EFFECT OF THE 'RED-TIDE' DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* ON RESPIRATION RATES OF THE COPEPOD *METRIDIA LONGA*

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Research on the interaction between zooplankton and toxic phytoplankton has centered on studies of the fate of toxins and on particle selection, ingestion, and behavioral responses. It is known that copepods can accumulate saxitoxins, the potent neurotoxins of the 'red-tide' dinoflagellate *Alexandrium* spp., and they can pass these toxins on to fish (White, Can. J. Fish. Aquat. Sci., 37: 2262-2265, 1980; Limnol. Oceanogr. 26: 103-109, 1981). When the toxic food is removed, the accumulated toxins gradually decline (White, 1981). There is a limited amount of data on the physiological response of zooplankton to toxic foods. Different species of dinoflagellate can evoke different responses in copepods ranging from rejection of particles to retching and rapid heartbeat (Sykes and Huntley, Mar. Biol., 94: 19-24, 1987). This latter study is one of few that allude to a physiological response (heartbeat) on the part of copepods to their potentially toxic diet, and the results suggest that there may be energetic costs to the presence of toxic dinoflagellates. Ives (J. Exp. Mar. Biol. Ecol., 112: 131-145, 1987) found that ingestion of the toxic dinoflagellate *Gonyaulax (= Alexandrium) tamarensis* declined proportionately with levels of toxin in different clones of the species. Furthermore, the decline in ingestion was due to the toxic effects of ingesting the cells and not to avoidance of the more toxic clones. Huntley et al. (Mar. Biol., 95: 103-113, 1987) also concluded that toxic cells were rejected only after an initial period of ingestion followed by a toxic reaction.

To test whether exposure to toxic algae affects respiration rates of copepods, the copepod *Metridia longa* was exposed to toxic and non-toxic isolates of the dinoflagellate *Alexandrium tamarense* at bloom concentrations (≈1000 cells ml^{-1}) for 24 h, and its subsequent respiration rates were measured. *M. longa* and *A tamarense* co-occur in the Gulf of Maine, and *M. longa* may be a significant grazer on the dinoflagellate during bloom formation.

Alexandrium tamarense isolates GtCA28 (toxic) and SP3B8-3 (non-toxic) were obtained through the lab of D. Anderson at Woods Hole. Cultures were maintained at 16°C on a 14/10 h light/dark cycle using L-growth medium (Keller et al., J. Phycol., 23: 633-638, 1985). A natural food assemblage for use as a control was prepared from seawater incubated with nutrient additions. Adult female *Metridia longa* were collected off the mouth of the Damariscotta River with a 100m - surface oblique haul, using a 350µm mesh, 3/4 m diameter plankton net. Copepods were sorted at the Darling Marine Center, and then returned to MDIBL. Prior to the start of the experiment, copepods were maintained in 4-l polycarbonate beakers in a flowing seawater tank at ≈12°C exposed to natural light. Water was changed daily, screened through a 73µm filter to provide a natural food source. At the start of the experiment, copepods were sorted into beakers and food was added. Initial respiration rates were determined on 2 groups of copepods, then the copepods were allowed to feed for 24-36 hr before treatment respiration rates were determined. Generally 4-6 replicate measurements were made for each treatment.

To measure respiration rates, copepods were first separated from food by pipetting three times into glass well-dishes containing 0.2 µm filtered seawater. Incubations were conducted for 1-2 h in a water-jacketed dual respiration cell at 12°C, with 2-3 copepods per 1.4 ml cell. Oxygen consumption was measured with Clark-type polarographic micro-cathode electrodes and a Cameron Instruments 2-channel oxygen meter, connected to a Macintosh Powerbook 150 and Vernier Software Serial Box Interface. Respiration rates were determined by linear regression fit to the data using Datalogger software (Vernier Software). Readings generally stabilized after 10-15 minutes, and a regression was fitted over the remaining 1-2 hour interval. Significant differences among treatments were determined by ANOVA (Statview Student). To test for

handling effects, the same copepods were used a second time in 10 incubations. Respiration rates declined on average by 11% in the 10 repeat incubations. However, 5 of the incubations had declines in respiration rate of >20%, while the other 5 had negligible changes. Because removal from the respiration cell is the most difficult handling procedure, it is likely that the declines in respiration rate were due to removal and not addition to the respiration cell.

Respiration rates among the control copepods declined significantly after the first day of the experiment, but remained stable for the following 8 days (Fig. 1). Following 24 h exposures to the

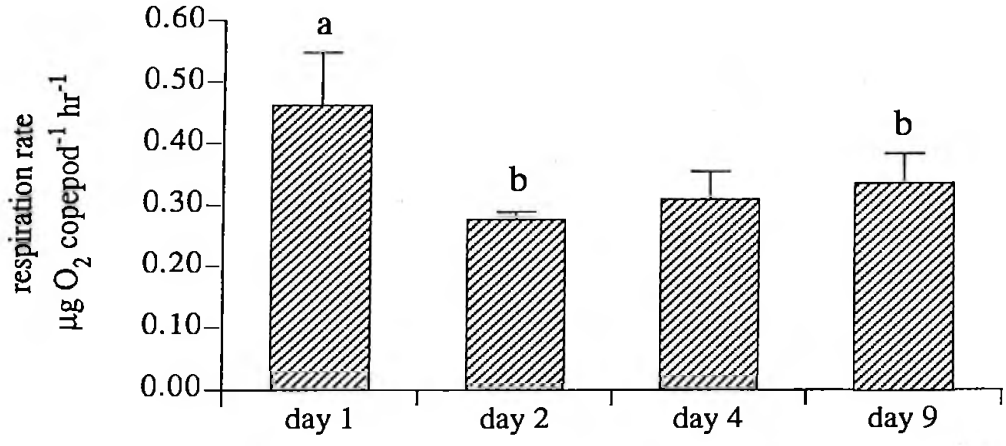

Figure 1. Respiration rates of *Metridia* in 1-2 hr incubations. Following day 1, copepods were switched to laboratory cultured food. a and b denote significant differences among treatments. Values are average ± standard deviation. Sig., $p<0.025$, F-test. Day 1 and 9, n=6; day 2 n=3; day 4 n=2.

different food sources, respiration rates were significantly lower for copepods fed both toxic and non-toxic isolates of *Alexandrium tamarense* (Fig. 2). Food was evident in the guts of *M. longa* feeding on the control food, and abundant fecal pellets were present. No fecal pellets were produced with the toxic GtCA28 isolate, and no food was evident in the gut, while with the non-toxic SP3B8-3 isolate, food was present in the gut but few fecal pellets were produced. Since *M. longa* did not appear to be consuming *A. tamarense*, a repeat experiment was conducted in which an additional treatment with no food was included. In this experiment (Fig. 3) the starved copepods had significantly lower respiration rates. Respiration rates for copepods feeding on the toxic *A. tamarense* isolate were not significantly different from the control, although the trend towards lower respiration rates with the toxic treatment was the same as in the first experiment.

These results suggest that the main effect of *Alexandrium tamarense* on the respiration rates of *Metridia longa* is due to a starvation effect as opposed to a response to the toxins. In experiment 1, *M. longa* avoided both the toxic and non-toxic isolates of *A. tamarense*, based on fecal pellet production, and *M. longa* had lower respiration rates after exposure to both isolates, a decline similar to that seen in the starved copepods. These results are in contrast to those obtained with bivalves, in which VO_2 for juveniles was the same after 1 h exposures to both toxic and non-toxic isolates of *A. tamarense*, despite differences in grazing rates (Marsden and Shumway, Comp. Biochem. Physiol., 106A: 769-773, 1993), a response that may reflect greater energy reserves in the bivalves and the short duration of exposure. The response to the non-toxic isolate was variable,

and may be due to varying lengths of exposure or the condition of the individuals at the start of the experiment. Thus the primary energetic cost to *Metridia longa* from a bloom of *Alexandrium tamarense* is likely to be due to lack of food rather than toxin content of the algae.

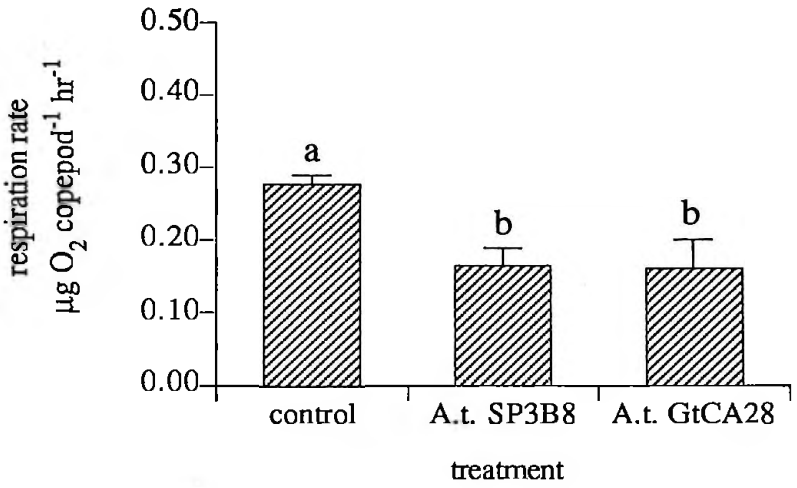

Figure 2. Respiration rates of *Metridia* following exposure to food treatments. Notations as in Fig. 1. Sig., $p<0.001$, F-test. Control n=3, SP3B8 n=5, GtCA28 n=4.

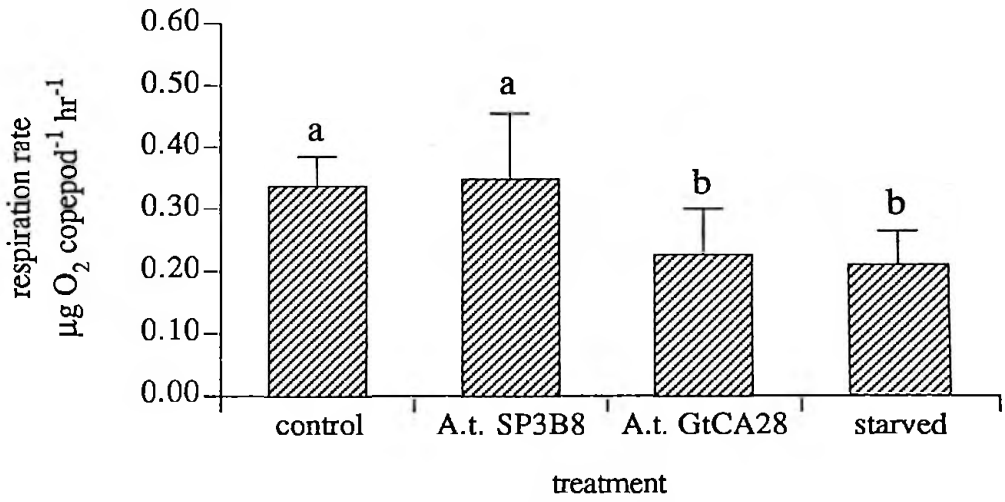

Figure 3. Respiration rates of *Metridia* following exposure to food treatments. 'Starved' copepods were in filtered seawater for 24 h. Notations as in Fig. 1. Sig., $p<0.04$, F-test. Control n=6, SP3B8 n=5, GtCA28 n=6, starved n=5.

This research was supported by a MDIBL New Investigator Award and NSF award #OCE-9402186. I would like to thank Kevin Lapham, captain of the RV Lee, University of Maine Darling Marine Center, for assistance in sampling.