Journal of Experimental Marine Biology and Ecology 446 (2013) 17–21

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Sex-specific tolerance to starvation in the copepod *Acartia tonsa*

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ARTICLE INFO

Article history:
Received 25 September 2012
Received in revised form 19 April 2013
Accepted 20 April 2013
Available online 19 May 2013

Keywords:
Acartia tonsa
Copepod
Egg production
Sex-specific
Survival
Tolerance to starvation

ABSTRACT

We define tolerance to starvation as the ability to survive in the absence of food. We tested the hypotheses that adult male *Acartia tonsa* copepods are less tolerant to starvation than adult female copepods, and that tolerance to starvation is affected by prior diet, age of individuals and duration spent in the adult stage. We also tested whether, during the period of starvation, egg production affected tolerance to starvation in female adults. Field-collected male and female copepods were fed non-limiting rations of the toxic dinoflagellate *Alexandrium fundyense*, non-toxic *Alexandrium tamarense*, the nutritionally insufficient dinoflagellate *Prorocentrum minimum*, or a standard non-toxic diet (a mixture of the diatom *Thalassiosira weissflogii*, the flagellate *Tetraselmis* sp., and the green microalga *Isochrysis galbana*) for 24 h before starvation. In separate experiments, male and female field-caught copepods were either starved or fed non-limiting rations of the standard non-toxic diet. Finally, tolerance to starvation was measured in adults raised in laboratory cohorts. Tolerance to starvation was lower for adult males than females in all experiments, with all starved copepods dying within fifteen days. We observed a similar pattern for field-caught and laboratory-raised animals: differences in adult survivorship between the sexes were immediately apparent under starvation. In contrast, adult survivorship in fed animals was relatively constant and independent of sex during the first fifteen days. Subsequently, male survivorship was lower. In the laboratory-cohort experiments differences in tolerance to starvation between the sexes were not affected by age or by duration within the adult stage. Taken together, the results of this study suggest that adult male *A. tonsa* are physiologically less tolerant to starvation compared to females, and may explain observations of female-skewed adult sex-ratios in food-limited field and laboratory experiments. Further, total egg production and survivorship during starvation were uncorrelated. We suggest that the former is controlled by previous feeding history and the latter by basal metabolism.

1. Introduction

Copepods are the most numerous metazoans in the ocean, and as such form integral links in aquatic ecosystems. Therefore, understanding the mechanisms that control copepod population growth is of great importance. Factors that affect either birth (e.g., food quantity and quality) or death (e.g., predation, starvation) rates control population growth. Interest in the factors affecting mortality rate has risen in the last two decades (Hirst and Kiørboe, 2002; Ohman and Wood, 1995). In particular, sex-specific mortality – which can result in skewed sex-ratios – is gaining attention as an important factor in understanding the dynamics of zooplankton populations (Kiørboe, 2007). There are multiple mechanisms that result in sex-specific mortality (i.e. predation, physiological, behavioral), but relatively few outside of predation have been rigorously tested.

Sex-specific predation can result in female- (Landry, 1978) or male- (Saito and Kiørboe, 2001) dominated sex-ratios. For example, many visual predators preferentially feed on egg-sac-carrying female copepods over males (Hairston et al., 1983; Maier et al., 2000; Svenson, 1997). In contrast, male copepods that sense their mates via pheromone trails, as opposed to hydromechanical signals, are more likely to be preyed upon as a result of increased searching time (Kiørboe, 2006; Kiørboe and Bagoien, 2005).

There are other sex-specific mechanisms of adult mortality that might be important. Male *Acartia tonsa* were found to have increased oxidative damage with age compared to females (Rodriguez-Grana et al., 2010). In laboratory studies, male *Acartia hudsonica* copepods were more susceptible to toxins produced by the dinoflagellate *Alexandrium fundyense* (Avery et al., 2008). Because these toxins physiologically incapacitate copepods (Ives, 1987), drastically reducing ingestion rates (Colin and Dam, 2003), it was suggested that males starve, and die, sooner than females (Avery and Dam, 2007; Colin and Dam, 2004). Indeed, physiological differences (i.e. lower tolerance of males to starvation) were suggested as the dominant mechanism for sex-specific mortality under severe food limitation (Hirst et al., 2010). If males die at higher rates because of starvation, this could explain the female-biased sex ratios observed in laboratory studies.

The ubiquitous copepod *A. tonsa* is often the dominant calanoid copepod species found in temperate estuaries. Because *Acartia* spp.

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0022-0981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jembe.2013.04.018

store minimal fats and lipids (Lee et al., 2006), convert ingested food to growth in a matter of hours (Tester and Turner, 1990), and also quickly stop growing in the absence of food (Dagg, 1977), it is a suitable genus to study in order to observe the immediate responses that copepods have to starvation. The main goal of this study was to test the hypothesis that male copepods (*A. tonsa*) have a lower tolerance to starvation than females; that is, males will die at a faster rate than females in the absence of food. We exposed field-caught copepods to four different diets prior to starvation. Separately, males and females were either starved or fed non-toxic food to evaluate the influence of diet on sex-specific tolerance to starvation. Further, we raised a cohort of *A. tonsa* in the laboratory to determine if age or duration in the adult stage affected tolerance to starvation. Lastly, we compared survival and fecundity during starvation.

2. Methods

To test whether prior diet influenced tolerance to starvation, adult male and female copepods (*A. tonsa*) were collected from Avery Point, Groton, CT, USA (latitude: 41.31519 N, longitude: 72.06352 W) using a 200 μm mesh size conical plankton net, equipped with a solid cod end, gently towed ~1–2 m below the surface. Copepods were immediately separated in the laboratory and placed into individual 100 × 60 mm Petri dishes filled with 75 ml of non-limiting concentrations (>600 μg C L^{-1}) (Besiktepe and Dam, 2002) of a food treatment, and maintained in an environmental chamber at 15 °C on a 12:12 light:dark cycle. Each food treatment had 20–25 individuals of each sex. The four food treatments consisted of the toxic dinoflagellate *A. fundyense* (Colin and Dam, 2003), the non-toxic congener species *Alexandrium tamarense* (Balech, 1990), the nutritionally insufficient dinoflagellate *Prorocentrum minimum* (Dam and Colin, 2005) and a mixture of non-toxic prey (*Thalassiosira weissflogii*, *Tetraselmis* sp., and *Isochrysis galbana*; standard diet) that we have used to consistently rear copepods for multiple generations (Colin and Dam, 2002; Feinberg and Dam, 1998). All algae were maintained in exponential growth phase as semi-continuous cultures on F/2 medium (Guillard, 1975). Experiments were done successively over a three-week time period in the following order: standard diet, *A. tamarense*, *P. minimum* and *A. fundyense*. Because experiments were conducted at different times, and hence we could not control for copepod condition factor, previous feeding history, etc., we did not make comparisons among experiments. In any case, the relevant comparisons for the purpose of this study are between sexes within an experiment.

After feeding for 24 h, copepods were removed from the food suspension, rinsed in 0.2 μm filtered seawater (FSW), and placed separately into 60 × 30 mm Petri dishes containing ~30 ml of 0.2 μm FSW. Copepod survival was checked daily, except on day 4 of the experiment with *A. tamarense*. Females were transferred daily to fresh FSW and eggs were counted, with the exception of the experiments with *P. minimum* (no egg counts on day 4) and *A. fundyense* (no egg counts on days 2 and 4). Males were transferred to fresh FSW every 1–2 days. Post-transfer microscopic verification ensured that the water contained no food. Day 1 in Fig. 1 represents the first full day of starvation. All copepods for these experiments were followed until death.

Further experiments were performed using field- and laboratory-raised copepods to determine the effect of age and the duration within adult stage of individuals on tolerance to starvation and adult longevity. Copepods were collected from Avery Point, CT, as described above. Adult males and females were immediately sorted and randomly placed into individual Petri dishes, as described above, filled with FSW (starvation-treatment), or non-limiting concentrations (>600 μg C L^{-1}) of the standard diet (fed-treatment). Approximately 30 individuals of each sex were assigned to each treatment. Starved animals were checked daily, with water changes every 1–2 days for males and every day for females. Fed animals were checked and transferred to fresh food daily.

At a later date, ~30 female copepods, collected from the same field location as above, were allowed to lay eggs for two days on non-limiting standard diet. All eggs were collected and incubated at 15 °C, and 12:12 light:dark conditions. Hatched eggs were raised under the same light and temperature conditions on the standard diet at non-limiting food concentrations. The starvation- and fed-treatments began upon maturation to the adult stage, between 18 and 23 days from egg-laying, with adults randomly assigned to either a fed (>600 μg C L^{-1} standard diet)

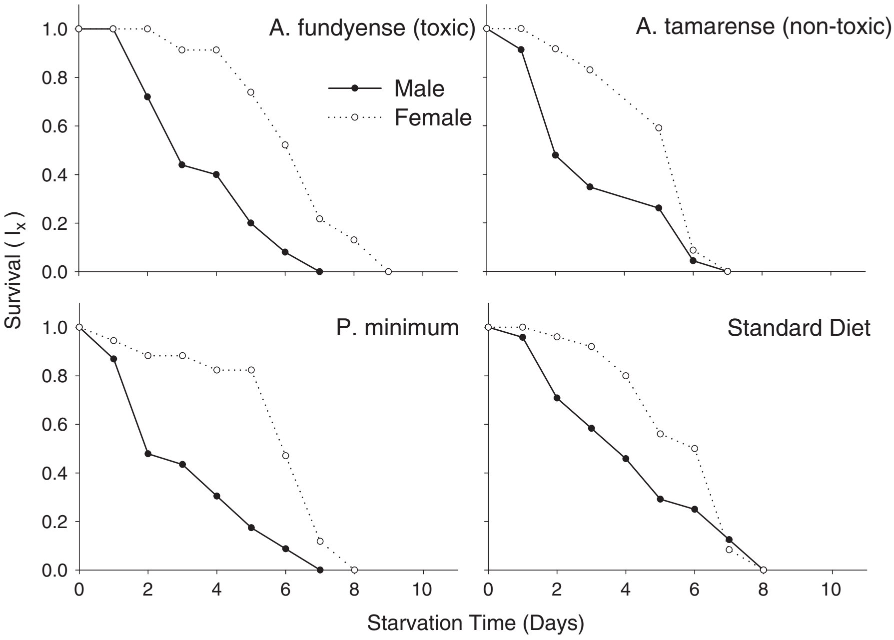

Fig. 1. Survival (fraction of individuals) versus starvation time for adult field-caught *Acartia tonsa* pre-fed four different diets then starved. The diets were: *Alexandrium fundyense* (a highly toxic clone), *Alexandrium tamarense* (non-toxic clone), *Prorocentrum minimum* (nontoxic, but nutritionally insufficient food), or a standard diet (mixed non-toxic algae). Day 1 represents the first full day of starvation. All copepods were followed until death. In all cases, males had lower tolerance to starvation than females; i.e., they died at faster rates (Gehan–Breslow test: *A. fundyense*: $p < 0.001$ (25 ♂, 23 ♀); *A. tamarense*: $p = 0.008$ (23 ♂, 24 ♀), *P. minimum*: $p < 0.001$ (23 ♂, 18 ♀); standard diet: $p = 0.034$ (24♂, 25♀)).