Does *Acartia hudsonica* exhibit an enzymatic stress response to toxic *Alexandrium* spp.?

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In the Gulf of Maine marine copepods, the primary component of the zooplankton, are regularly exposed to blooms of toxic *Alexandrium* spp.[12]. Copepods are known to accumulate toxins[6,11] and pass them up the food chain[13]. Ingestion rates decline with cell toxicity, a physiological effect and not selection[6]. *Alexandrium* can produce rapid heart beat and "retching" behavior in copepods[10]. Ingestion of toxic *Alexandrium* may also reduce fecundity in *Acartia*, a common genus of nearshore waters[3]. Not all copepods, however, are affected by toxicity. *A. hudsonica* readily ingests *Alexandrium*, with both survival and oxygen consumption unaffected, while two other copepods, *Calanus finmarchicus* and *Metridia lucens*, avoid ingesting *Alexandrium*[5]. The variable response of copepods to toxic *Alexandrium* raises the question of whether some copepods have defense mechanisms, such as detoxifying enzymes, that allow them to safely ingest *Alexandrium*.

During experiments in which plasma membranes were prepared from *Acartia hudsonica*[2] specific activities of Na^+/K^+-ATPase (NKA) and NADPH-dependent cytochrome c reductase (NCCR) (used as enzyme markers of biological membranes) were found to be nearly 2-3 times lower following 5 days of acclimation to the diatom *Thalassiosira weissflogii* than they were in animals recently sorted and held overnight in large tanks (Fig. 1). NCCR has been used as a biomarker of exposure to toxins in the euphausid *Meganyctiphanes norvegica*[4]. NKA has been found to increase in fish gill in response to environmental stress[7]. Since toxic algal species, including *Alexandrium*, are frequently found in the Gulf of Maine and Frenchman Bay during the summer, the observed decline in these two enzymes during the acclimation treatment may be the result of removing the animals from an environment containing toxic algal species in the phytoplankton. In the present study we test the hypothesis that the presence of toxic *Alexandrium* spp. induces higher activities of these enzymes.

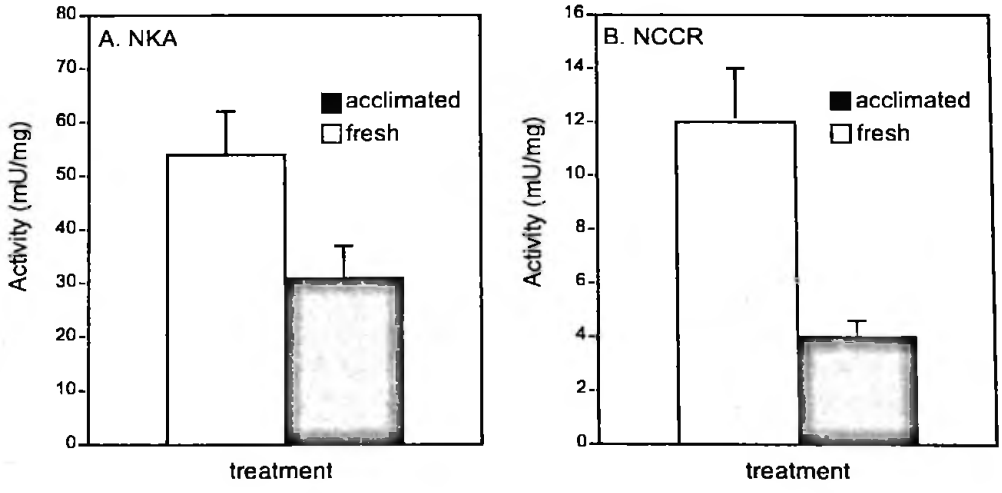

Fig. 1. Activities of A. Na^+/K^+ ATPase (NKA) and B. NADPH-dependent cytochrome C reductase (NCCR) of *Acartia hudsonica* on freshly caught animals (24 h after collection) and following 5 d acclimation to the diatom *Thalassiosira weissflogii*.

Acartia hudsonica were collected from Frenchman Bay with a 202μm ringnet towed at 2-3 m below the surface. Contents of the net cod end were added to a 20g cooler and copepods were

presorted by attracting *Acartia* to a lighted corner of the cooler. Adult females were then sorted by microscope and held in 4 l containers for experimental treatments. Algal stocks were obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton (CCMP), Bigelow Laboratory for Marine Sciences (Boothbay Harbor, ME) and maintained in L2 culture media (also from CCMP). *Thalassiosira weissflogii*, toxic *Alexandrium fundyense* CCMP1719, and non-toxic *Alexandrium tamarense* CCMP115 were used as food sources. Stocks were incubated at 18°C on a 14h light/10h dark cycle. Copepods were acclimated for 2 days on *T. weissflogii* at 1000 cells ml^{-1}, with concentrations monitored by in vivo fluorescence (Turner handheld fluorometer). Following the initial acclimation period CCMP1710 and CCMP115 were added to two of the 3 treatments at concentrations of 10 cells ml^{-1}, comparable to densities observed during *Alexandrium* blooms. *T. weissflogii* was maintained at 1000 cells ml^{-1} in all 3 treatments during this period. After an additional 2 days copepods were sorted from the treatments and prepared for enzyme analysis. Feeding activity was confirmed by changes in fluorescence in the containers as well as by cell counts of *Alexandrium* and appearance of *Alexandrium* in copepod guts (apparent by changes in color).

Animals from each treatment were homogenized in a Ten-Broeck ground-glass homogenizer using 25 mM Hepes/1 mM EDTA (pH 7.6) and stored in liquid nitrogen until enzymatic assays were performed. NKA and NCCR were assayed according to Barnett[1] and Masters *et al.* [8], respectively. Enzymes were assayed in duplicate at 25°C. To obtain protein-specific activities of the enzymes, protein contents were determined by the bicinchoninic acid method[9].

Ingestion of *Thalassiosira weissflogii*, as measured by change in fluorescence, was comparable in all 3 treatments. Ingestion of *Alexandrium* was evident from low concentrations remaining in the containers as well as the color of copepod guts and fecal pellets, although the low concentrations used in the acclimations precluded an accurate assessment of ingestion rates between the two *Alexandrium* treatments. Neither activity of NKA nor NCCR changed significantly with either of the two *Alexandrium* treatments (Fig. 2).

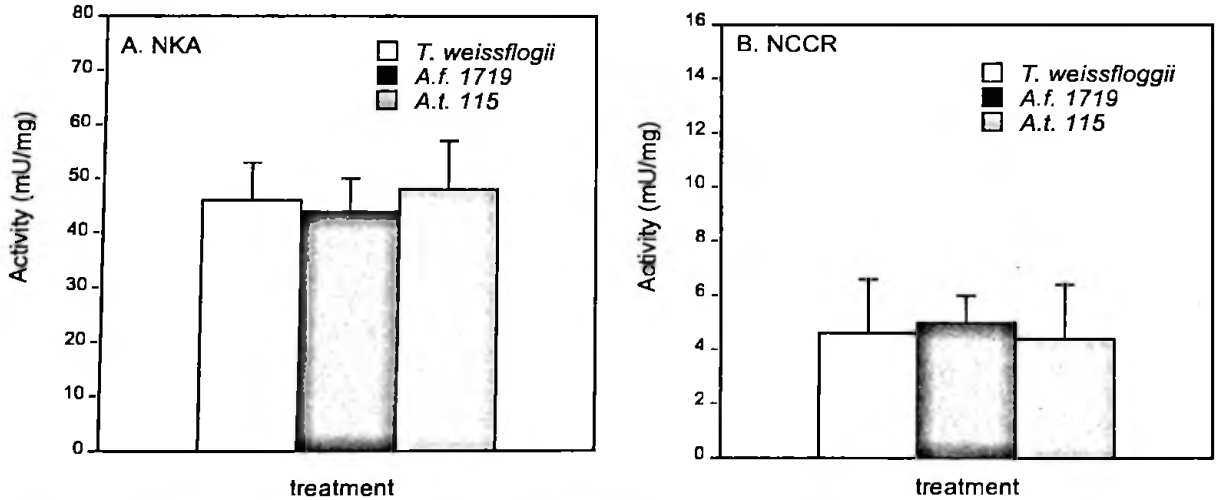

Fig. 2. Activities of A. Na^+/K^+ ATPase (NKA) and B. NADPH-dependent cytochrome C reductase (NCCR) of *Acartia hudsonica* following 2 d acclimation to the diatom *Thalassiosira weissflogii*, and the dinoflagellates *Alexandrium fundyense* CCMP1719 (A.f. 1719) and the non-toxic strain *Alexandrium tamarense* CCMP115 (A.f. 115).

In conclusion, there is no evidence for an effect of *Alexandrium* (at concentrations typically seen during blooms of this dinoflagellate) on activities of NKA and NCCR in *Acartia hudsonica*. Activities

of NKA and NCCR following exposure to all 3 food treatments were comparable to activities observed the previous year following acclimation (5 days) to *Thalassiosira weissflogii*. *Alexandrium* densities even 10-fold higher do not affect oxygen consumption rates of *A. hudsonica*[5], and given the probable importance of NKA in the animals' energy budget, it is unlikely that NKA is affected at these high cell densities. Oxygen consumption of *A. hudsonica* and other copepod species is elevated for several hours after handling before declining to a level that remains stable for at least 4-7 days[5]. The high enzyme activities observed initially (measured on copepods that had been held overnight) may be a transient stress response to collection.

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