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Sex-related differential mortality of a marine copepod exposed to a toxic dinoflagellate

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Abstract

In this study we hypothesized that the sexes of the copepod *Acartia hudsonica* experience different mortalities when exposed to the toxic dinoflagellate *Alexandrium fundyense*. In laboratory experiments, we manipulated toxin dose and measured sex-specific mortality. We also used a split-family design to determine whether there were genetic influences on sex determination, and therefore on sex ratio. When the dose of the toxic alga was high (25% of diet by carbon content), population mortality was high, and the fraction of males surviving to adulthood was low (20–30% of the adult population). No such effect was observed when *A. fundyense* comprised only 10% of the diet. Adult male copepods were also more susceptible to the toxic alga, though prior selection for resistance to toxins also had an effect on mortality. No genetic variation for sex ratio was detected in the split-family experiment. Males of *A. hudsonica* are more sensitive to the toxins of *A. fundyense* than are females. Resistance may be sex-linked, and copepod populations exposed to a bloom of toxic *Alexandrium* sp. may experience shifts in sex ratios away from males and toward females. Differential mortality and skewed sex ratios are feedback mechanisms potentially affecting both the population dynamics of grazers and the development of toxic algal blooms.

Under most circumstances a population of sexually reproducing organisms ought to have an equal sex ratio, one male for every female. If, for some reason, the sex ratio deviates from 1 : 1, frequency-dependent selection should restore the ratio to 1 : 1 (Fisher 1930). Nevertheless, skewed sex ratios are known to occur. For example, when mothers control the sex ratio of a brood and fitness differs between sexes in the brood, the sex ratio of the offspring should be skewed toward the sex that will provide the mother with the highest relative fitness gains. In such a case, the sex ratio is considered adaptive (Charnov 1982). However, not all skewed sex ratios should be considered adaptive; some may simply be the result of transient shifts away from a normal 1 : 1 ratio (Charnov 1982).

Sex ratios that deviate from one male for every female are often observed in populations of free-living pelagic copepods. Several reasons have been suggested for these observations, including sex change (Fleminger 1985; Lee et al. 2003), differential development rates or mortality rates (Conover 1965; Lee and McAlice 1979), and environmental sex determination (ESD) influenced by nutrition (Irigoien et al. 2000). Most often females are found to be favored in copepod populations.

Sex ratios skewed toward females may have either positive or negative consequences for these populations. For instance, if the sex ratio at birth is biased toward females, then constraints on permissible egg mortality are relaxed (Dam and Tang 2001). Also, if mating encounters and subsequent fertilization are not limited, then a preponderance of females will enhance population growth rate. On the other hand, skewed sex ratios may have negative consequences. For example, copepods are obligatorily sexual, and fertilization is internal. Therefore, finding a mate and copulating is necessary for mating success and production of viable eggs. Any factor that skews sex ratios toward females and away from males might adversely affect population growth rate through mating limitation. Kiørboe (2007) found that at least one species of copepod could encounter mating limitation, a situation in which male copepods fertilized only one third of females. He suggested that unmated females are the source of nonviable eggs often observed in the field. In another study, Rey-Rassat et al. (2004) attributed periods of low population egg production in *Calanus helgolandicus* to unfertilized females.

In order to understand the ramifications of skewed sex ratios, we must know the inherent sex-determining mechanism of organisms (Bull 1983). Sex determination among harpacticoid copepods has been studied (Voordouw and Anholt 2002*a,b*; Voordouw et al. 2005), but such studies among other groups are fewer. In the calanoid genus *Acartia,* a chromosomal (genotypic) mechanism of sex determination has been reported. Males are heterogametic (XO) and females homogametic (XX) (Goswami and Goswami 1974; Lecher et al. 1995). Such a mechanism of sex determination is predicted to yield a sex ratio at fertilization of 1 : 1 (Bull 1983). Therefore, species in the genus *Acartia* sp. are promising models to understand changes in sex ratio that occur after fertilization.

We have been using the calanoid copepod *Acartia hudsonica* as a model organism to investigate copepod

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resistance to the toxic dinoflagellate *Alexandrium* sp. Many of our previous experiments compared populations of *A. hudsonica* raised on diets with and without a toxic *Alexandrium* strain. In these experiments, we often noted adult sex ratios that were skewed significantly in favor of females (Colin and Dam 2004). We also noted that exposure to a toxic diet affected time to maturity in male copepods differently than in female copepods (Avery and Dam 2007). The simplest explanation for these results is that male copepods are more susceptible to the neurotoxins than are female copepods.

In this study, we therefore test the hypothesis that exposure to the toxic dinoflagellate *Alexandrium fundyense* results in a biased sex ratio in the copepod *A. hudsonica* at adulthood. Further, we present evidence that differential mortality between sexes is responsible for the skewed sex ratios that we observed. Finally, we argue that resistance to the toxin may be a sex-linked trait.

Methods

Culture of copepods and algae—The experimental copepod populations used in this study are the same lines reported on in Colin and Dam (2004) and Avery and Dam (2007), where a complete description of the rearing procedures can be found. In general, two laboratory populations of *A. hudsonica* were used: a control line that was never exposed to *Alexandrium* sp. and a line selected for resistance to a diet containing *Alexandrium* sp. Both lines had been kept in the laboratory for several years before the start of the experiment. They originated from individuals captured from Great Bay, New Jersey, in 1999. The copepod cultures were maintained in 20-liter pails at 15°C and ambient day length under fluorescent lighting. Water was changed once per week. Three times a week, the control line was fed a standard diet consisting of a mixture of nontoxic algae (*Thalassiosira weissflogii* [~11 μm diameter, ~4.5×10^{-5} μgC cell^{-1}], *Isochrysis galbana* [~4 μm diameter, ~4×10^{-6} μgC cell^{-1}], and *Tetraselmis* sp [~7 μm diameter, ~4×10^{-5} μgC cell^{-1}]).

The selected line was also fed the standard diet three times a week. In addition, the selected line received the toxic *A. fundyense* (isolate BF-5—equivalent to NB05 in Colin and Dam 2003; ~25 μm diameter, ~3.5×10^{-3} μgC cell^{-1}) once per week. The total concentration of phytoplankton in the water immediately after feeding was approximately 10^3 μgC L^{-1}, a concentration above grazing rate saturation and chosen to maintain high copepod biomass in the pails. The concentration of *A. fundyense* cells in the pails immediately after feeding was approximately 10^4 cells L^{-1}, equivalent to a severe bloom in nature (Townsend et al. 2001; Anderson et al. 2005).

The phytoplankton cultures used to feed the copepods were grown in semicontinuous culture using F/2 growth medium (Guillard and Ryther 1962). The 2-liter cultures were diluted with growth medium approximately once each week. They were kept at 18°C in an environmental chamber equipped with fluorescent lights set to a 12 : 12 light : dark (LD) photoperiod.

Experimental design—Cohort experiments: Two different cohort experiments were conducted: one in which the toxic diet treatment consisted of a low dose of toxin (10% by carbon content; $n = 60$ initial nauplii per treatment), and one in which the toxic diet treatment contained a higher dose of toxin (25% of the total ration; $n = 90$ initial nauplii per treatment). The cohort experiment with the high dose of toxin is the same experiment reported in Avery and Dam (2007). In that paper, the reproductive success of females was analyzed and reported. Here we report on survivorship and include male copepods in our analysis. The low-toxin cohort experiment is new to this study.

To begin the cohort experiments, a random subsample of eggs and nauplii, density unknown, from each line was transferred from the mass rearing pails to separate nearby beakers, fed the standard diet, and allowed to mature (F_1 generation). When the copepods matured, approximately 50 gravid female copepods from these sublines were isolated, fed the standard diet, and allowed to produce eggs over 24 h. After the eggs hatched (F_2 generation), individual nauplii were collected from these cohorts and placed in 60 × 15-mm petri dishes containing one of two food mixtures, standard and toxic. This procedure was used to reduce potential maternal effects.

Two mixtures of algae were used as food. The standard diet mixture consisted of *T. weissflogii*, *I. galbana*, and *Tetraselmis* sp. in equal proportions (by carbon) at a total of approximately 600 μgC L^{-1}, a nonlimiting concentration (Colin and Dam 2003). The cell sizes and cell concentrations were measured with an Elzone 280 particle counter. The average carbon content of each algal clone was estimated from calculated cell volume and carbon volume relationships established in previous experiments in our lab. In the low-dose experiment, the toxic diet consisted of a combination of the nontoxic algae mentioned and the toxic *A. fundyense*. The toxic diet also totaled 600 μg C L^{-1}, but approximately 10% of it was from *A. fundyense*. In the high-dose experiment, the toxic diet consisted of approximately 25% *A. fundyense*. The toxic cell concentration was approximately 40 cells mL^{-1} in the high-toxin experiment and 15 cells mL^{-1} in the low-toxin experiment. The toxin content of the *A. fundyense* cells was determined by high-pressure liquid chromatography after extraction in 0.1 mol L^{-1} acetic acid (Anderson et al. 1990). Saxitoxin derivatives measured included saxitoxin, neosaxitoxin, gonyautoxins 1 and 4, and gonyautoxins 2 and 3. The toxin content of BF-5 was measured at the beginning or at the end of an experiment. The average cellular toxin content of the stock *A. fundyense* cultures during the high-toxin experiment was 11.3 pg saxitoxin equivalents (SD = 0.94, $n = 4$). During the low-toxin experiment, it was 19.1 pg saxitoxin equivalents (SD = 6.6, $n = 3$).

Copepod survivorship was tracked by examining each petri dish under a dissecting microscope every day or two. The food medium in the petri dishes was refreshed every other day for juveniles or daily for adult copepods. The petri dishes containing copepods were kept in translucent plastic containers with lids. The plastic containers were kept on shelves in a walk-in environmental chamber at 15°C and at a 12 : 12 LD photoperiod with diffuse

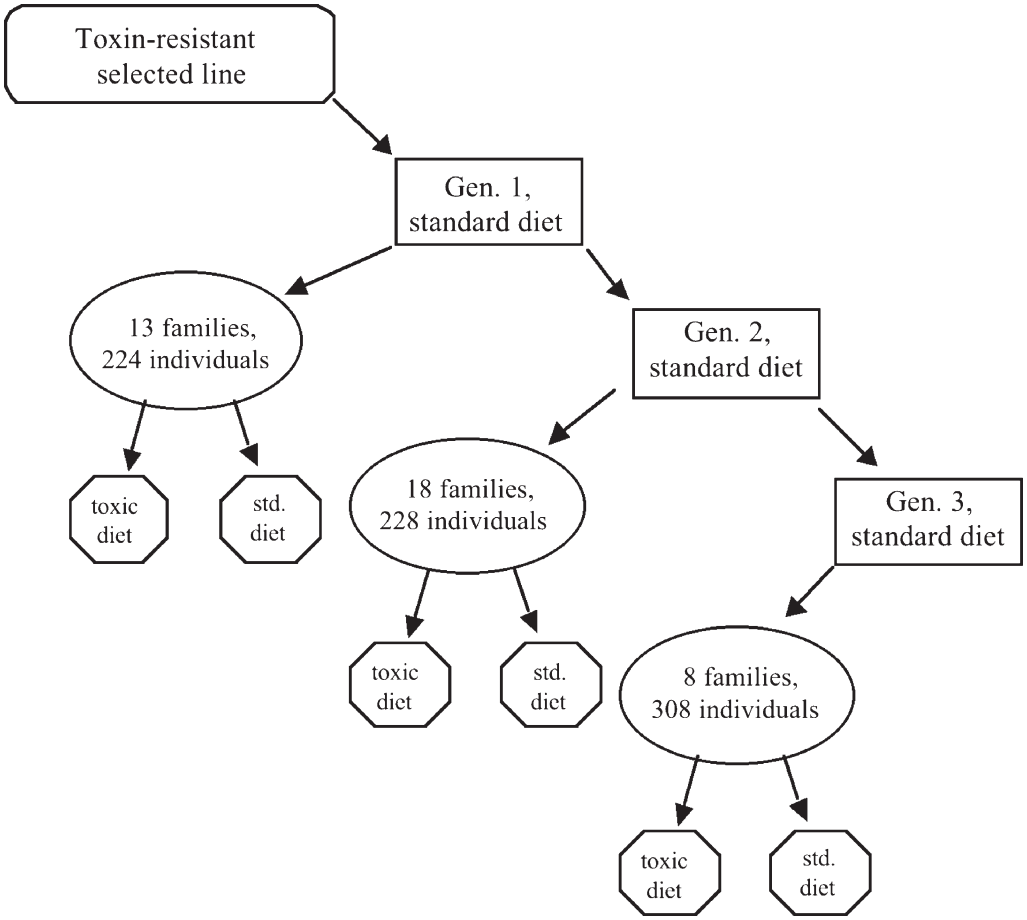

Fig. 1. Design of split-family experiment. The original source population of *A. hudsonica* was selected for resistance to toxic *A. fundyense* for many generations. Sex ratios of families were measured on two diets: a standard nontoxic diet and a diet containing toxic *A. fundyense*.

fluorescent lighting. Data for each experiment were analyzed separately using a chi-square test for significant differences between the percentages of male copepods occurring in the standard diet as compared to the toxic diet. Throughout this study we use the term "sex ratio" as synonymous with percentage of male copepods (50% male equals a 1 : 1 ratio), as is customary in most of the literature concerning sex ratios (Hardy 2002).

Adult survivorship experiment: The adult survivorship experiment was also conducted using the control and selected laboratory lines described above. Naupliar copepods were removed at random from the zooplankton culture containers and allowed to grow up on a standard diet described above. At one time approximately 20 male and 20 female copepods from each treatment were selected at random and placed in individual petri dishes. They were fed one of two diets at maximum ration: the standard diet described above or a diet that replaced half (by C content) of the standard diet with toxic *A. fundyense*. Food was renewed, and the copepods checked for mortality approximately daily. The toxin content of the cells during this experiment was 18.6 pg saxitoxin equivalents (SD = 1.0, n = 2). The data were analyzed using the survivorship tests of STATVIEW (version 5.01, SAS Institute) and the Mantel-Cox test of significance.

Split-family experiment: To determine whether there was a detectable genetic component to the observed sex ratios, we conducted an experiment in which we could partition variance among environmental influences and genetic influences. In the split-family experiment, only selected-line copepods were used. A group of female copepods was removed from the selected-line rearing chambers. They were fed a nontoxic diet. Some individuals from this group were isolated to begin the experiment. Their offspring were generation 1 (Fig. 1). The rest were allowed to continue to reproduce and grow on the standard nontoxic diet. Subsequent generations (2 and 3), isolated in the same way, would come from this group.

During each generation of the split-family experiment, individual female copepods were isolated and their eggs collected over several days. When the eggs hatched, early nauplii were split into two groups of approximately 20 copepods each. One group was raised on a toxic diet (10% *A. fundyense* by carbon content), the other on the standard diet alone. Both diets were provided at maximum ration. Each nauplius was raised alone in a petri dish and its development

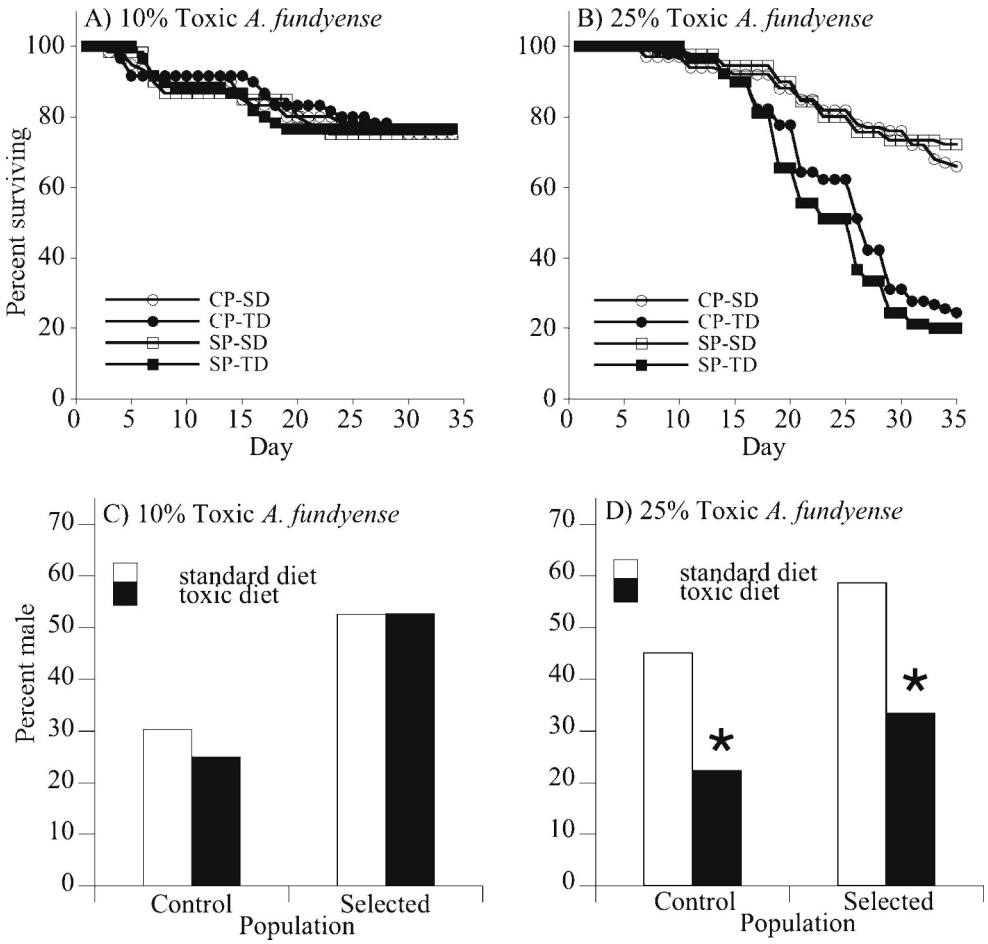

Fig. 2. (A, B) Survivorship curves for two cohort experiments and (C, D) resulting sex ratios of animals reaching maturity in each experimental treatment. In (A), the toxic diet treatment (TD) was composed of toxic *A. fundyense* (10% by carbon) and a mixture of nontoxic algae (90%). In (B) (previously published in Avery and Dam 2007), a higher toxin dose was used (25% by carbon was toxic *A. fundyense,* 75% nontoxic mixed algae). All diets were offered at nonlimiting concentrations. SD, standard, nontoxic diet; CP, control population; SP, selected population. Asterisks indicate sex ratios that are significantly different from the sex ratio on the standard diet.

tracked approximately daily. They were fed every day or two by removing approximately half of the volume of the petri dish and replacing it with fresh growth medium. The experiment was carried out until nearly all copepods had either matured or died. Although sex is morphologically distinguishable at copepodite stage 4, only adult sex ratios were analyzed. The trials using generations 2 and 3 were carried out in a similar manner. In all, the experiment utilized 13 families and 224 individuals in generation 1, 18 families and 228 individuals in generation 2, and 8 families and 308 individuals in generation 3. The reader should note that the copepods in the experimental families in generation 2 are not the direct descendants of those in the experimental families of generation 1 (likewise generation 3 vis-à-vis generation 2). They are the offspring of the other copepods in the population that were allowed to continue to reproduce and grow on the standard nontoxic diet (Fig. 1). The toxin content of our *A. fundyense* cultures was measured several times during this experiment and averaged 22.4 pg saxitoxin equivalents (SD = 12.5, n = 3).

Data collected from the split-family experiment included time to death and sex. The data were analyzed by nested ANOVA (family and diet within generation) using SAS (PROC GLM, version 9, SAS Institute). By removing the selected-line copepods from the toxic diet that they had been reared on for several generations, we were, in effect, relaxing the selection for resistance that we had imposed in previous generations. Consequently, these data can be interpreted as the population response to the relaxation of selection pressure for toxin resistance.

Results

Cohort experiments—In the cohort experiments, a greater proportion of male copepods died when exposed to a high concentration of toxin. To begin, copepod

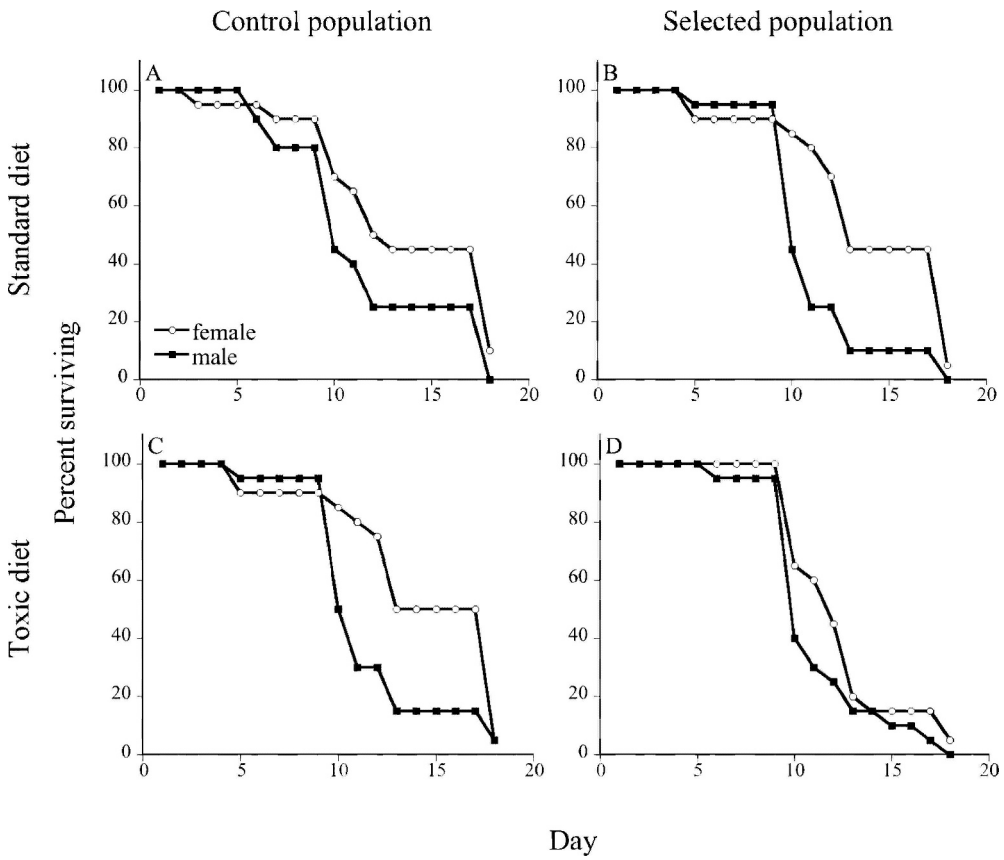

Fig. 3. Results of adult survivorship experiment. Survivorship curves in standard diet for (A) control population and (B) selected population and survivorship curves in toxic diet for (C) control population and (D) selected population. Male survivorship curves differed significantly from female curves in panels (B) and (C). The toxic diet consisted of 50% standard diet plus 50% *A. fundyense*. $n = 20$ for each sex. Experimental copepods were selected at random.

mortality was clearly related to the concentration of toxic algae present in the diet of the experimental copepods (Fig. 2). When the diet consisted of 10% *A. fundyense*, only 25% of the individuals died, the same percentage as in the standard nontoxic diet treatment (Fig. 2A). When the diet consisted of 25% *A. fundyense*, approximately 80% of copepods, regardless of population of origin, died (Fig. 2B). This pattern of mortality was accompanied by a peculiar pattern in sex ratio. When mortality on the toxic diet was not different from mortality on the standard diet (Fig. 2A), the adult sex ratio did not favor one sex over the other (Fig. 2C; $\chi^2 = 0.030$, df = 1, $p > 0.05$). When mortality was higher on the toxic diet than on the standard diet (Fig. 2B), the adult sex ratio favored females significantly in both the selected and the control populations (Fig. 2D; $\chi^2 = 3.843$, df = 1, $p < 0.05$).

Adult survivorship experiment—The survivorship of male copepods relative to female copepods was also affected in some treatments of the adult survivorship experiments. When copepods from the control population were fed the standard nontoxic diet, there was no difference in survivorship between the sexes (Fig. 3A; $\chi^2 = 0.2838$, df = 1, $p > 0.05$). By contrast, when copepods from the control population were fed the toxic diet, survivorship curves differed significantly between the sexes (Fig. 3C; $\chi^2 = 8.657$, df = 1, $p = 0.0033$), with males succumbing sooner than females. When copepods from the selected population were fed the standard nontoxic diet, survivorship curves differed significantly (Fig. 3B; $\chi^2 = 8.657$, df = 1, $p = 0.0033$). Males died sooner than females. Survivorship curves were similar when the animals were fed the toxic diet (Fig. 3D; $\chi^2 = 0.936$, df = 1, $p > 0.05$).

Split-family experiment—In the split-family experiments, we tracked the sex ratio of the selected population over several generations after removing the selective pressure of the toxic diet (Fig. 1). The survivorship curve of generation 1 revealed an unusual pattern (Fig. 4A). On the standard nontoxic diet, only 1 copepod of 112 (all families pooled) survived to maturity, whereas approximately 50% of those raised on the toxic diet survived (Fig. 4A). In subsequent generations, there was no difference between survivorship on the toxic diet compared to that on the standard diet (Fig. 4B,C). Sex ratio varied greatly over the course of these three generations, ranging from more than 65% males in generation 1 (Fig. 4D) to approximately 10% in generation 2 and to approximately 40% in generation 3. Only the toxic diet treatment is shown in generation 1 because of the low survivorship on the standard diet.

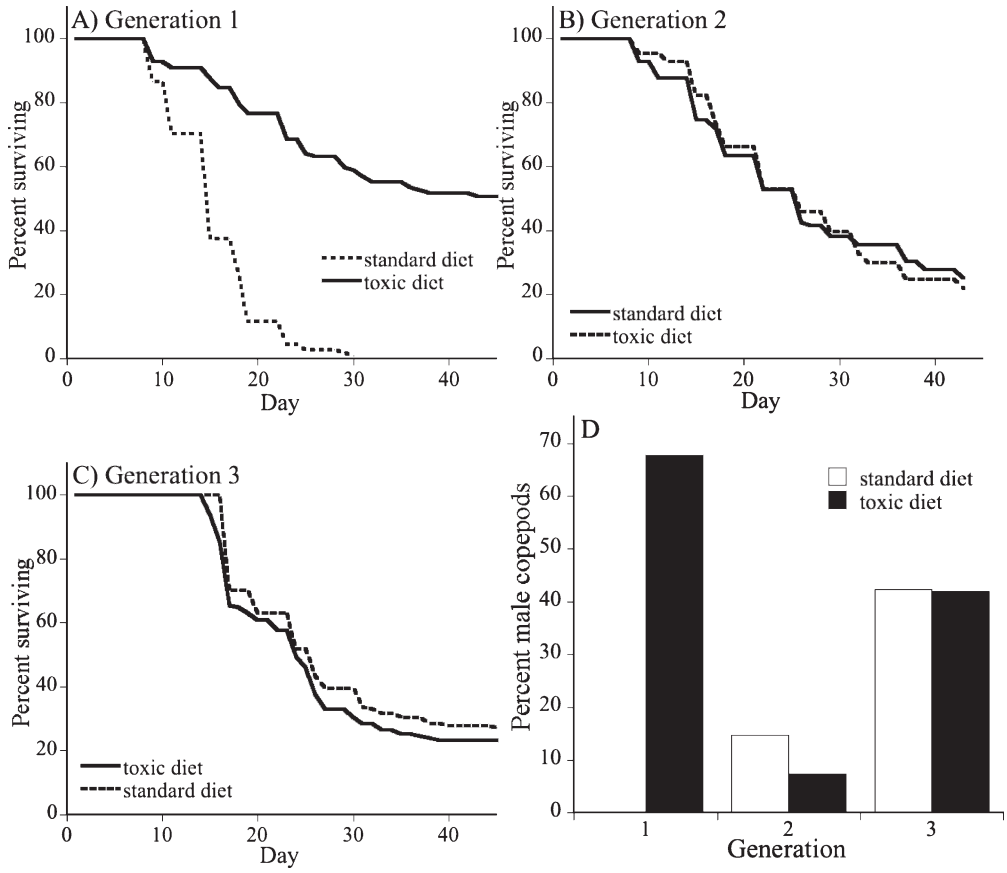

Fig. 4. (A–C) Survivorship curves for 3-generation split-family experiment and (D) the adult sex ratios in those generations. No sex ratio is reported for the standard diet in generation 1 because only one male copepod survived. Experimental copepods originated from the selected population. They were removed from the selective pressure of toxic *A. fundyense* for one, two, and three generations, respectively.

The split-family experiment also allowed us to partition variance in sex ratio among several factors: generation, family, and diet. A nested ANOVA (family, diet, and [family × diet] nested within generation) revealed a significant effect of generation on the number of male copepods produced, but no effect of diet or family, and no interaction (Table 1). Generation 1 was omitted from this ANOVA because only one copepod survived to maturity in the standard diet treatment.

Discussion

Our results are consistent with the hypothesis that differential mortality causes the skewed sex ratios of *A. hudsonica* in our experiments. Our experiments also indicate that other factors, such as resistance to the effects of the toxic alga, also exert an influence on sex ratio. Other hypotheses such as facultative sex change or parasites that affect sex determination (Hardy 2002), cannot explain all of our results. However, we did not test any of these hypotheses explicitly, so they cannot be ruled out at this time.

Differential mortality—If differential mortality leads to skewed sex ratios, then males would be underrepresented when total mortality in the population is high. We observed this result in the cohort experiment (Fig. 2). At the low dose of toxin (Fig. 2A,C), the two diets resulted in equal and low mortality and the sex ratios on both diets were equal. At the higher dose of toxic *A. fundyense* (Fig. 2B,D) mortality was high, and the frequency of males was low on the toxic diet. This pattern held for both the control population and the population selected for resistance to the toxin. We think that it is important to note that, in this experiment, the animals from the selected population had been removed from the toxic diet for two generations. Recombination occurring in these generations may have caused the line selected for resistance to revert to the genetic composition of the control population (Avery and Dam 2007).

The results of the adult survival experiments also support the differential mortality hypothesis (Fig. 3), but other factors also appear to contribute to the sex ratios observed in them. First, the toxic alga kills male copepods sooner than females (Fig. 3C), but only if they come from the control population. Male copepods from the selected population were not killed sooner than female copepods (Fig. 3D). These results suggest that the populations differ in their genetic compositions with respect to resistance to the toxic alga, consistent with the results of others who

Table 1. Nested ANOVA table for the number of male copepods observed in a split-family experiment. Successive generations of copepods (*A. hudsonica*) were drawn from one resistant source population after the selective pressure for resistance was relieved. The two diets tested were a standard nontoxic diet of mixed algae and a mixture of algae 10% of which was toxic *A. fundyense*. MS = mean square error.

Source of variation	df	MS	*F*	*p*
Generation	1	1.009	4.98	0.0282
Family (generation)	20	0.074	0.36	0.994
Diet (generation)	2	0.1038	0.51	0.601
Family × diet (generation)	13	0.1672	0.82	0.633

found that a line selected for resistance diverged from a control population in just a few generations (Colin and Dam 2004). These results also suggest that it is not just normal male physiology—higher metabolic rate or lesser lipid stores—that leads to differential mortality, but that resistance is somehow involved. It is important to note that, in contrast to the animals used in the cohort experiment, copepods from the selected population used in the adult survival experiments had been removed from chronic exposure to the toxic alga for only one generation. Consequently, they had not yet undergone sufficient recombination to eliminate sex-specific population differences.

Working with the data of Schnack (1978), Kiørboe (2006) modeled the population dynamics and the adult sex ratios of *Acartia bifilosa* in the Kiel Bight. In that study, which did not concern red tide toxins at all, adult sex ratios varied seasonally between zero and two males for every female. Moreover, the data demonstrated that during periods of high population growth, males became more numerous, whereas during periods of low population growth, males became more rare. Our results may explain how the sex ratios observed in this study became skewed. When mortality from starvation was low (spring and summer), males probably survived relatively better than when mortality from starvation was high (winter, a period of food limitation). It is our contention that resistance to toxins also confers resistance to starvation (Avery and Dam 2007), which we discuss further below.

ESD—Other hypotheses may be invoked to explain skewed sex ratios—ESD, for example (Voordouw and Anholt 2002*a*). ESD related to the presence of *A. fundyense* cannot explain our results. In the split-family experiment, the sex ratios of successive generations were tracked. During this time, the environment with respect to temperature, salinity, presence of toxic alga, density of toxic alga, density of total food, and density of copepods was constant, but sex ratio fluctuated a great deal (Fig. 4D). Instead of ESD, we believe that the differences observed from generation to generation in this experiment reflect the recombination of sex-linked resistance alleles present in the selected population. With each generation, the frequency of sex-linked resistance alleles changes. Shifting genotype frequencies may account for the differences between Figs. 2A and 4B—experiments with similar conditions of generation and toxin concentration, but conducted at different times. The survivorship depicted in Fig. 4B is obviously different than the survivorship in Fig. 2A.

ESD may evolve if male organisms and female organisms experience different fitnesses in a patchy environment (Bull 1983). Perhaps predictably seasonal variation selected for the apparent ESD observed in *Calanus* spp. (Irigoien et al. 2000). However, if the environment is unpredictably variable, it is generally thought that genotypic (chromosomal) sex determination would be favored over ESD (Bull 1983). For most populations of copepods, the occurrence of toxic *Alexandrium* sp. is not predictable, so ESD related to *Alexandrium* sp. blooms is not likely. Further, sex in the genus *Acartia* has been related to an XX–XO chromosomal mechanism (Goswami and Goswami 1974; Lecher et al. 1995), and it is morphologically obvious at copepodite stage 4. These facts argue against ESD, but cannot rule out environmental modification of genotypic sex determination under some circumstances (Bull 1983).

Genetic sex determination—Autosomal genetic factors may also affect sex ratio by determining sex or by modifying chromosomal sex determination (Bull 1983). Genetic factors modifying sex ratio may be revealed by significant variance among families in a split-family experiment (Voordouw and Anholt 2002*b*). In our split-family experiment, we saw no effect of family or diet in the analysis of variance (Table 1). Instead, only generation showed a significant effect, indicating that recombination of alleles related to resistance and linked to sex determination was occurring as the population responded to the relaxation of the selective pressure of chronic toxin exposure.

Sex linkage of resistance—The results of some of our experiments indicate that resistance is sex-linked. For example, in the adult survivorship experiments, male copepods from the selected population actually died sooner than females on the standard nontoxic diet (Fig. 3B). Male copepods carrying resistance alleles that have a high cost could be suffering from that cost when fed the standard nontoxic diet (Avery and Dam 2007). The same was not true for males of the control population (Fig. 3A). In essence, resistance alleles made male copepods less fit than females in the absence of the toxin. Importantly, in toxicological studies, male copepods of the species *Acartia tonsa* were found to be twice as sensitive as females to the insecticide cypermethrin (Medina et al. 2002). Cypermethrin affects the voltage-gated sodium channels of nerve cells as do the paralytic shellfish poisoning (PSP) toxins of *Alexandrium* sp. Such sex-specific sensitivity may be expected if a gene important to the sodium channel is located on a sex chromosome. At least one gene encoding the sodium channel alpha subunit is located on the X chromosome in some arthropods (Ramaswami and Tanouye 1989).

Simultaneous resistance to starvation—Some have hypothesized that the toxic effects of *Alexandrium* spp.

interfere with the acquisition or assimilation of energy from food. Dutz (1998) hypothesized that *Alexandrium* sp. interfered with assimilation when it was ingested by *Acartia clausi*. Avery and Dam (2007) suggested that resistance to the PSP toxins of *Alexandrium* spp. also served as resistance to starvation. They further hypothesized that it could be a common trait in *Acartia* sp. populations. If resistance alleles are common, and they help copepods resist starvation, then a link to the data from Kiel Bay (Schnack 1978) is apparent. During winter, food is limiting and males are less able to resist starvation than are females, so the population's adult sex ratio at this time reflects the differential mortality. Males may eventually be eliminated from the population in winter. In summer, food is more plentiful; males survive better than they do in winter, and the sex ratio responds accordingly. Males may even outnumber females at this time.

Kiørboe (2006) modeled the population dynamics of *A. bifilosa* in Kiel Bay over the course of 2 yr. He assumed male mortality to be twice that of female mortality because males are at a higher risk of predation as they search for mates. The modeled sex ratios, however, ranged between only 0.6 and 1.0 males for every female, and failed to capture the complete range of observed values. The author suggested that other factors governing sex ratio or sex determination must be considered. Our results indicate that adjusting sex-specific mortalities to reflect sex-specific sensitivities to food limitation could solve the problem.

Skewed sex ratios related to the food environment are not unique to *Acartia* spp. Irigoien et al. (2000) found that higher food concentrations apparently increased the representation of male *Calanus* spp., consistent with our interpretation of sex-specific sensitivity to food limitation. Perhaps skewed sex ratios of *Acartia* sp. and other calanoids observed in the field can be used to infer past periods of food limitation.

In conclusion, male copepods of the species *A. hudsonica* are more sensitive than female copepods to the presence of PSP toxins in their diets, succumbing at higher rates, provided that the dose of toxin is high enough or the population has not been selected for resistance. In addition, male copepods from a population selected for resistance to the toxins also appear to be more sensitive to the absence of the toxins than are females, reflecting differential costs of resistance. These costs could affect the recovery of copepod grazers after a bloom of *Alexandrium* sp. by causing a time lag from bloom termination to population recovery. Also, toxin resistance may be common and linked to starvation resistance. If male copepods are more sensitive than females to food limitation, past periods of food limitation could be revealed by skewed sex ratios.

The differential survivorship of males and females demonstrated in this study may influence the development or fate of *Alexandrium* sp. blooms in nature. First, if, by virtue of male sensitivity to the toxins, the population shrinks, then population grazing on the toxic alga will be reduced directly through cell mortality (Colin and Dam 2007). Reduced population size of grazers may also influence *Alexandrium* sp. blooms indirectly through cascading effects (Turner and Graneli 2002). Finally, if the timing is correct, an absence of male copepods could limit fertilization success in the population (Kiørboe 2007), possibly exacerbating the effects of the toxins on a grazer population and further reducing grazing pressure.

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