Environmental sex determination in a splash pool copepod

M. J. VOORDOUW* and B. R. ANHOLT

Department of Biology, University of Victoria, PO Box 3020, Victoria, B.C., Canada, V8W 3 N5

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. © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society,* 2002, **76**, 511–520.

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 mech-

anism 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

(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 expectation (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 dH₂O).

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 off-

spring. 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 (Δl_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 (Δl_x) between temperature treatments is significantly different from zero (Table 1). The change in survivorship (Δl_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

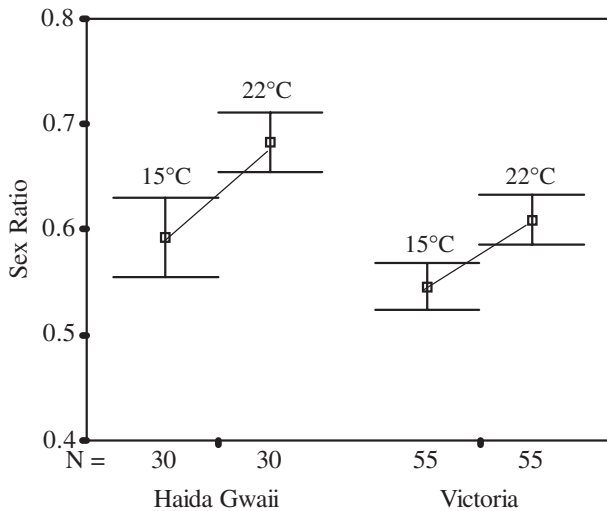| Corrected	Expt	Pop	N	Mean TSD ± SE	t	df	P
No	1	HG	30	+0.09 ± 0.038	2.411	29	**0.022**
		VIC	55	+0.06 ± 0.021	3.067	54	**0.003**
	2	Hot	36	+0.04 ± 0.030	1.334	35	0.191
		Cold	40	+0.08 ± 0.024	3.238	39	**0.002**
Yes	1	HG	56	−0.03 ± 0.022	1.427	55	0.159
		VIC	57	+0.05 ± 0.018	2.876	56	**0.006**
	2	Hot	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-
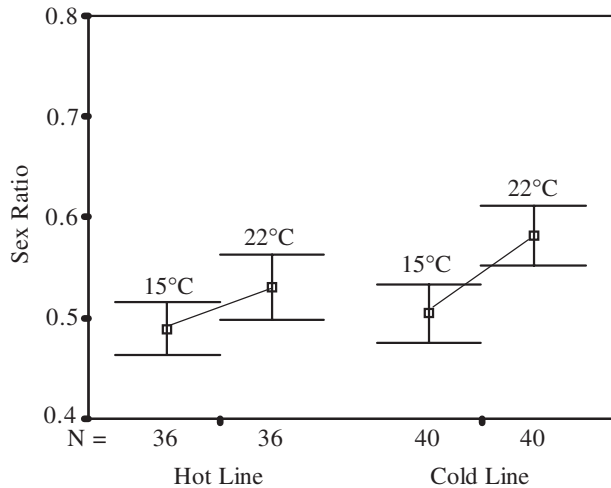
Figure 2. Temperature-dependent sex determination (TSD) in two laboratory populations; hot line and the cold line. Clutch sex ratios were not corrected for larval mortality. Shown are the mean and standard error.

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.

ACKNOWLEDGEMENTS

REFERENCES

Adams J, Greenwood PJ, Naylor C. 1987. Evolutionary aspects of environmental sex determination. *International Journal of Invertebrate Reproduction and Development* **11:** 123–136.

Albert AYK, Borkent CJ, Duquette SL, Voordouw MJ, Anholt BR. 2001. Effects of an introduced mosquito on juvenile *Tigriopus californicus* (Copepoda: Harpacticoidea) in supratidal pools. *Archiv Fur Hydrobiologie* **152:** 203–213.

Ansell AD, Barnett PRO, Bodoy A, Masse H. 1986. Upper temperature tolerances of some European molluscs. I. *Tellina fibula and Tellina tenuis. Marine Biology* **58:** 33–39.

Bergmans M. 1981. A demographic study of the life cycle of *Tisbe furcata* (Baird, 1827) (Copepoda: Harpacticoida). *Journal of the Marine Biological Association of the United Kingdom* **61:** 691–705.

Blackmore MS, Charnov EL. 1989. Adaptive variation in environmental sex determination in a nematode. *American Naturalist* **134:** 817–823.

Bull JJ. 1980. Sex determination in reptiles. *Quarterly Review of Biology* **55:** 3–21.

Bull JJ. 1981. Sex ratio evolution when fitness varies. *Heredity* **46:** 9–26.

Bull JJ. 1983. *Evolution of sex determining mechanisms.* Reading, MA: Benjamin/Cummings

Bull JJ. 1985. Sex ratio and nest temperature in turtles: comparing field and laboratory data. *Ecology* **66:** 1115–1122.

Bull JJ, Charnov EL. 1988. How fundamental are Fisherian sex ratios? *Oxford Surveys of Evolutionary Biology* **5:** 96–135.

Bull JJ, Vogt RC. 1979. Temperature-dependent sex determination in turtles. *Science* **206:** 1186–1188.

Bull JJ, Vogt RC, Bulmer MG. 1982a. Heritability of sex ratio in turtles with environmental sex determination. *Evolution* **36:** 333–341.

Bull JJ, Vogt RC, Mccoy CJ. 1982b. Sex determining temperatures in turtles: a geographic comparison. *Evolution* **36:** 326–332.

Bulmer MG, Bull JJ. 1982. Models of polygenic sex determination and sex ratio control. *Evolution* **36:** 13–26.

Bulnheim HP. 1978a. Interaction between genetic, external and parasitic factors in sex determination of the crustacean amphipod *Gammarus duebeni. Helgolander Meeresuntersuchungen* **31:** 1–33.

Bulnheim HP. 1978b. Variability of the modes of sex determination in littoral amphipods. In: Battaglia B, Beardmore JA, eds. *Genetics, ecology, and evolution.* New York: Plenum Press, 529–548.

Burton RS. 1985. Mating system of the intertidal copepod *Tigriopus californicus. Marine Biology* **86:** 247–252.

Chalker-Scott L. 1995. Survival and sex ratios of the intertidal copepod, *Tigriopus californicus,* following ultraviolet-B (290–320 nm) radiation exposure. *Marine Biology* **123:** 799–804.

Charnov EL. 1982. *The theory of sex allocation.* Princeton, NJ: Princeton University Press.

Charnov EL, Bull JJ. 1977. When is sex environmentally determined? *Nature* **266:** 828–830.

Charnov EL, Bull JJ. 1989a. Non-Fisherian sex ratios with sex change and environmental sex determination. *Nature* **338:** 148–150.

Charnov EL, Bull JJ. 1989b. The primary sex ratio under environmental sex determination. *Journal of Theoretical Biology* **139:** 431–436.

Conover DO, Heins SW. 1987a. Adaptive variation in environmental and genetic sex determination in a fish. *Nature* **326:** 496–498.

Conover DO, Heins SW. 1987b. The environmental and genetic components of sex ratio in *Menidia menidia* (Pisces, Atherinidae). *Copeia:* 732–743.

Conover DO, Kynard BE. 1981. Environmental sex determination – interaction of temperature and genotype in a fish. *Science* **213:** 577–579.

Conover DO, Van Voorhees DA, Ehtisham A. 1992. Sex ratio selection and the evolution of environmental sex determination in laboratory populations of *Menidia menidia. Evolution* **46:** 1722–1730.

Dybdahl MF. 1995. Selection on life-history traits across a wave exposure gradient in the tidepool copepod *Tigriopus californicus* (Baker). *Journal of Experimental Marine Biology and Ecology* **192:** 195–210.

Egami N. 1951. A note on the sex-differentiation of the marine copepod, *Tigriopus japonicus. Annotationes Zoologicae Japonenses* **24:** 131–136.

Egloff DA. 1966. Biological aspects of sex ratio in experimental field populations of the marine copepod *Tigriopus californicus.* Unpublished PhD thesis, Stanford University.

Environment Canada AES. 1982. *Canadian climate normals: Vol. 2, Temperature.* Ottawa: Atmospheric Environment Service, Environment Canada.

Falconer DS. 1989. *Introduction to quantitative genetics.* New York: John Wiley and Sons.

Ferguson MWJ, Joanen T. 1982. Temperature of egg incubation determines sex in *Alligator mississippiensis. Nature* **296:** 850–853.

Gaston KJ, Spicer JI. 1998. Do upper thermal tolerances differ in geographically separated populations of the beach-flea *Orchestia gammarellus* (Crustacea: Amphipoda)? *Journal of Experimental Marine Biology and Ecology* **229:** 265–276.

Gaudy R, Guerin JP, Moraitouapostolopoulou M. 1982. Effect of temperature and salinity on the population dynamics of *Tisbe holothuriae* Humes (Copepoda: Harpacticoida)

fed on two different diets. *Journal of Experimental Marine Biology and Ecology* **57:** 257–271.

Haderlie EC, Abbott DP, Caldwell RL. 1980. Three other crustaceans: a copepod, a leptostracan, and a stomatopod. In: Haderlie EC, Abbott DP, Caldwell RL, eds. *Intertidal invertebrates of California*. Stanford: Stanford University Press, 631–635.

Hagiwara A, Lee CS, Shiraishi DJ. 1995. Some reproductive characteristics of the broods of the harpacticoid copepod *Tigriopus japonicus* cultured in different salinities. *Fisheries Science* **61:** 618–622.

Hicks GRF, Coull BC. 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanography and Marine Biology* **21:** 67–175.

Hipeau JR. 1988. Environmental sex determination in a crustacean parasite. *International Journal of Invertebrate Reproduction and Development* **14:** 11–24.

Huizinga HW. 1971. Cultivation, life history and salinity tolerance of the tidepool copepod, *Tigriopus californicus* Baker 1912, in artificial sea water. *Transactions of the Illinois State Academy of Science* **64:** 230–236.

Hurlbert SJ. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54:** 187–211.

Hurst GDD, Johnson AP, von der Schulenburg JHG, Fuyama Y. 2000. Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density. *Genetics* **156:** 699–709.

Igarashi S. 1963a. On the stability of sex ratio and the prediction of its abnormality depending on the color of the ovisac in *Tigriopus japonicus* Mori. *Scientific Report Tohoku University Series IV (Biology)* **29:** 83–90.

Igarashi S. 1963b. The primary sex ratio of a marine copepod, *Tigriopus japonicus*. *Scientific Report Tohoku University Series IV (Biology)* **29:** 73–81.

Igarashi S. 1964. A possibility of cytoplasmic inheritance of the male dominancy in *Tigriopus japonicus*. *Scientific Report Tohoku University Series IV (Biology)* **30:** 77–84.

Janzen FJ. 1992. Heritable variation for sex ratio under environmental sex determination in the common snapping turtle (*Chelydra serpentina*). *Genetics* **131:** 155–161.

Korpelainen H. 1990. Sex ratios and conditions required for environmental sex determination in animals. *Biological Reviews of the Cambridge Philosophical Society* **65:** 147–184.

Lagomarsino IV, Conover DO. 1993. Variation in environmental and genotypic sex-determining mechanisms across a latitudinal gradient in the fish, *Menidia menidia*. *Evolution* **47:** 487–494.

Lecher P, Defaye D, Noel P. 1995. Chromosomes and nuclear DNA of Crustacea. *Invertebrate Reproduction and Development* **27:** 85–114.

Legrand JJ, Legrandhamelin E, Juchault P. 1987. Sex determination in Crustacea. *Biological Reviews of the Cambridge Philosophical Society* **62:** 439–470.

Miliou H. 1993. Temperature, salinity and light induced variations on larval survival and sex ratio of *Tisbe holothuriae* Humes (Copepoda: Harpacticoidea). *Journal of Experimental Marine Biology and Ecology* **173:** 95–109.

Naylor C, Adams J, Greenwood P. 1988a. Population dynamics and adaptive sexual strategies in a brackish water crustacean, *Gammarus duebeni*. *Journal of Animal Ecology* **57:** 493–507.

Naylor C, Adams J, Greenwood PJ. 1988b. Variation in sex determination in natural populations of a shrimp. *Journal of Evolutionary Biology* **1:** 355–368.

Powlik JJ. 1998. Seasonal abundance and population flux of *Tigriopus californicus* (Copepoda: Harpacticoida) in Barkley Sound, British Columbia. *Journal of the Marine Biological Association of the United Kingdom* **78:** 467–481.

Rigaud T, Antoine D, Marcade I, Juchault P. 1997. The effect of temperature on sex ratio in the isopod *Porcellionides pruinosus*: Environmental sex determination or a by-product of cytoplasmic sex determination? *Evolutionary Ecology* **11:** 205–215.

Spicer JI, Gaston KJ. 1999. *Physiological diversity and its ecological implications*. Oxford: Blackwell Science.

Takeda N. 1950. Experimental studies on the effect of external agencies on the sexuality of a marine copepod. *Physiological Zoology* **23:** 288–301.

Vacquier VD. 1962. Hydrostatic pressure has a selective effect on the copepod *Tigriopus*. *Science* **135:** 724–725.

Vacquier VD, Belser WL. 1965. Sex conversion induced by hydrostatic pressure in the marine copepod *Tigriopus californicus*. *Science* **150:** 1619–1621.

Vernberg FJ, Tashian RE. 1959. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. I. Thermal death limits. *Ecology* **40:** 589–593.

Vernberg WB, Vernberg FJ. 1972. *Environmental physiology of marine animals*. New York: Springer-Verlag.

Vittor BA. 1971. Effects of the environment on fitness-related life history characters in *Tigriopus Californicus*. Unpublished PhD thesis, University of Oregon.

Watt PJ, Adams J. 1994. Adaptive variation in sex determination in a crustacean, *Gammarus duebeni*. *Journal of Zoology* **232:** 109–116.

Webb DG, Parsons TR. 1988. Empirical analysis of the effect of temperature on marine harpacticoid copepod development time. *Canadian Journal of Zoology – Revue Canadienne de Zoologie* **66:** 1376–1381.