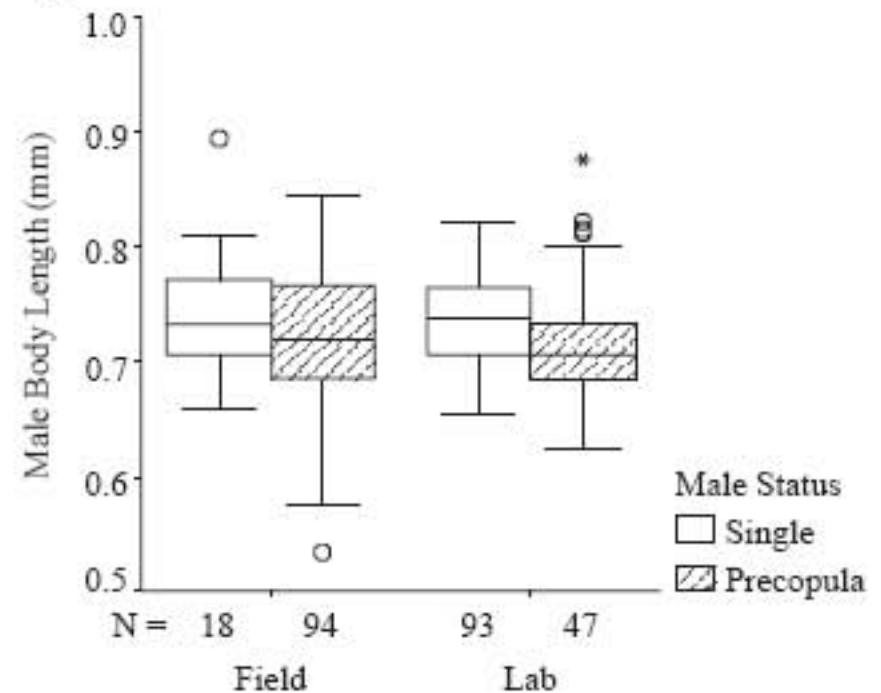
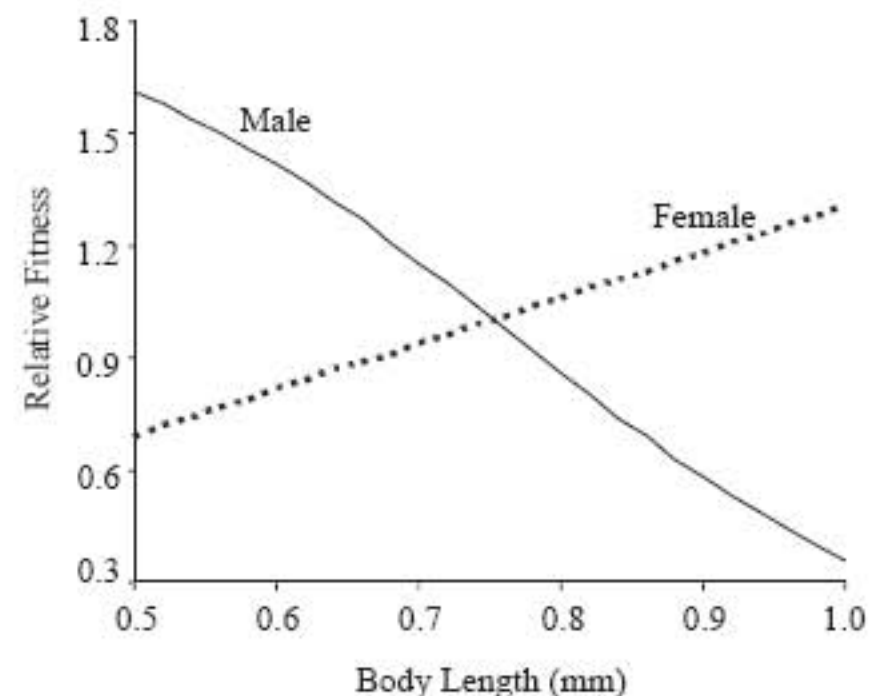


Fig. 4. Relative fitness versus body length for male (solid line) and female (stippled line) *T. californicus*.



siblings. Hence, our density treatments provided a relevant manipulation of the opportunity for future growth as required by the Charnov–Bull model of adaptive ESD.

Egloff (1966) used nauplii from 80 egg sacs and assigned them at random to one of three density treatments. In contrast, we used a paired design in which nauplii from the same full-sib family were reared under both low and high density. The advantage of this paired design is that it allows one to assess the among-family variation in the primary sex ratio (Cook 2002; Yusa 2004), which is clearly significant in

*T. californicus* (i.e., the probability of differentiating into a male ranges from  $<0.20$  to  $>0.90$ ; Fig. 1). Voordouw and Anholt (2002a) also found that differences among families accounted for much more of the variation in the primary sex ratio than differences in temperature. Genetic variation for the primary sex ratio seems to be a common phenomenon in organisms with ESD (Bull et al. 1982; Conover and Heins 1987b; Watt and Adams 1994), suggesting that ESD might play some role in maintaining this variation (Bulmer and Bull 1982).

The mean proportion of males was significantly male-biased in both experiment 1B and experiment 1C. In contrast to the highly female-biased sex ratios observed in calanoid (Irigoiien et al. 2000), vent (Tsurumi et al. 2003), and harpacticoid copepods (Hicks and Coull 1983), the primary sex ratio in *T. californicus* and closely related species is often male-biased (Battaglia 1958; Igarashi 1963; Voordouw and Anholt 2002a). Adaptive ESD theory predicts a primary sex ratio that is biased towards the sex produced in the lower quality environment (Bull 1981; Charnov and Bull 1989). If ESD plays a role in the sex determination of *T. californicus*, then the male-biased primary sex ratio suggests that males are the "cheaper" sex, as we have previously argued (Voordouw and Anholt 2002a). Recent work has led us to suspect that an unidentified paternally transmitted genetic element, rather than ESD, is responsible for the male-biased primary sex ratio in *T. californicus* (Voordouw et al. 2005). With respect to the rearing methodology in future experiments, one encouraging conclusion that can be drawn from Voordouw and Anholt (2002a) and the present study is that the sex-ratio trait in *T. californicus* appears to be robust to substantial variation in temperature and food availability.

The second objective of this study was to determine whether larval-density-induced variation in adult body size has different fitness consequences between the sexes. In laboratory-reared copepods (experiments 1B and 1C), and in contrast to our predictions, large, low-density females did not produce significantly more eggs than their smaller, high-density sisters but large, low-density males had significantly higher mating success than their smaller, high-density brothers. This pattern of selection was reversed in the field sample (Fig. 4), where large females produced significantly more eggs and small males were significantly more likely to be found in the precopula state. What accounts for these contradictory results between the laboratory and the field?

In the field-sampled females (experiment 2), the first (field-born) clutch was much smaller than the second (laboratory-born) clutch, suggesting that egg production is food-limited in the field (Fig. 2). That a statistically significant relationship was found between female body size and the size of the field-born clutch but not between female body size and the size of the laboratory-born clutch further suggests that fecundity selection on female body size is more likely to be detected in food-limited environments. Similarly, while laboratory-reared females (experiments 1B and 1C) in the high-density treatment were initially food-limited, the Tetramin flake food supplement (at the third copepodite stage) may have subsequently obscured differences in egg production related to body size. With respect to the laboratory-reared females, another possible confounding factor is that the high-density females may have benefited from scaveng-

ing their dead siblings. Scavenging is inferred from the observation that, typically, fewer dead individuals were recovered in the high-density treatments than in the solitary, low-density treatments.

The conflicting role of male body size in this study may reflect the possibility that experiments 1 and 2 are actually measuring different aspects of sexual selection. In the male-male competition trials of experiment 1, the gradient of the male body size effect (Table 2) reflects the efficacy of the larval density treatments. In experiment 1A, males were visually selected to maximize size asymmetry, and male mating success was significantly related to male body size ( $p = 0.016$ ). In experiment 1B, the average difference in body size between low- (0.89 mm) and high-density males (0.84 mm) was small, which made selection more difficult to detect ( $p = 0.060$ ). Finally, in experiment 1C, there was no effect of larval density on male body size and it is therefore not surprising that we failed to detect sexual selection on this variable ( $p = 0.883$ ).

If body size does influence male mating success in *T. californicus*, the results are consistent with other studies that have shown the importance of male body size in crustaceans with precopulatory mate-guarding (Jormalainen 1998). Sexual selection on male body size has been reported in a calanoid copepod (Grad and Maly 1988, 1992) and several amphipods (Ward 1988; Bertin and Cezilly 2003). In amphipods and isopods, large males are capable of displacing smaller males from their mates (called a take-over, Jormalainen et al. 1994) and are better at subduing females that actively struggle against male clasping attempts (Jormalainen and Merilaita 1993, 1995). In addition, female mate choice may have influenced male mating success in experiment 1 if virgin adult females are more likely to resist smaller, less attractive males, as has been shown in isopods (Jormalainen and Merilaita 1993, 1995). By contrast, in experiment 2, female resistance was unlikely to play a role because the female copepodite is less than half the size of the adult male.

In experiment 2, we found that smaller males were more likely to be in the precopula state than larger males (in both field and laboratory samples). Several authors have pointed out that when sexually receptive females are rare, male mating success may depend more on encounter rates than on male-male interactions (Trivers 1972; Ghiselin 1974; Reiss 1989). Under this type of scramble competition, selection will favor small, mobile males over large, dominant ones (Fairbairn and Preziosi 1994). However, the mating success of smaller males is not necessarily guaranteed if larger males are capable of take-overs (Jormalainen 1998). Recent trials using dyed males have shown that take-overs do in fact occur in *T. californicus*, although they are not common (G. Stebbins, personal observation). Our results therefore suggest conflicting selection pressures on male body size: small males are more likely to find and clasp sexually immature females, but larger males are more likely to win mating competitions.

In conclusion, we found no compelling evidence that larval density plays a biologically significant role in the sex determination of *T. californicus*. If ESD plays a role in structuring the male bias and the among-family sex-ratio variation in *T. californicus*, this study shows that larval den-

sity is unlikely to be the cause. Hence, the factors that maintain the sex-ratio trait in *T. californicus* and other harpacticoid copepods have yet to be elucidated. In our experiments, the power to detect selection on adult body size was often limited by measurement error (i.e., 48% in the case of adult body length). Despite these limitations, and to our knowledge, this study is the first attempt at measuring sex-specific differences in the strength of selection on adult body size in any copepod and the data can be used to inform models of ESD, sexual size dimorphism, protandry, and other phenomena that play a prominent role in the biology of copepods and other crustaceans. Future experiments measuring body size selection should focus on reducing this measurement error and on searching for natural variation in body size among populations of *T. californicus* and other copepods.

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## References

- Adams, J., Greenwood, P.J., and Naylor, C. 1987. Evolutionary aspects of environmental sex determination. *Int. J. Invertebr. Reprod. Dev.* **11**: 123–136.
- Anholt, B.R. 1991. Measuring selection on a population of damselflies with a manipulated phenotype. *Evolution*, **45**: 1091–1106.
- Baker, B.S., and Ridge, K.A. 1980. Sex and the single cell. I. On the action of major loci affecting sex determination in *Drosophila melanogaster*. *Genetics*, **94**: 384–423.
- Battaglia, B. 1958. Selezione e rapporto sessi nel Copepode Harpacticoido *Tisbe gracilis* (T. Scott). *Atti Accad. Naz. Lincei, Rend.* **24**: 327–335.
- Becheikh, S., Michaud, M., Thomas, F., Raibaut, A., and Renaud, F. 1998. Roles of resource and partner availability in sex determination in a parasitic copepod. *Proc. R. Soc. Lond. B Biol. Sci.* **265**: 1153–1156.
- Bertin, A., and Cezilly, F. 2003. Sexual selection, antennae length and the mating advantage of large males in *Asellus aquaticus*. *J. Evol. Biol.* **16**: 698–707.
- Blackmore, M.S., and Charnov, E.L. 1989. Adaptive variation in environmental sex determination in a nematode. *Am. Nat.* **134**: 817–823.
- Bull, J.J. 1981. Sex ratio evolution when fitness varies. *Heredity*, **46**: 9–26.
- Bull, J.J. 1983. Evolution of sex determining mechanisms. Benjamin/Cummings Publ. Co., Menlo Park, Calif.
- Bull, J.J., and Charnov, E.L. 1988. How fundamental are Fisherian sex ratios? *Oxf. Surv. Evol. Biol.* **5**: 96–135.
- Bull, J.J., Vogt, R.C., and Bulmer, M.G. 1982. Heritability of sex ratio in turtles with environmental sex determination. *Evolution*, **36**: 333–341.
- Bulmer, M.G., and Bull, J.J. 1982. Models of polygenic sex determination and sex ratio control. *Evolution*, **36**: 13–26.
- Bulnheim, H.P. 1967. On the influence of the photoperiod on sex realization in *Gammarus duebeni*. *Helgol. Wiss. Meeresunters.* **16**: 69–83.
- Burton, R.S. 1985. Mating system of the intertidal copepod *Tigriopus californicus*. *Mar. Biol. (Berl.)*, **86**: 247–252.
- Charnov, E.L., and Bull, J.J. 1977. When is sex environmentally determined? *Nature (Lond.)*, **266**: 828–830.
- Charnov, E.L., and Bull, J.J. 1989. The primary sex ratio under environmental sex determination. *J. Theor. Biol.* **139**: 431–436.
- Conover, D.O. 1984. Adaptive significance of temperature-dependent sex determination in a fish. *Am. Nat.* **123**: 297–313.
- Conover, D.O., and Heins, S.W. 1987a. Adaptive variation in environmental and genetic sex determination in a fish. *Nature (Lond.)*, **326**: 496–498.
- Conover, D.O., and Heins, S.W. 1987b. The environmental and genetic components of sex ratio in *Menidia menidia* (Pisces, Atherinidae). *Copeia*, 1987: 732–743.
- Conover, D.O., and Kynard, B.E. 1981. Environmental sex determination: interaction of temperature and genotype in a fish. *Science (Wash., D.C.)*, **213**: 577–579.
- Cook, J.M. 2002. Sex determination in invertebrates. *In Sex ratios: concepts and research methods. Edited by I.C.W. Hardy.* Cambridge University Press, Cambridge. pp. 178–194.
- Egloff, D.A. 1966. Biological aspects of sex ratio in experimental field populations of the marine copepod *Tigriopus californicus*. Ph.D. thesis, Department of Biological Sciences, Stanford University, Stanford, Calif.
- Fairbairn, D.J. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annu. Rev. Ecol. Syst.* **28**: 659–687.
- Fairbairn, D.J., and Preziosi, R.F. 1994. Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis*. *Am. Nat.* **144**: 101–118.
- Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.
- Ghiselin, M.T. 1974. The economy of nature and the evolution of sex. University of California Press, Berkeley, Calif.
- Gilbert, J.J., and Williamson, C.E. 1983. Sexual dimorphism in zooplankton (Copepoda, Cladocera, and Rotifera). *Annu. Rev. Ecol. Syst.* **14**: 1–33.
- Grad, G., and Maly, E.J. 1988. Sex size ratios and their influence on mating success in a calanoid copepod. *Limnol. Oceanogr.* **33**: 1629–1634.
- Grad, G., and Maly, E.J. 1992. Further observations relating sex size ratios to mating success in calanoid copepods. *J. Plankton Res.* **14**: 903–913.
- Haderlie, E.C., Abbott, D.P., and Caldwell, R.L. 1980. Three other crustaceans: a copepod, a leptostracan, and a stomatopod. *In Intertidal invertebrates of California. Edited by E.C. Haderlie, D.P. Abbott, and R.L. Caldwell.* Stanford University Press, Stanford, Calif. pp. 631–635.
- Hatcher, M.J., and Dunn, A.M. 1997. Size and pairing success in *Gammarus duebeni*: can females be too big? *Anim. Behav.* **54**: 1301–1308.
- Hicks, G.R.F., and Coull, B.C. 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. Mar. Biol. Annu. Rev.* **21**: 67–175.
- Hopcroft, R.R., and Roff, J.C. 1998. Zooplankton growth rates: the influence of female size and resources on egg production of tropical marine copepods. *Mar. Biol. (Berl.)*, **132**: 79–86.
- Igarashi, S. 1963. The primary sex ratio of a marine copepod, *Tigriopus japonicus*. *Sci. Rep. Tohoku Univ. Fourth Ser. (Biol.)*, **29**: 73–81.

- Irigoin, X., Obermuller, B., Head, R.N., Harris, R.P., Rey, C., Hansen, B.W. et al. 2000. The effect of food on the determination of sex ratio in *Calanus* spp.: evidence from experimental studies and field data. *ICES J. Mar. Sci.* **57**: 1752–1763.
- Janzen, F.J. 1992. Heritable variation for sex ratio under environmental sex determination in the common snapping turtle (*Chelydra serpentina*). *Genetics*, **131**: 155–161.
- Janzen, F.J. 1994. Climate change and temperature-dependent sex determination in reptiles. *Proc. Natl. Acad. Sci. U.S.A.* **91**: 7487–7490.
- Jormalainen, V. 1998. Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. *Q. Rev. Biol.* **73**: 275–304.
- Jormalainen, V., and Merilaita, S. 1993. Female resistance and precopulatory guarding in the isopod *Idotea baltica* (Pallas). *Behaviour*, **125**: 219–231.
- Jormalainen, V., and Merilaita, S. 1995. Female resistance and duration of mate-guarding in three aquatic peracarids (Crustacea). *Behav. Ecol. Sociobiol.* **3**: 43–48.
- Jormalainen, V., Merilaita, S., and Tuomi, J. 1994. Male choice and male–male competition in *Idotea baltica* (Crustacea, Isopoda). *Ethology*, **96**: 46–57.
- Kiorboe, T., and Sabatini, M. 1995. Scaling of fecundity, growth and development in marine planktonic copepods. *Mar. Ecol. Prog. Ser.* **120**: 285–298.
- Korpelainen, H. 1990. Sex ratios and conditions required for environmental sex determination in animals. *Biol. Rev. Camb. Philos. Soc.* **65**: 147–184.
- Krackow, S., and Tkadlec, E. 2001. Analysis of brood sex ratios: implications of offspring clustering. *Behav. Ecol. Sociobiol.* **50**: 293–301.
- Lagomarsino, I.V., and Conover, D.O. 1993. Variation in environmental and genotypic sex-determining mechanisms across a latitudinal gradient in the fish, *Menidia menidia*. *Evolution*, **47**: 487–494.
- McCabe, J., and Dunn, A.M. 1997. Adaptive significance of environmental sex determination in an amphipod. *J. Evol. Biol.* **10**: 515–527.
- Michaud, M., de Meeus, T., and Renaud, F. 2004. Environmental sex determination in a parasitic copepod: checking heterogeneity and unpredictability of the environment. *Mar. Ecol. Prog. Ser.* **269**: 163–171.
- Naylor, C., Adams, J., and Greenwood, P. 1988a. Population dynamics and adaptive sexual strategies in a brackish water crustacean, *Gammarus duebeni*. *J. Anim. Ecol.* **57**: 493–507.
- Naylor, C., Adams, J., and Greenwood, P.J. 1988b. Variation in sex determination in natural populations of a shrimp. *J. Evol. Biol.* **1**: 355–368.
- Orzack, S.H. 2002. Using sex ratios: the past and the future. *In* Sex ratios: concepts and research methods. *Edited by* I.C.W. Hardy. Cambridge University Press, Cambridge. pp. 383–398.
- Powluk, J.J. 1998. Seasonal abundance and population flux of *Tigriopus californicus* (Copepoda: Harpacticoida) in Barkley Sound, British Columbia. *J. Mar. Biol. Assoc. U.K.* **78**: 467–481.
- Reiss, M.J. 1989. The allometry of growth and reproduction. Cambridge University Press, Cambridge.
- Rhen, T., and Lang, J.W. 1998. Among-family variation for environmental sex determination in reptiles. *Evolution*, **52**: 1514–1520.
- Trivers, R.L. 1972. Parental investment and sexual selection. *In* Sexual selection and the descent of man, 1871–1971. *Edited by* B.G. Campbell. Aldine, Chicago. pp. 136–179.
- Tsurumi, M., de Graaf, R., and Tunnicliffe, V. 2003. Distributional and biological aspects of copepods at hydrothermal vents on the Juan de Fuca Ridge, north-east Pacific Ocean. *J. Mar. Biol. Assoc. U.K.* **83**: 469–477.
- Voordouw, M.J., and Anholt, B.R. 2002a. Environmental sex determination in a splash pool copepod. *Biol. J. Linn. Soc.* **76**: 511–520.
- Voordouw, M.J., and Anholt, B.R. 2002b. Heritability of sex tendency in a harpacticoid copepod, *Tigriopus californicus*. *Evolution*, **56**: 1754–1763.
- Voordouw, M.J., Robinson, H.E., and Anholt, B.R. 2005. Paternal inheritance of the primary sex ratio in a copepod. *J. Evol. Biol.* In press. doi: 10.1111/j.1420-9101.2005.00922.x
- Ward, P.I. 1988. Sexual selection, natural selection, and body size in *Gammarus pulex* (Amphipoda). *Am. Nat.* **131**: 348–359.
- Watt, P.J., and Adams, J. 1994. Adaptive variation in sex determination in a crustacean, *Gammarus duebeni*. *J. Zool. (Lond.)*, **232**: 109–116.
- Williams, G.C. 1979. The question of adaptive sex ratio in outcrossed vertebrates. *Proc. R. Soc. Lond. B Biol. Sci.* **205**: 567–580.
- Wilson, K., and Hardy, I.C.W. 2002. Statistical analysis of sex ratios: an introduction. *In* Sex ratios: concepts and research methods. *Edited by* I.C.W. Hardy. Cambridge University Press, Cambridge. pp. 48–92.
- Yamahira, K., and Conover, D.O. 2003. Interpopulation variability in temperature-dependent sex determination of the tidewater silverside *Menidia peninsulae* (Pisces: Atherinidae). *Copeia*, **2003**: 155–159.
- Yusa, Y. 2004. Brood sex ratio in the apple snail *Pomacea canaliculata* (Gastropoda: ampullariidae) is determined genetically and not by environmental factors. *J. Mollusc. Stud.* **70**: 269–275.

## Appendix A

*Tigriopus californicus* follows the typical mode of copepod development (Haderlie et al. 1980), with six naupliar stages (N1–N6) and six copepodite stages (C1–C6). In previous grazing experiments on *T. californicus*, we determined that at 20 °C, the average stage I nauplius (N1) consumes 9000 *Isochrysis galbana* cells per day and the average adult (C6) consumes 455 000 *I. galbana* cells per day. We modeled the stage-specific daily per capita algal consumption rate ( $Q$ ) at 20 °C as an exponential growth equation:  $Q_{n+i} = Q_n e^{ri}$ , where  $Q_{n+i}$  and  $Q_n$  are the daily algal consumption rates of the  $(n+i)^{\text{th}}$  and  $n^{\text{th}}$  stages, respectively;  $r$  is the stage-specific instantaneous rate of increase in the daily algal consumption rate; and  $i$  is the number of stages between the  $(n+i)^{\text{th}}$  and  $n^{\text{th}}$  stages. At 20 °C,  $r = \ln(Q_{C6}/Q_{N1}) / (C6 - N1) = \ln(455\,000 / 9000) / (11 \text{ stages}) = 0.3566$  per stage. To calculate the cumulative number of algal cells consumed from birth to adulthood at 20 °C, we assumed that the average individual spent 1 day in each of the six naupliar stages (N1–N6) and 2 days in each of the five copepodite stages (C1–C5) for a total of 16 days from N1 to C6. The average individual consumes  $1.9 \times 10^6$  cells of *I. galbana* from birth to adulthood (Table A1).

**Table A1.** For *Tigriopus californicus* at 20 °C, this table shows the stage-specific daily per capita *Isochrysis galbana* consumption rates, the duration of each stage, and the total number of *I. galbana* cells consumed during each stage.

Stage	Consumption rate (10 <sup>3</sup> cells/day)	Stage duration (days)	Total consumption (10 <sup>3</sup> cells)
N1	9.0	1.0	9.0
N2	13.0	1.0	13.0
N3	18.0	1.0	18.0
N4	26.0	1.0	26.0
N5	37.0	1.0	37.0
N6	54.0	1.0	54.0
C1	76.0	2.0	153.0
C2	109.0	2.0	219.0
C3	156.0	2.0	312.0
C4	223.0	2.0	446.0
C5	318.0	2.0	637.0
C6	455.0	—	—
Total		16.0	1924.0

**Note:** Daily stage-specific algal consumption rates for stage 1 nauplii (N1) and stage 6 copepodites (C6) were obtained from grazing experiments. Stage-specific algal consumption rates for the other 10 stages were interpolated using the exponential growth model in Appendix A.