Food limitation in *Chironomus tepperi*: Effects on survival, sex ratios and development across two generations

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A B S T R A C T

Species from the Dipteran family Chironomidae are widely used in laboratory and field studies to identify toxicity in freshwater environments. However, toxicity assessments can be influenced by food availability, which can alter endpoints in assays including viability, sex ratios and development time. The aim of this study was to determine if food limitation affected the endpoints used in toxicity tests with the Australian model organism, *Chironomus tepperi*, including responses in offspring. First instar larvae were subjected to food treatments with larval density controlled and offspring were either raised under the same food conditions as their parents or under standard conditions. In lower food density treatments adults in the F_0 generation experienced delayed emergence and females produced fewer egg masses. F_0 diet affected the performance of F_1 to continued exposure and there was evidence that the quality of the offspring was compromised. Although sex ratios were not skewed, males and females responded differently to food limitation, especially in the F_1 generation where female development was more delayed. These results demonstrate that endpoints used in toxicity evaluation in *C. tepperi* also respond to food availability, highlighting the need to control for food in both laboratory and field toxicity studies. Multiple generations should ideally be exposed to stressors under laboratory conditions to elucidate likely long term effects in the field.

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Additional information

The effects of food limitation were investigated over two generations in the Australian Chironomidae species, *Chironomus tepperi*, which is used in laboratory toxicity testing and field biomonitoring. Food rations were selected to avoid mortality so sublethal effects could be investigated. In the F_0 generation a reduced food ration had no effect on adult sex ratios but caused significant delays in the development of larvae. Both sexes experienced delays in development, although their responses were different. In the second generation, F_1 were either exposed to the same food limited conditions as their parents or to standard conditions. There was some evidence that F_0 diet affected F_1 responses, such as reduced survival of low food ration offspring, regardless of what nutrition these offspring were fed. Offspring of food-limited parents experienced delayed development under continued food limitation, whereas offspring reared under standard conditions developed at a rate similar to chironomids exposed to standard conditions in both generatons. In both generations fewer egg masses were laid by females that experienced greater food limitation, while hatching was unaffected.

These results demonstrate how endpoints used in ecotoxicology with *Chironomus tepperi* are affected by changes in food limitation, and highlight the need to control for changes to food availability (such as via density changes due to mortality or adult emergence) in toxicity studies. Parental exposure to a stressor can affect the sensivity of offspring to that stressor or other stressors, and can alter the quality of those offspring. We found evidence of this in the current study, which emphasises the importance of including multigenerational effects in ecotoxicology. Multigenerational effects become particularly important when laboratory results are being extrapolated to field conditions, where multiple generations of a species may be exposed to stressors; however few laboratory tests are conducted to examine multigenerational effects. We conclude that food limitation and multigenerational effects are important to consider in ecotoxicology and may help in the interpretation of field data.

1. Introduction

In aquatic ecotoxicology invertebrate taxa are commonly used to indicate biological impairment from contaminant exposure. The Chironomidae represents one species diverse family within the Diptera that is frequently used in field surveys to assess aquatic ecosystem health (e.g., Hawkins et al., 2000; EPA Victoria,

2003), in field-based experimental approaches to isolate the effects of factors such as sediment pollution from hydrology and habitat (e.g., O'Brien et al., 2010), and in laboratory toxicity testing (e.g., Rakotondravelo et al., 2006). In these types of studies any biological responses are usually assumed to be the result of the stressor/s of interest. However, the responses measured may be confounded by other factors including nutrition.

Changes to resource availability can potentially exert effects on chironomids that resemble those due to toxicity, affecting viability and delaying development. In natural systems and field-based experiments, food availability can vary considerably between sites and environmental samples collected. This heterogeneity can result in differences in the availability of food items for different chironomid species, which may consume detritus, algae and organic matter, other macroinvertebrates and even wood (Oliver, 1971). For example, the abundance algae can be directly influenced by pollutants such as nutrients and herbicides, leading to indirect effects on algal grazers including chironomids. The effect of these changes and ability to mimic macroinvertebrate toxicity can also depend on the density of the chironomid populations (e.g., Postma et al., 1994).

Mortality, adult emergence, development and reproduction are commonly used endpoints in *Chironomus* tests, but these can be susceptible to the effects of food limitation as demonstrated in other taxa (e.g., Anger and Dawirs, 1981; Juliano, 1986). In *C. riparius* high larval densities reduced survival, adult emergence, development rate, fecundity and adult body weight as a result of decreased resource availability (Hooper et al., 2003). Because food limitation can affect the sexes differently, there is also the potential to influence sex ratio. For example, female Chinese mantids *Tenodera sinensis* (Saussure) were more susceptible to starvation than males because they were larger and had greater food requirements (Moran and Hurd, 1994). Female larvae and pupae of several chironomid species also tend to be larger and have greater energy reserves (Atchley, 1971; Servia et al., 2006; Benbow, 2008). Therefore chironomids may be susceptible to sex specific effects of food limitation, skewing sex ratios in populations in the same way that chemical stressors affect sex specific endpoints in chironomids (for example, Hahn et al., 2001; Rakotondravelo et al., 2006).

Both toxicity and nutrition effects may extend across generations by influencing resources that parents allocate towards offspring. For instance, females of two fish species that provision their young through yolk content produced larger offspring with greater energy reserves when they were fed a low food ration (Reznick et al., 1996). In chironomids, multigenerational effects occur following contaminant exposure, and in some cases the magnitude of effects appear greater in later generations (Janssens de Bisthoven et al., 2001). However, later generations can also show increased tolerance towards contaminants, implying that previously exposed generations confer an adaptive advantage to their offspring (Vogt et al., 2007). There is little information on the potential multigenerational effects of food limitation in chironomids even though this could confound the detection of toxicity in the field where multiple generations are exposed.

In Australia the chironomid *Chironomus tepperi* is used in field surveys (e.g., Carew et al., 2007) and toxicity studies (e.g., Stevens, 1992), but the effects of food limitation in this species have not been studied. The aims of this study are to provide data on the effects of food limitation on *C. tepperi* and to test the importance of controlling food availability in *Chironomus* toxicity tests. The experiments were conducted over two generations to determine if parental condition affects the quality and performance of offspring. We hypothesised that under limited food availability adult emergence would be delayed, and that this effect would be greater in female chironomids, which are likely to have greater

developmental energy requirements because of their larger size and egg provisioning. We also hypothesised that the offspring of food-limited parents would be of poor quality even when raised using recommended feeding levels.

2. Materials and methods

2.1. Experimental design

The experimental protocol was adapted from the guidelines recommended by OECD (2004a, 2004b). *Chironomus tepperi* were obtained from three different laboratory cultures originating from the University of Melbourne, Latrobe University and CSIRO Land and Water, South Australia. Inbreeding depression is a problem with laboratory experiments, so to increase the genetic variability of the laboratory population and mask potential inbreeding depression the cultures were crossed. First instar larvae for the experiment were obtained by collecting several egg ropes from the mixed laboratory population. First instar larvae from the egg ropes were collected within 24 h of hatching and were added to the test vessels (below).

Each food treatment was represented by three replicate test vessels. Each vessel consisted of a 600 mL Pyrex beaker with an artificial substrate and approximately 400 mL of Martin's solution, a reconstituted water solution that is a suitable medium for rearing *Chironomus tepperi* (Martin, 2009). The reconstituted water had a pH of 6.43, dissolved oxygen content of 92.0% saturation, conductivity of 237 $\mu S\, cm^{-1}$, general hardness of 53.7 ppm, carbonate hardness of 17.9 ppm and ammonia of 0 ppm at 22 °C. The artificial substrate was two squares of toilet tissue (Coles Soft & Strong Toilet Tissue, Coles Australia) previously rinsed in ethanol, dried and shredded. Toilet tissue was selected rather than other substrates like sediments to reduce food availability from alternative sources. Test vessels were rinsed in 10% nitric acid then distilled water before use. After the addition of substrate and reconstituted water, the food treatment was added to each vessel followed by 20 first instar larvae. Each vessel was securely covered by a piece of translucent white nylon cloth. After an initial 24 h the test vessels were aerated. Food treatments consisted of finely ground TetraMin Tropical Flakes (Tetra, Germany). The treatments were designated as "standard" (upper limit of 1 mg larva^{-1} day^{-1} recommended by OECD (2004a, 2004b) for *C. riparius* larvae), "2.0" (2 mg larva^{-1} day^{-1}, twice the standard amount), "0.50" (the lower limit recommended by the OECD guidelines of 0.5 mg larva^{-1} day^{-1}), "0.25" (0.25 mg larva^{-1} day^{-1}) and "0.10" (0.1 mg larva^{-1} day^{-1}). Early instar larvae were fed half the experimental quantity, as recommended in the OECD guidelines. Larvae were counted twice weekly by gently searching through the substrate with a clear plastic pipette. Food was added to each test vessel twice weekly. The amount of food added was calculated based on the number of larvae remaining in the test vessel to avoid increased food availability with decreasing larval density. The experiment was run at 20 °C under 16 h light: 8 h dark in a temperature controlled cabinet. Water and substrate were renewed weekly.

Emerging adults were collected daily using an aspirator and their sex was noted. Adults from all replicates of a treatment were pooled into one breeding tank (clear Perspex, 24 cm depth × 30 cm width × 30 cm height courtesy of J. Martin) with a tray of reconstituted water at the bottom for reproduction. Adult *Chironomus tepperi* live for three to five days with males emerging one to two days earlier than females. F_1 treatments were set up within five days of first emergence from a treatment. This meant that stressed treatments with delayed emergence were set up a few days later than other treatments. Egg masses were removed from the tray daily, placed in petri dishes with reconstituted water under experimental conditions and checked daily for hatching.

A multigenerational experiment was conducted using these egg masses. Newly hatched larvae from several egg masses of a treatment were mixed together before the experiment to decrease relatedness of the larvae. Larvae were then selected and were either raised under the same food conditions as their parents or were raised under standard conditions. The experiment was conducted following the same experimental procedure described above. F_1 adults were also allowed to breed, and the number and hatching success of egg masses noted.

Water quality was measured prior to water and substrate renewal using a field water quality meter (TPS WP-81, Australia). Water quality was similar among all treatments with temperatures between 19 °C and 20 °C, pH between 5.7 to 6.7, and conductivity between 210 and 274 $\mu S\, cm^{-1}$. Dissolved oxygen was not measured due to a faulty probe; however it was not expected to significantly differ between treatments because in the treatment where it was most likely to decrease (i.e., 2.0 food treatment) other water quality parameters were unaffected, there was no evidence of excessive decomposition or microbial growth, and *C. tepperi* larvae from this treatment were large, red, "healthy" and similar in appearance to larvae from the standard and 0.5 food treatments (K.T. personal observation).

2.2. Statistical analyses

All tests were conducted in PASW 18 (SPSS Chicago, IL) and parametric tests were used because data were normally distributed (Kolmogorov–Smirnov tests,