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Harmful Algae 4 (2005) 371–382
HARMFUL ALGAE
www.elsevier.com/locate/hal

Experimental study on the impact of dinoflagellate *Alexandrium* species on populations of the rotifer *Brachionus plicatilis*

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Received 28 January 2004; received in revised form 3 April 2004; accepted 18 June 2004

Abstract

To investigate harmful effects of the dinoflagellate *Alexandrium* species on microzooplankton, the rotifer *Brachionus plicatilis* was chosen as an assay species, and tested with 10 strains of *Alexandrium* including one known non-PSP-producer (*Alexandrium tamarense*, AT-6). HPLC analysis confirmed the PSP-content of the various strains: *Alexandrium lusitanicum*, *Alexandrium minutum* and *Alexandrium tamarense* (ATHK, AT5-1, AT5-3, ATCI02, ATCI03) used in the experiment were PSP-producers. No PSP toxins were detected in the strains *Alexandrium* sp1, *Alexandrium* sp2.

Exposing rotifer populations to the densities of 2000 cells ml^{-1} of each of these 10 *Alexandrium* strains revealed that the (non-PSP) *A. tamarense* (AT-6) and two other PSP-producing algae: *A. lusitanicum*, *A. minutum*, did not appear to adversely impact rotifer populations. Rotifers exposed to these three strains were able to maintain their population numbers, and in some cases, increase them. Although some increases in rotifer population growth following exposures to these three algal species were noted, the rate was less than for the non-exposed control rotifer groups.

In contrast, the remaining seven algal strains (*A. tamarense* ATHK, AT5-1, AT5-3, ATCI02, ATCI03; also *Alexandrium* sp1 and *Alexandrium* sp2) all have adverse effects on the rotifers. Dosing rotifers with respective algal cell densities of 2000 cells ml^{-1} each, for *Alexandrium* sp1, *Alexandrium* sp2, and *A. tamarense* strains ATHK and ATCI03 showed mean lethal time (LT_{50}) on rotifer populations of 21, 28, 29, and 36h, respectively. The remaining three species (*A. tamarense* strains AT5-1, AT5-3, ATCI02) caused respective mean rotifer LT_{50}s of 56, 56, and 71 h, compared to 160 h for the unexposed ''starved control'' rotifers. Experiments to determine ingestion rates for the rotifers, based on changes in their Chlorophyll *a* content, showed that the rotifers could feed on *A. lusitanicum*, *A. minutum* and *A. tamarense* strain AT-6, but could graze to little or no extent upon algal cells of the other seven strains. The effects on rotifers exposed to different cell densities, fractions, and growth phases of *A. tamarense* algal culture were respectively compared. It was found that only the whole algal cells had lethal effects, with strongest impact being shown by the early exponential growth phase of *A. tamarense*.

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1568-9883/$ – see front matter
doi:10.1016/j.hal.2004.06.014

The results indicate that some toxic mechanism(s), other than PSP and present in whole algal cells, might be responsible for the adverse effects on the exposed rotifers.

Keywords: *Alexandrium* spp.; *Brachionus plicatilis*; Toxicity

1. Introduction

The dinoflagellate *Alexandrium* spp. is widely distributed in China and throughout the world coasts and is responsible for the occurrence of paralytic shellfish poisoning (PSP) in a variety of hydrographical regions ranging from temperate to tropical areas (Hallegraeff, 1995). PSP toxins are sodium channel blocking toxins, which can have lethal neurotoxic effects on humans and other mammals. There is copious information on the effects of *Alexandrium* species on scallop, fish, and crustacea (Shumway and Cucci, 1987; Bricelj and Shumway, 1998; Chen and Chou, 2001; Cembella et al., 2002; Tan et al., 2002; Suzuki et al., 2003), as well as well-documented intoxication events involving humans (Shumway, 1990). Toxigenic *Alexandrium* species can also adversely affect other components of the marine food web, including finfish and marine mammals, probably via a zooplankton vector (White, 1979, 1980, 1981; Geraci et al., 1990; Colin and Dam, 2002a, 2002b, 2003).

Herbivorous zooplankton populations that come in contact with toxic algae can be profoundly affected by the occurrence of harmful algal blooms. Studies on the effects of *Alexandrium* species on zooplankton have concluded that these algae causes adverse impacts to the zooplankton including reduced survival, reduced hatching of zygotes, inhibited grazing and changes in behavior patterns (Huntley et al., 1986; Hansen, 1989; Robineau et al., 1991; Hansen et al., 1992; Teegarden and Cembella, 1996; Teegarden, 1999; Frangópulos et al., 2000). Most studies have been focused on metazoans such as copepods, while relatively few have examined microzooplankton grazers.

The rotifer *Brachionus plicatilis* is identified as a polyphagous microplanktonic filter feeder, which feeds on particles such as microalgae, yeast, bacterial species, and nonliving particles (Hino and Hirano, 1980). It is an important aquaculture species used as live food for aquaculture organisms including fish larvae (Balompapueng et al., 1977). Previous studies found that the dinoflagellate *Pfiesteria piscicida* decreased the grazing rate of *B. plicatilis* (Chotiyaputta and Hirayama, 1978); while *Heterocapsa circularisquama* and *Gymnodinium* sp. had lethal effects on *B. plicatilis* (Abe and Hirayama, 1979; Kim et al., 2000). However, the effects of *Alexandrium* species on *B. plicatilis* remain unknown. In present study, we chose 10 strains of *Alexandrium* species including one known non-PSP-producer, to investigate their influences on the rotifer *B. plicatilis*, and the ingestion ability of rotifers, on these algae. The *Alexandrium tamarense* strain ATHK, a know PSP-producer, was selected to further explore the relationship of its toxicity to algal density, as well as to the different fractions and different growth phases of its various culture stages.

2. Materials and methods

2.1. Algae and rotifers

The dinoflagellate *Alexandrium* sp1, *Alexandrium* sp2 and five strains of *A. tamarense*: ATHK, AT5-1, AT5-3, ATCI02, ATCI03 were collected from the South China Sea and provided by Jinan University. *Alexandrium minutum* was provided by Dr. H. Chou in Taiwan. *Alexandrium lusitanicum* was obtained from CCMP (Provasoli-Guillard National Center for Culture of Marine Phytoplankton, USA) as a strain of 1888. A non-PSP-producing *A. tamarense* (AT-6) was provided by Dr. D. Anderson, Woods Holes Oceanographic Institution, USA. *Chlorella* sp. was provided by the Algal Culture Center of our institute. All algae were grown in f/2 medium in flasks, at temperature 20 $\pm$ 0.5 °C, with irradiance at 56 $\mu Em^{-2} S^{-1}$ and 12 h light:12 h dark photoperiod. The rotifer *B. plicatilis* was supplied by the Culture Center in our institute and cultured under the same above conditions as *Chlorella* sp.