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Comparative dynamics of paralytic shellfish toxins (PST) in a tolerant and susceptible population of the copepod *Acartia hudsonica*

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ABSTRACT

In common-environment experiments, we measured the uptake, accumulation, retention, transformation and depuration of paralytic shellfish toxins (PSTs) of the dinoflagellate *Alexandrium fundyense* in adult females of two geographically separated populations (Maine and New Jersey, USA) of the copepod *Acartia hudsonica*. These populations were previously shown to differ in their history of exposure to *Alexandrium* blooms, and in their degree of adaptation to this dinoflagellate. Toxin accumulation was measured at several times in incubations lasting up to 72 h. Toxin depuration was assessed after a 60 h incubation of females with toxic *A. fundyense* and subsequent 60 h incubation in a sole diet of the non-toxic green flagellate *Tetraselmis* sp. As previously observed, the Maine population had significantly higher toxin ingestion rates. By contrast, both toxin accumulation (up to ~2.5 ng STX eq. female^{-1}) and the depuration rate (~0.73 d^{-1}) were not significantly different between populations. Hence, faster depuration is ruled out as a tolerance mechanism in the Maine population. Some toxin transformation during both accumulation and depuration was evident in both populations. However, differential toxin transformation does not appear to be a tolerance mechanism either. In contrast to these results, toxin retention (ratio of accumulation/cumulative ingestion) was significantly lower in the Maine population. The retentions of gonyautoxin 3 (GTX3) and of two of the most potent toxins, neosaxitoxin (NEO) and saxitoxin (STX), were also significantly lower in that population. At steady state, toxin absorption efficiency was estimated to be 6% for the Maine population and 9% for the New Jersey population. These results suggest that lower toxin absorption is a possible tolerance mechanism for the Maine population. The results of the present study also suggest that copepod adaptation to toxic dinoflagellates does not necessarily lead to higher toxin transfer up the food web.

1. Introduction

Blooms of the dinoflagellate genus *Alexandrium* cause serious threats to coastal ecosystems and human health (Anderson, 1997; Van Dolah, 2000) through the production of paralytic shellfish toxins (PSTs), the suite of toxins that include saxitoxin and its derivatives (Anderson et al., 1990; Strichartz and Castle, 1990). It is widely accepted that *Alexandrium* is grazed by a variety of organisms and the ingested PSTs may be transferred to higher trophic levels (e.g., zooplankton: White, 1981; shellfish: Shumway, 1990; whales: Durbin et al., 2002 and fish: Samson et al., 2008).

PSTs may inhibit grazing (as reviewed in Turner and Tester, 1997) although that may not be the only function of the toxins (e.g., pheromones: Wyatt and Jenkinson, 1997), nor are PSTs the only toxic substances produced by *Alexandrium* (Juhl et al., 2008). In any case, the susceptibility of grazers to PSTs varies. For example, work with bivalve mollusks has reported not only cross-genera, but also interspecific variation in sensitivity to PSTs (Shumway and Cucci, 1987; MacQuarrie and Bricelj, 2000; Bricelj et al., 2005). Although size and age play a role in eliciting these differences, the primary driver of interspecific variation to PSTs is an animal's history of exposure, largely a function of geography (Twarog, 1974). Recent work with another grazer, the boreal planktonic copepod *Acartia hudsonica*, has also demonstrated geographically-underlain interspecific variation to PSTs. Specifically, adult female *A. hudsonica* from regions exposed (Casco Bay, Maine) to recurrent blooms of toxic *Alexandrium* have significantly higher ingestion and fecundity rates (Colin and Dam, 2002) and population fitness, lambda (Colin and Dam, 2004) on a diet of toxic *Alexandrium fundyense* than female *A. hudsonica* from regions (Great Bay, New Jersey) not exposed to these toxic blooms. Furthermore, ingestion of *A. fundyense* decreases respiration rates of copepods from unexposed regions, but not those of copepods from exposed regions (Colin and Dam, 2003). These results were obtained after raising exposed and

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unexposed copepods (hereafter referred to as the Maine and New Jersey populations, respectively) in the same environment for many generations and are thus consistent with the hypothesis that copepods from the Maine population have evolved tolerance to toxic *Alexandrium*.

Here, we draw a distinction between toxin tolerance and toxin resistance. Although the definitions and distinctions between tolerance and resistance are not always consistent within the ecotoxicological literature, for the purpose of this work, we adopted the conventions used within the plant–pathogen/herbicide community. Thus, PST resistance would suggest a mechanism that prevents the copepod from experiencing damaging effects from the toxins. By contrast, tolerance implies that some damage is sustained, but the copepod has mechanisms to compensate for the incurred damage with no loss to its fitness (Baucom and Mauricio, 2004; Råberg et al., 2009).

Common toxin tolerance mechanisms either decrease the quantity of toxin reaching the site it affects (e.g., restricted uptake or accumulation), or reduce the response to the toxin by some cellular mechanism (e.g., biotransformation pathways, sequestration, or depuration). While previous studies on PSTs in grazers have dealt with the processes of toxin accumulation (uptake minus elimination: White, 1981; Boyer et al., 1985; Bricelj et al., 1990; Turriff et al., 1995; Teegarden et al., 2003), retention (accumulation/toxin ration: White, 1981; Teegarden and Cembella, 1996), depuration (elimination: Teegarden, 1999; Guisande et al., 2002) and transformation (modification by the grazer of the toxin ingested: Shimizu, 1978; Hamasaki et al., 2003; Teegarden et al., 2003), the mechanisms that confer tolerance to the Maine population are not known and may have a significant impact on the role of copepods as grazers of toxic algae as well as in the transfer of toxins through the food web.

In this study, our approach was to compare as many parameters as possible of PST dynamics (dissolved and particulate uptake, depuration, accumulation, retention and transformation) in the Maine and New Jersey populations of the copepod *A. hudsonica*.

2. Materials and methods

2.1. Algal and copepod cultures

The toxic dinoflagellate *A. fundyense* (strain NB-05) and the non-toxic green flagellate *Tetraselmis* sp. (EPA strain, RI, USA) were grown in *f*/2 medium (Guillard, 1975) without silica at 15 °C on a 12 h light: 12 h dark cycle and maintained in exponential growth phase by diluting the cultures by ~50% weekly with growth medium.

The estuarine copepod *A. hudsonica* was collected from Casco Bay, Maine (43°41′N, 70°15′W) and Great Bay, New Jersey (39°48′N, 74°50′W). Casco Bay experiences recurrent blooms of toxic *Alexandrium* spp. whereas Great Bay has never experienced such blooms (see summary in Colin and Dam, 2002). After collection, samples were immediately transported to the laboratory, where 1000–1500 individuals from each population were isolated for long-term culture (Colin and Dam, 2002). Copepod cultures were reared on a mixed diet of *Thalassiosira weissflogii*, *Isochrysis galbana* and *Rhodomonas lens* (Feinberg and Dam, 1998), at a concentration of 400–500 μg C L^{-1}. This concentration is near the saturation level of the functional and numerical responses of *A. hudsonica* (Colin and Dam, 2007). Cultures were refreshed annually with new individuals from their source location. Adults (500–1000) were kept in 20 L vessels to minimize genetic drift within populations (Falconer, 1996). The light cycle and temperature for the copepod cultures and all experiments were the same as for the algal cultures.

2.2. Dissolved toxin uptake

To verify the assumption that direct uptake of dissolved toxins is negligible in both the tolerant and non-tolerant copepod populations, we ran a single experiment. Approximately 20 adult female copepods were placed in 500 mL 0.2-μm filtered seawater spiked with gonyautoxin certified reference standard to a final concentration of 20 μM. This concentration was chosen based on the per cell toxin available in a 400 μg C L^{-1} diet of *A. fundyense*, as determined from preliminary toxin analyses. The copepods were removed after 8 h and processed for toxin analyses as per the protocol below.

2.3. Toxin accumulation

Two separate experiments were performed using healthy, adult female copepods that had been individually picked from the rearing cultures. Twenty-four hours prior to the experiment, copepods were fed a suspension of *Tetraselmis* sp. (~400 μg C L^{-1}) and acclimated to experimental conditions. To verify that differences in ingestion and toxin accumulation between Maine and New Jersey female copepods were not due to differences in animal size, three replicates of 30 copepods from each population were collected and filtered onto combusted (500 °C, 8 h) GF/F-filters and dried. Dry weights were determined to the nearest μg on a Cahn microbalance.

At time zero (t = 0), triplicate samples of 12 females from each population were isolated into 1.5 ml microcentrifuge tubes. Excess seawater was removed with a fine-gauge needle and syringe. Copepods were frozen (−80 °C) in 125 μl 0.1 M acetic acid (0.2 μm-filtered) for later toxin extraction (Frangópulos et al., 2000).

Copepods were exposed to toxic *A. fundyense* for different time intervals to measure ingestion rates and toxin accumulation over time. To ensure that cell removal was no more than 25% of the initial concentration, the length of the incubation determined the appropriate bottle size. Triplicate sets of 20 females each were incubated for 8, 16, 24, 36, 48 and 60 h in 250, 250, 500, and 1000 ml bottles, respectively. The bottles were filled with filtered seawater, *f*/2 growth medium and a suspension of *A. fundyense* (400 μg C L^{-1}). In a second experiment, incubation times were 12, 24, 48, 60 and 72 h. The longer incubation time was to resolve the point at which toxin accumulation reached a relatively constant level. In all experiments, three treatment (with copepods) and two control bottles (no copepods) were used. Treatment and control bottles were incubated in a walk-in environmental chamber and rotated end to end at 1.3 rpm on a plankton wheel. At the conclusion of each exposure time, females were gently removed from their bottles by wet-sieving onto a 200 μm sieve and aliquots of the *A. fundyense* suspensions were taken for cell counts and preserved in 0.5% acid Lugol's solution. Cell concentrations were determined by microscopic cell counts using the Utermöhl method (1958). Cell ingestion rates (μg C copepod^{-1}) were calculated according to Frost (1972). Cumulative ingestion of *Alexandrium* was calculated as the total number of cells eaten from t = 0 up to a specific time point. Copepods were transferred to 1.5 ml microcentrifuge tubes and prepared for toxin extraction as described above. In this study we define toxin accumulation as the toxins present in the gut and tissues of the grazer, as has been done in previous studies (e.g., Bricelj et al., 1990; Teegarden and Cembella, 1996; Hamasaki et al., 2003).

2.4. Toxin depuration

To compare the depuration of PSTs between Maine and New Jersey copepods, triplicates of 100 adult females from each