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# Environmental sex determination in a splash pool copepod

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The sex-determining mechanism has important demographic and genetic consequences by virtue of its effect on the population sex ratio. Here we investigate the effect of temperature dependent sex determination (TSD) on the primary sex ratio of the harpacticoid copepod, *Tigriopus californicus*. At the two experimental temperatures (15° and 22°C) used in this study, the primary sex ratio is almost always biased in favour of males. Higher temperatures induce masculinization and the change in sex ratio is not caused by differential mortality of the sexes. The mean level of TSD in the population is small (proportion of males increases by ~5% between 15° and 22°C) because only one-third of the families actually exhibit a significant sex-ratio response while the rest of the population is insensitive to temperature. A comparison of the primary sex ratio and the level of TSD between two locations reveals few differences among populations. Finally, individuals still exhibited TSD after having been maintained under constant temperature conditions in the lab for several generations. In addition the proportion of temperature-sensitive individuals remained unchanged. This suggests that the observed level of TSD is not an artefact of testing field-captured individuals in a novel laboratory environment. At this point the adaptive significance of temperature-dependent sex determination in *T. californicus* remains unknown.

ADDITIONAL KEYWORDS: harpacticoid copepod – phenotypic plasticity – primary sex ratio – temperature-dependent sex determination – *Tigriopus californicus*.

## INTRODUCTION

In sexually reproducing organisms there is a wide variety of sex-determining mechanisms (Bull, 1983). The significance of this diversity becomes evident when we consider how sex-determining mechanisms affect population processes. The sex-determining mechanism drives the population sex ratio and sets the effective population size. These in turn have important consequences for the demographic and genetic properties of sexually reproducing populations (Bulmer & Bull, 1982; Bull & Charnov, 1988). This is particularly true of organisms with environmental sex determination (ESD) where sex is determined sometime after conception by an environmental factor (Adams, Greenwood & Naylor, 1987; Bull, 1983; Korpelainen, 1990). In these systems, the population sex ratio is driven by the sensitivity of the sex-determining mechanism to the environmental factor and by the range of environmental variation (Bulmer & Bull, 1982). Extreme environmental fluctuations can result in highly biased sex ratios that may predispose the population towards extinction (Bulmer & Bull, 1982). This raises the question of why such sex-determining mechanisms exist and how they manage to persist over time. The fact that these mechanisms have persisted suggests that ESD may have some adaptive benefit.

Charnov & Bull (1977) were the first to propose an adaptive explanation for ESD. They pointed out that if the environment is patchy and if one sex has a higher fitness in certain patches than the other, selection would favour those individuals that develop into the optimum sex for that particular patch. Two other conditions of their model are that individuals have no control over the patch in which they are born and mating takes place among individuals from all patches (Charnov & Bull, 1977). The term 'patch' suggests that the different environments are separated on a spatial scale but the stratification may also be temporal

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(Adams *et al.*, 1987). The distinction between spatial and temporal patches is useful because the two types of ESD tend to be associated with different life histories. ESD based on spatial patches is relatively common in parasitic organisms where the size or quality of the host has major fitness consequences for the parasite (Hipeau, 1988; Blackmore & Charnov, 1989). In contrast, temporally based ESD appears to be restricted to organisms living in seasonal habitats where the recruitment date determines the potential for future growth (Conover & Heins, 1987b; Naylor, Adams & Greenwood, 1988a, b; Watt & Adams, 1994).

ESD is found across a wide range of taxa but is relatively rare (Bull, 1983). One group in which ESD is potentially widespread is the harpacticoid copepods. The harpacticoid copepods lack sex chromosomes (Lecher, Defaye & Noel, 1995), a necessary precondition for the evolution of ESD (Bull, 1980), and systems of ESD have been reported for a variety of species (Hicks & Coull, 1983).

Field workers have found substantial variation in the adult sex ratio of natural populations of the harpacticoid *Tigriopus* (Igarashi, 1963b; Egloff, 1966; Vittor, 1971; Powlik, 1998). Differences in the population sex ratio occur on both a spatial (between pools) and a temporal scale (between seasons). To date it remains unclear whether this variation is the result of biased sampling protocols, differential mortality of the sexes in the field or the sex determining mechanism.

Our study organism, *Tigriopus californicus* (Baker) is found in the splash pools on the Pacific coast from Alaska to Baja, California. Population densities of *T. californicus* are highest in the summer (Powlik, 1998) and lower in the winter but reproduction occurs year round (Haderlie, Abbott & Caldwell, 1980; Burton, 1985). Females mate once and produce up to a dozen egg sacs over their life-time; each egg sac containing 40–80 offspring (Burton, 1985; Haderlie *et al*., 1980). In the lab, individuals can live up to 70 days and we have found no evidence of sex-specific mortality differences (unpublished data).

The environment in which it lives is characterized by extreme variation in temperature, salinity and irradiation. Between winter and summer, splash pool temperatures and salinities range between 5°C to 35°C and from 0 to over 100 ppt, respectively (Egloff, 1966; Dybdahl, 1995; Albert *et al.*, 2001). Out of the collection of relevant environmental factors we chose temperature as it changes predictably across seasons and has met with some success in earlier studies (Egloff, 1966; Vittor, 1971). Hence one objective of this study is to determine whether temperature-dependent sex determination (TSD) does in fact exist in *T. californicus*. Another is to measure the primary sex ratio of *T. californicus* over a range of relevant temperatures because of its effect on demography.

In most studies concerned with variation in the primary sex ratio (including ours), reliable identification of the sex phenotype is only possible at some later stage of development (often sexual maturity). Inevitably, some individuals die before sexual maturity and so their sex phenotype is unknown. It therefore becomes important to rule out differential mortality of the sexes as an alternative explanation. Following the example of Bull & Vogt (1979), Conover & Kynard (1981), and Bull, Vogt & Bulmer (1982a), we assign dead, missing or unidentified individuals (e.g. juveniles) to the rarer sex. This protocol, hereafter referred to as the 'larval mortality correction', is conservative because it biases the observed sex ratio towards the null expection (50 : 50 sex ratio).

An important point to consider in the study of ESD (or any plastic response) is the distinction between the individual vs. the population response. From quantitative genetics it is well known that different genotypes may not respond in the same way to changed environments (Falconer, 1989). In the extreme case, genotypes may exhibit opposite phenotypes across a range of environments. Averaged across the population there is no response to the environment when in fact the opposite is true. In this study we show that the sex-ratio response differs among families and this had not been shown in previous ESD studies on *Tigriopus* (Vacquier, 1962; Vacquier & Belser, 1965; Egloff, 1966; Vittor, 1971; Chalker-Scott, 1995).

Another objective of this study was to determine whether there was variation in the sex-ratio response to temperature between populations from different locations. Although we only use two locations, this study marks the first attempt to quantify variation in TSD among populations of *Tigriopus*.

Most studies investigating ESD collect gravid females from the field and rear their offspring in a laboratory setting (Bull *et al*., 1982a, b; Janzen, 1992). It is possible that the observed level of ESD is influenced by rearing offspring from field-captured individuals in a novel environment. In this study we address this problem by comparing the sex ratio response between field-captured and lab-reared individuals.

## MATERIAL AND METHODS

### General overview of experiments 1 and 2

In the summer and fall of 2000 we conducted two separate experiments to investigate temperature-dependent sex determination (TSD) and its effect on the primary sex ratio in *Tigriopus* at two experimental temperature treatments. In both experiments we quantified the variation among families in the sex ratio response to temperature. In the first experiment

we compared the primary sex ratio and the level of TSD between populations from two different locations. In the second experiment we determined whether populations still exhibited TSD after having been maintained under constant temperature conditions in the lab for several generations.

In experiments 1 and 2 we quantified TSD by comparing a female's clutch sex ratio between 15°C and 22°C temperature treatments. Each female and her offspring represent a family and the difference in clutch sex ratio between the two temperature treatments is an estimate of the level of TSD for that family. The 15 and 22°C treatments were chosen as representative of field conditions (see intro). The choice of these two treatments also reflects a compromise between increased larval mortality at higher temperatures and slow development at lower temperatures. At 22°C, *Tigriopus* develops almost one week faster than at 15°C (Haderlie *et al*., 1980; Webb & Parsons, 1988).

For a sample of gravid females we estimated the clutch sex ratio of each individual by raising her offspring in the lab and counting the number of males and females at sexual maturity (sixth copepodite stage). At this stage males are easy to distinguish from females by their large, geniculate antennae. For every female (family) at each temperature, estimates of clutch sex ratio were based on approximately 40 offspring.

## Experiment 1: variation in TSD among locations

To determine if there is variation in the sex ratio response to temperature among locations we compared the primary sex ratio and the level of TSD between a population from Arbutus Cove (48°28′36″, 123°18′00″), Victoria, British Columbia, Canada and one from Low Island (52°55′00″, 131°32′30″), Haida Gwaii, British Columbia, Canada. We obtained samples of *Tigriopus* from Arbutus Cove on 31 May, 2000; the Low Island samples were collected by T. Reimchen on 25 May 2000. Samples were taken by haphazardly scooping a small quantity of splash pool water into a 250-ml nalgene bottle. We selected 60 gravid females from each population and individual females were isolated in their own well within 24 well tissue culture plates. Wells were stocked with 2.5 ml of filtered sea water and one drop (~0.5 ml) of a Tetramin™ flake solution (100 mg of ground up Tetramin flakes suspended in 50 ml of $dH_2O$).

From each of the 60 females we collected two egg sacs. Egg sacs were removed from the female's urosome with the use of a thin needle under a dissecting scope (Vittor, 1971). Removing egg sacs in this way prevents females from cannibalizing their offspring. Egg sacs were left to hatch at room temperature in glass spot plates stocked with 2 ml of filtered sea water and 3 drops of an *Isochrysis galbana* culture. In the majority of cases, the transfer of recently hatched nauplii from the spot plates to a temperature treatment was accomplished within 24 h.

For every female in the experiment, each of her two egg sacs was split into two groups of approximately 20 nauplii (no group contained less than 18 nauplii). The two groups, hereafter referred to as 'sibships', were randomly assigned to either the 15°C or the 22°C treatment. Sibships were placed in 100 ml plastic vials containing 40 ml of filtered sea water and 10 ml of the *Isochrysis* culture. These vials were capped with lids to prevent contamination and reduce evaporation and were incubated without light. We added 5 mg of Tetramin flakes and 2.5 ml of *Isochryis* every five days to ensure that the sibships had sufficient food for maximal development.

## Experiment 2: TSD in lab-reared populations

At the end of experiment 1 we used the offspring from the sex ratio assay to create several laboratory stocks on 19 July 2000. Two of these stocks; hereafter referred to as the cold line and the hot line, were initiated with offspring from the Victoria population (originally sampled from Arbutus Cove on 31 May 2000). Both the hot and cold line were stocked with 20 different sibships from the Victoria population. The cold and hot lines were maintained in culture for four months and were kept in incubation fridges without light at 15°C and 22°C, respectively. We estimate that over this period of time the cold line went through four generations and the hot line went through six generations.

We obtained a haphazard sample of 50 gravid females from each line on 26 October 2000. As in the first experiment, we isolated two egg sacs per females; split each egg sac into two groups (sibships) and subsequently assigned these sibships to either the 15°C or the 22°C temperature treatment. For the hot line we obtained 36 gravid females of which 20 produced a second egg sac. For the cold line we obtained 42 females of which 36 produced a second egg sac.

To eliminate mortality (which was a problem in the first experiment) nauplii from the first egg sac were reared in isolation in 24-well tissue culture plates. Unfortunately, the method is time-consuming and so nauplii from the second egg sac were reared in vials (as in the first experiment). Hence nauplii from the first egg sac developed in isolation (1 nauplius/2.5 ml well) while those from the second egg sac developed in groups (20 nauplii/50 ml vial). In both rearing methods the density was the same (~1 nauplius/2.5 ml).

We were concerned that the difference in rearing

methods between the first and second egg sac might have an effect on clutch sex ratio in experiment 2. To determine whether this was in fact the case we used a paired *t*-test to compare clutch sex ratio between egg sacs reared in tissue culture plates (first egg sac) vs. those reared in vials (second egg sac). This analysis showed that there was no effect of rearing method (and/or parity) on clutch sex ratio (at 15°C, $t = 1.594$, df = 49, $P = 0.117$; at 22°C, $t = 1.484$, df = 49, $P = 0.144$).

## STATISTICAL METHODS

### Temperature effects on sex and survivorship

For every family*temperature combination we pooled the number of sons and daughters across the two egg sacs. Sex ratio is defined here as the proportion of males and was calculated for each family (at each temperature) by dividing the number of sexually mature males by the total number of sexually mature adults (males and females). Survivorship ($l_x$) was calculated as the percentage of live individuals recovered relative to the number of nauplii with which the vial(s) had been stocked.

To determine if the primary sex ratio of *T. californicus* is significantly biased (in either direction) we used a one sample *t*-test where the null expectation was 0.5 (a balanced sex ratio). To investigate differences in the primary sex ratio between locations (at each temperature treatment) we used a two-sample *t*-test.

The family clutch-sex-ratios at 15° and 22°C represent paired observations and are therefore not independent (Zar, 1999). For each family, we can subtract the sex ratio at 15°C from the sex ratio at 22°C and use this difference as an independent estimate of the level of TSD in that population (Conover, Van Voorhees & Ehtisham, 1992; Watt & Adams, 1994). Similarly, the change in survivorship ($\Delta l_x$) was calculated as $l_{x\ 22}$°C – $l_{x\ 15}$°C. To determine whether temperature affected sex ratio we performed a one-way *t*-test on the sample of TSDs (equivalent to a paired *t*-test on the original data). We used a two-sample *t*-test to determine whether the mean level of TSD differed between locations in experiment 1, and between the hot and cold line in experiment 2. We used a similar approach to test for temperature-dependent variation in survivorship among locations. The averaged pair-wise differences were normally distributed for both the sex ratio and the survivorship data in experiments 1 and 2.

To investigate the intrapopulation variation in the sex ratio response to temperature we classified families as either temperature insensitive or temperature sensitive. For each family we used the trial size and sex ratio at 15°C to determine whether the increase in the proportion of males observed at 22°C could have occurred by chance alone (i.e. perform a one-sided test). Families were classified as temperature insensitive if the *P*-value >0.05 and temperature sensitive if the *P*-value <0.05. If temperature has no effect on sex-determination, the expected frequency of temperature sensitive genotypes is equal to the type I error rate ($\alpha = 0.05$). Families were classified as having 'reverse temperature sensitivity' if the proportion of males observed at 22°C represented a statistically significant decrease from that at 15°C.

We analysed both the original and the larval-mortality-corrected data and present both. For the uncorrected data we excluded all families where the sex ratio at 15° or 22°C was based on fewer than 10 offspring. All means are reported with their standard error.

## RESULTS

### Experiment 1: differences in survivorship among locations

Survivorship in the Haida Gwaii population is much higher at 15°C than at 22°C (Table 1). In comparison, survivorship in the Victoria population is relatively insensitive to temperature (Table 1). In both sites, the change in survivorship ($\Delta l_x$) between temperature treatments is significantly different from zero (Table 1). The change in survivorship ($\Delta l_x$) was also significantly different between the two locations ($t = 9.17$, df = 98, $P < 0.001$). This difference between locations was mostly the result of poor survivorship of Haida Gwaii nauplii at 22°C (Table 1).

Differences in survivorship complicate the comparison of the primary sex ratio and TSD among locations. This is because the larval mortality correction essentially removes all the variation in clutch sex ratio at 22°C for the Haida Gwaii population. Hence we emphasize from the outset that the results from the comparison among locations are weak.

### Experiment 1: differences in the primary sex ratio and TSD among locations

*Primary sex ratio:* The mean clutch sex ratio of both the Haida Gwaii and the Victoria population are significantly male-biased at both temperature treatments (Table 2). The primary sex ratio remains significantly male biased after correcting the data for larval mortality for all location*temperature combinations except the Victoria population at 15°C (Table 2).

At 15°C, *T. californicus* from Haida Gwaii produced a clutch sex ratio (0.59 ± 0.038) that is slightly but not significantly more male-biased than that of Victoria (0.55 ± 0.022; $t = 1.133$, df = 83, $P = 0.261$). Similarly, at 22°C, the clutch sex ratio from Haida Gwaii

**Table 1.** Survivorship for populations (Pop) from Haida Gwaii (HG) and Victoria (VIC) in experiment 1 and for the hot and cold lines in experiment 2. In each experiment families are reared at two different temperatures (15° and 22°C). Shown are the number of families (*N*) and the mean survivorship ± the standard error (SE). The paired-sample *t*-statistic tests whether survivorship is significantly different between temperature treatments for each population; *t* = *t*-statistic, df = degrees of freedom, *P* = *P*-value

| Exp | Pop | Temp | *N* | Survivorship ± SE | *t* | df | *P* |
|---|---|---|---|---|---|---|---|
| 1 | HG | 15°C | 56 | 86.4 ± 2.15 | 14.3 | 55 | **<0.001** |
| | | 22°C | 56 | 31.1 ± 3.31 | | | |
| | VIC | 15°C | 57 | 93.3 ± 1.41 | 4.7 | 56 | **<0.001** |
| | | 22°C | 57 | 81.0 ± 2.15 | | | |
| 2 | Hot | 15°C | 36 | 96.7 ± 1.23 | –0.1 | 35 | 0.895 |
| | | 22°C | 36 | 96.8 ± 0.97 | | | |
| | Cold | 15°C | 42 | 95.8 ± 0.93 | –0.8 | 41 | 0.424 |
| | | 22°C | 42 | 96.5 ± 0.83 | | | |

**Table 2.** The primary sex ratio (proportion of males) for populations (Pop) from Haida Gwaii (HG) and Victoria (VIC) in experiment 1 and for the hot and cold lines in experiment 2. In each experiment families are reared at two different temperatures (15° and 22°C). Shown are the number of families (N) and the mean primary sex ratio (MSR) ± the standard error (S.E). The one-sample t-statistic tests whether the primary sex ratio is significantly different from 0.5; *t* = t-statistic, df = degrees of freedom, *P* = *P*-value. Corrected refers to whether the data were adjusted for larval mortality

| Corrected | Expt | Pop | Temp | *N* | MSR ± SE | *t* | df | *P* |
|---|---|---|---|---|---|---|---|---|
| No | 1 | HG | 15°C | 30 | 0.59 ± 0.038 | 2.435 | 29 | **0.021** |
| | | | 22°C | 30 | 0.68 ± 0.028 | 6.503 | 29 | **<0.001** |
| | | VIC | 15°C | 55 | 0.55 ± 0.022 | 2.057 | 54 | **0.045** |
| | | | 22°C | 55 | 0.61 ± 0.024 | 4.562 | 54 | **<0.001** |
| | 2 | Hot | 15°C | 36 | 0.49 ± 0.027 | –0.390 | 35 | 0.699 |
| | | | 22°C | 36 | 0.53 ± 0.032 | 0.928 | 35 | 0.360 |
| | | Cold | 15°C | 40 | 0.50 ± 0.029 | 0.150 | 39 | 0.882 |
| | | | 22°C | 40 | 0.58 ± 0.029 | 2.789 | 39 | **0.008** |
| Yes | 1 | HG | 15°C | 56 | 0.56 ± 0.022 | 2.703 | 55 | **0.009** |
| | 2 | | 2°C | 56 | 0.53 ± 0.008 | 3.335 | 55 | 0.002 |
| | | VIC | 15°C | 57 | 0.53 ± 0.019 | 1.589 | 56 | 0.118 |
| | | | 22°C | 57 | 0.58 ± 0.016 | 5.164 | 56 | **<0.001** |
| | 2 | Hot | 15°C | 36 | 0.49 ± 0.024 | –0.498 | 35 | 0.621 |
| | | | 22°C | 36 | 0.54 ± 0.029 | 1.365 | 35 | 0.181 |
| | | Cold | 15°C | 41 | 0.52 ± 0.026 | 0.841 | 40 | 0.405 |
| | | | 22°C | 41 | 0.59 ± 0.026 | 3.354 | 40 | **0.002** |

(0.68 ± 0.028) is slightly but not significantly more male-biased than that of Victoria (0.61 ± 0.024; $t = 1.914$, df = 83, $P = 0.059$). After correcting the clutches for larval mortality (Table 2) the differences are even smaller. Hence we have no evidence that the primary sex ratio differs among locations at either temperature treatment.

*TSD:* In both sites, significantly more males were produced at higher temperatures (Table 3; Fig. 1). After correcting the clutches for larval mortality, there is no longer a significant increase in the proportion of males in the Haida Gwaii population but the response remains significant in the Victoria population (Table 3). The increase was slightly, but not significantly, larger in Haida Gwaii (0.09 ± 0.038) than in the Victoria population (0.06 ± 0.021; $t = 0.690$, df = 83, $P = 0.492$; Fig. 1).

## Experiment 1: variation in TSD among families

Compared to the sex ratio observed at 15°C, 10 out of 30 families from the Haida Gwaii population were significantly more male-biased (i.e. exhibited tempera-

**Table 3.** Temperature-dependent sex determination (TSD) for populations (Pop) from Haida Gwaii (HG) and Victoria (VIC) in experiment 1 and for the hot and cold lines in experiment 2. For each family, TSD is calculated as the proportion of males in a clutch at 22°C minus the proportion of males in a clutch at 15°C. Shown are the number of families (N) and the mean level of TSD ± the standard error (S.E). The one-sample t-statistic tests whether the proportion of males increases at 22°C; *t* = t-statistic, df = degrees of freedom, *P* = *P*-value. Corrected refers to whether the data was adjusted for larval mortality

| Corrected | Expt | Pop | *N* | Mean TSD ± SE | *t* | df | *P* |
|---|---|---|---|---|---|---|---|
| No | 1 | HG | 30 | +0.09 ± 0.038 | 2.411 | 29 | **0.022** |
| | | VIC | 55 | +0.06 ± 0.021 | 3.067 | 54 | **0.003** |
| | 2 | Hot | 36 | +0.04 ± 0.030 | 1.334 | 35 | 0.191 |
| | | Cold | 40 | +0.08 ± 0.024 | 3.238 | 39 | **0.002** |
| Yes | 1 | HG | 56 | –0.03 ± 0.022 | 1.427 | 55 | 0.159 |
| | | VIC | 57 | +0.05 ± 0.018 | 2.876 | 56 | **0.006** |
| | 2 | Hot | 36 | +0.05 ± 0.025 | 2.034 | 35 | 0.050 |
| | | Cold | 41 | +0.06 ± 0.022 | 2.964 | 40 | **0.005** |

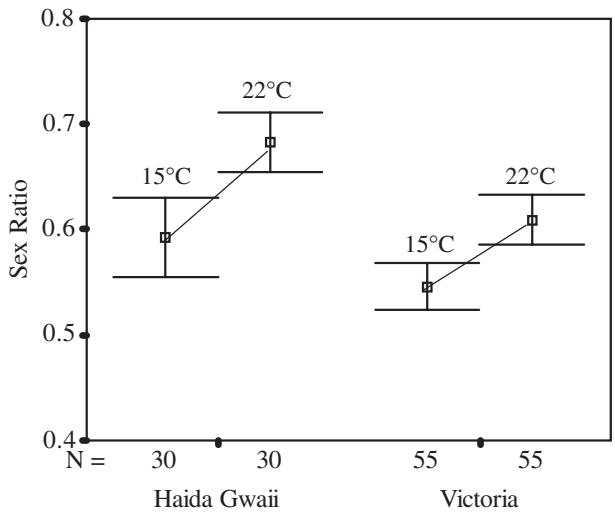


**Figure 1.** Temperature-dependent sex determination (TSD) in two different populations; Haida Gwaii and Victoria. Clutch sex ratios were not corrected for larval mortality. Shown are the mean and standard error.

ture-sensitivity) and 3 out of 30 were significantly less male-biased at 22°C (i.e. exhibited reverse temperature sensitivity). For the Victoria population, 18 out of 55 families were significantly more male-biased at 22°C (temperature-sensitive) compared to the sex ratio observed at 15°C. Only 2 out of 55 Victoria families were significantly less male-biased. Across both populations, approximately one-third of the families (28/85) were clearly temperature-sensitive (i.e. exhibited statistically significant male-biased TSD). Even with the larval mortality correction, 25 out of 85 families were still temperature-sensitive.

### Experiment 2: TSD in lab-reared populations

*Primary sex ratio:* The mean clutch sex ratio in the cold line is significantly male-biased at 22°C but not at 15°C (Table 2). The hot line is not significantly different from 0.5 at either temperature (Table 2). Survivorship in both lines (hot and cold) at each temperature treatment is close to 100% (Table 1). The results are therefore unchanged by the larval mortality correction (Table 2).

*TSD:* After 4 months of inhabiting a constant temperature environment both the hot and the cold lines still exhibit TSD in the predicted direction (proportion of males increases at 22°C). However the level of TSD is only statistically significant for the cold line (Table 3; Fig. 2). The mean level of TSD was larger in the cold line (0.08 ± 0.024) than in the hot line (0.04 ± 0.030), but this difference between lines was not statistically significant ($t = 0.961$, df = 74, $P = 0.340$; Fig. 2). These results are unchanged by the larval mortality correction (Table 3). The level of TSD in the Victoria population in experiment 1 (Table 3, Fig. 1) was comparable to that observed in the hot and cold lines in experiment 2 (Table 3, Fig. 2).

### Experiment 2: variation in TSD among families

Across both lines (hot and cold), 22 out of 76 families (28.9%) were significantly more male-biased at 22°C compared to 15°C (i.e. exhibited temperature sensitivity). In contrast, only 3 out of 76 families (3.9%) were significantly less male-biased at 22°C relative to 15°C (exhibited reverse temperature sensitivity). When corrected for larval mortality only one of the 22 families with male-biased TSD lost its statistical sig-

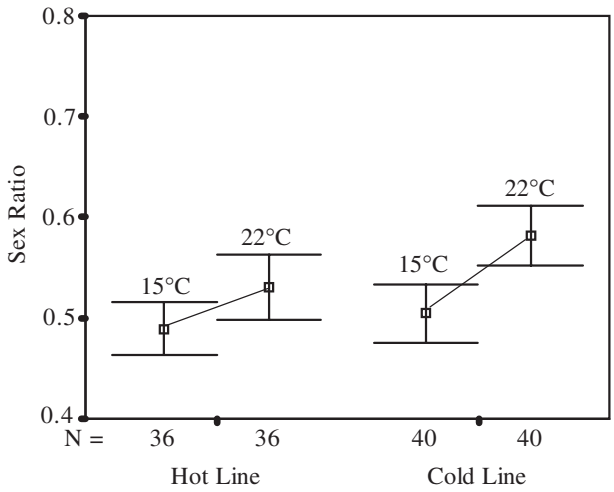


**Figure 2.** Temperature-dependent sex determination (TSD) in two laboratory populations; hot line and the cold line. Clutch sex ratios were not corrected for larval mortality. Shown are the mean and standard error.

nificance. Hence the proportion of temperature-sensitive families remained relatively constant over four to six generations in the laboratory environment.

## DISCUSSION

### The primary sex ratio in *Tigriopus*

Out of eight population*temperature combinations in both experiments only one was not male biased (hot line at 15°C; Table 2). In general the primary sex ratio of *T. californicus* under laboratory conditions is male-biased (Figs 1 and 2). Egloff (1966) also obtained a male-biased primary sex ratio in his work on *T. californicus* and similar observations have been made for *T. japonicus*. In Egami's (1951) work on sex conversion in *T. japonicus* only 6 out of 62 control samples were female biased and the same is true for Takeda's (1950) work. Igarashi (1963b) showed that the primary sex ratio in *T. japonicus* is always male biased and that this is true regardless of the geographical origin of the stock.

In systems of ESD, biased sex ratios are frequently observed and are expected from theory (Charnov, 1982). ESD models predict a primary sex ratio that is biased towards the sex that develops in the poorer patches (Bull, 1981; Charnov, 1982; Charnov & Bull, 1989a, b). Hence the observation that the primary sex ratio is generally male-biased in *T. californicus* suggests that males are making the best of a bad lot at warmer temperatures. This assumes however, that adaptive TSD exists in *T. californicus* for which we have no evidence. To address these questions we need information on sex-specific fitness curves as a function of temperature.

### TSD and ESD in crustaceans

Environmental sex determination is relatively common in crustaceans (Korpelainen, 1990; Legrand, Legrandhamelin & Juchault, 1987) and has been reported for a variety of harpacticoid copepods (Hicks & Coull, 1983). Many of these studies fail to account for differential mortality of the sexes and are therefore ambiguous examples of ESD (Bergmans, 1981; Gaudy, Guerin & Moraitouapostolopoulo, 1982; Miliou, 1993; Hagiwara, Lee & Shiraishi, 1995). In contrast, our use of the larval mortality correction allows us to rule out differential mortality of the sexes. The disadvantage of the larval mortality correction is that it increases Type II error. However, we still detected statistically significant TSD in half of the location*temperature combinations (Victoria population in experiment 1 and the cold line in experiment 2).

These experiments show that sex determination in *T. californicus* is affected by temperature and that high temperatures induce masculinization in this species. The level of plasticity in the sex-ratio response is much smaller than the systems of TSD observed in reptiles (Bull, 1985; Bull & Vogt, 1979; Ferguson & Joanen, 1982) and fish (Conover & Heins, 1987a; Lagomarsino & Conover, 1993) where high temperatures typically increase the proportion of one sex by more than 50%. In comparison, higher temperatures increased the proportion of males by only five to 10 percent in *T. californicus* (Table 2, Figs 1 and 2). The mean population response is weak because only 30% of the families had statistically significant TSD. In contrast, photoperiod has a statistically significant effect on brood sex ratio in more than 90% of the families examined in *Gammarus duebeni* (Bulnheim, 1978a). Hence compared to other examples, both the magnitude and the incidence of TSD are relatively low in *T. californicus*.

Egloff (1966) found that high temperatures increased the proportion of males by as much as 30% in *T. californicus*. Survivorship was 98% or higher, so that he was able to rule out differential mortality. Vittor (1971) also found that the proportion of males increased with temperature in 100% seawater, but that the relationship was reversed in 50% seawater. However, Vittor (1971) points out that differential male and female survivorship may have influenced his results. Other environmental factors that influence sex determination in *T. californicus* include UV-B irradiation (Chalker-Scott, 1995), salinity (Egloff, 1966), light (Egloff, 1966) and hydrostatic pressure (Vacquier, 1962; Vacquier & Belser, 1965). Application

of hydrostatic pressure treatments (500 atmospheres) to different developmental stages indicated that sex was irreversibly determined at the first copepodite stage (Vacquier & Belser, 1965).

Other apparent examples of ESD are caused by cytoplasmic factors such as bacteria and viruses. Cytoplasmic inheritance has been well documented in crustaceans including *Gammarus duebeni* (Bulnheim, 1978a, b), terrestrial isopods (Rigaud *et al.*, 1997) and *Tigriopus japonicus* (Igarashi, 1963a, 1964). In these systems thelygenous (100% female) broods are common (Bull, 1983) however, in some cases the penetrance of these pathogens depends on temperature and other environmental conditions (Bulnheim, 1978a, b; Hurst *et al.*, 2000). Unfortunately, our experimental design does not allow us to rule out this phenomenon as an alternative explanation of our results.

### Differences in survivorship, the primary sex ratio and TSD among locations

The comparison between the Haida Gwaii and Victoria populations was a preliminary attempt to detect latitudinal variation in the primary sex ratio and the level of TSD among populations of *Tigriopus*. Such comparisons have given insight into the adaptive significance of ESD in other systems (Bull *et al.*, 1982b; Conover & Heins, 1987a; Naylor *et al.*, 1988b; Blackmore & Charnov, 1989; Watt & Adams, 1994). We recognize that because we only sampled one pool for each location (HG vs. VIC), the effect of latitude is confounded with all other sources of variation (e.g. shade, aspect, pool size, etc.) that contribute to differences between two splash pools (Hurlbert, 1984).

Geographic clines in thermal tolerance are well known in marine animals (Vernberg & Vernberg, 1972; Spicer & Gaston, 1999). Southern populations of the supra-tidal fiddler crab, *Uca rapax*, are more tolerant of higher temperatures than individuals from northern latitudes (Vernberg & Tashian, 1959). Similar patterns have been observed in bivalve mollusks (Ansell *et al.*, 1986) and beachfleas (Gaston & Spicer, 1998). The pattern of survivorship observed in our experiments is consistent with this cline in thermal tolerance. Survivorship of nauplii from the northern population (Haida Gwaii) was substantially lower at the warmer temperature (22°C) compared to that of the southern (Victoria) population (Table 3). Still, the magnitude of the difference in survivorship was surprising for two reasons. First, *T. californicus* has been shown to tolerate a wide range of temperatures (Huizinga, 1971) and second, mean daily minimum and maximum temperatures are very similar between Haida Gwaii and Victoria (Environment Canada, 1982).

If the environmental cue varies across the range of the organism we would expect the sex-determining response (i.e. the threshold) to be adjusted accordingly (Bull *et al.*, 1982b; Blackmore & Charnov, 1989). For example, under TSD individuals from northern populations are expected to differentiate into the high temperature sex at lower threshold temperatures than individuals from southern (warmer) locales (Bull *et al.*, 1982b). At both temperatures, the northern Haida Gwaii population produced more males than the more southern Victoria population. Although this pattern is consistent with Bull *et al.*'s (1982b) prediction it is not statistically significant. Similarly, the magnitude of TSD is not significantly different between the two populations although such variation has been detected in other systems (Conover & Heins, 1987a; Naylor *et al.*, 1988a, b; Watt & Adams, 1994).

### Maintenance of TSD in lab-reared populations

After several generations of exposure to constant temperature conditions in the lab, the cold line still produced significantly more males at higher temperatures, although the hot line did not. The level of TSD in both lines (in experiment 2) was similar to that observed in the Victoria population in experiment 1 (Table 3). Similarly, the proportion of temperature-sensitive families remained unchanged between experiments 1 and 2. Hence both the level and the incidence of TSD remained stable over the 4 months that the two lines inhabited their constant temperature environments. This suggests that the observed response is not simply an artefact of testing field-captured genotypes in the lab.

## CONCLUSION

We show that the primary sex ratio of *T. californicus* is almost always male-biased and provides evidence for moderate TSD in this species. Only a third of the families actually exhibited TSD; the rest did not respond to temperature. This is one reason why the mean level of TSD in the population is so low. There is no difference in the primary sex ratio or the level of TSD between populations from Haida Gwaii and Victoria. Populations reared under constant temperature conditions for several generations still exhibited a sex ratio response to temperature. At this point, the adaptive significance of a temperature-based cue is not clear and it is possible that other factors (e.g. photoperiod, maternal condition, cytoplasmic factors) are more important in the sex determination of this organism.

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