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NOTE

Reduction in the toxicity of the dinoflagellate Gymnodinium catenatum when fed on by the heterotrophic dinoflagellate Polykrikos kofoidii

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ABSTRACT: To investigate the ability of the heterotrophic dinoflagellate Polykrikos kofoidii to reduce the toxicity produced by the dinoflagellate Gymnodinium catenatum (toxicity = 2.0 to 3.9 Mouse Units [MU]/10^5 G. catenatum cells), we used the mouse bioassay to measure the toxicity retained in a population of P. kofoidii, originally fed G. catenatum for 2 d (ingestion rate = 5.6 cells grazer^{-1} d^{-1}) and then starved. As a control, we measured the toxicity retained in a population of P. kofoidii originally fed a non-toxic strain of Scrippsiella trochoidea. The toxicity retained in a population of P. kofoidii at Hour 0 (P. kofoidii starved for 0 to 48 h after being fed) was 17.3 MU/10^5 P. kofoidii cells. With increasing elapsed time after starvation the toxicity rapidly decreased to 3.6 MU/10^5 P. kofoidii cells at Hour 24, slowly decreased between Hours 36 and 81, and was not detectable at Hour 96. The decay constant in the exponential equation of the regression line for the toxicity in 10^5 P. kofoidii cells between Hours 0 and 96 was 0.059. No toxicity was detected in P. kofoidii fed S. trochoidea. This evidence suggests that the starvation of P. kofoidii fed G. catenatum may provide dissipation of the toxicity caused by this prey in marine food webs.

KEY WORDS: Harmful algal bloom · Ingestion · Protist · Red tide · Toxin · Zooplankton

Blooms dominated by toxic phytoplankton can upset the balance of food webs and cause large-scale mortalities in fish and shellfish (e.g. ECOHAB 1995) and are thus of concern for people in the aquaculture industry, fish consumers, government officers, and marine scientists. Interactions between toxic phytoplankton and their zooplankton grazers are important in understanding the dynamics of toxic phytoplankton in marine food webs. However, the interactions and roles of the zooplankton in toxin dynamics are complex and obscure (Turner & Tester 1997).

Toxic phytoplankters may be actively ingested (Teegarden & Cembella 1996), consumed as less preferred prey, or not eaten at all by zooplankton (Huntley et al. 1986, Turriff et al. 1995). Some grazers ingest toxic phytoplankton without apparent harm (Turner & Anderson 1983, Teegarden & Cembella 1996, Costa & Fernández 2002), whereas others are adversely affected (Ives 1985, Gill & Harris 1987, Costa & Fernández 2002). Many studies have reported that some zooplankters, in particular copepods, that feed on toxic phytoplankters without apparent harm usually accumulate toxins inside their bodies and thus play a role in transferring the toxins to the higher trophic levels (White 1977, 1979, Robineau et al. 1991, Turriff et al. 1995, Teegarden & Cembella 1996). This transport of toxins in food webs has often caused mortality of fish, sea birds, marine mammals, and humans (White 1980, Turner et al. 2000). However, if a zooplankter has the ability to reduce toxicity after ingesting toxic phytoplankton, the toxin should partially or completely dissipate from food webs and thus may prevent mortality of higher trophic level organisms.

There have been many studies on the roles of metazooplankters in toxin dynamics (Robineau et al. 1991, Turriff et al. 1995, Teegarden & Cembella 1996), but few studies on those of heterotrophic protists. Many heterotrophic and/or mixotrophic protists do not ingest toxic phytoplankton (Jeong et al. 1999a,b), while oth-

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ers sometimes showed abnormal swimming patterns or mortality when toxic phytoplankton or phytoplankton exudates were provided (Hansen 1989, 1995, Hansen et al. 1992, Kamiyama & Arima 1997). However, some heterotrophic protists have been known to feed effectively on a toxic phytoplankton (Jeong et al. 2001a,b, Stoecker et al. 2002). Jeong et al. (2001a) suggested that the heterotrophic dinoflagellate Oxyrrhis marina might have the ability to reduce the toxicity produced by the toxic dinoflagellate Amphidinium carterae, based on the feeding responses of copepods fed O. marina that had been satiated with A. carterae and then starved. Still, little is known about changes in the toxicity retained in the body of heterotrophic protistan grazers after ingesting toxic phytoplankton.

The heterotrophic dinoflagellate Polykrikos kofoidii feeds effectively on the toxic dinoflagellate Gymnodinium catenatum (Matsuyama et al. 1999, Jeong et al. 2001b), a harmful algal species that causes paralytic shellfish poisoning in many areas (Anderson et al. 1989, Hallegraeff & Fraga 1998). Among diverse dinoflagellate prey P. kofoidii had a maximum growth rate (1.2 d $^{-1}$) when fed G. catenatum (Jeong et al. 2001b). Considering this result, P. kofoidii may be able to reduce the toxicity produced by the prey and/or be insensitive to it. G. catenatum has many types of toxins (i.e. STX, neoSTX, GTX1-6, C1-4, deSTX, deneoSTX, deGTX1-4, 11-$\alpha$-hydroxySTX, 11-$\beta$-hydroxySTX) and one type can change to another under certain conditions (Sako et al. 2001, Holmes et al. 2002). Therefore, to investigate whether P. kofoidii has the ability to reduce the toxicity produced by G. catenatum and, if so, to quantify the reduction rate of the toxicity, we measured total toxicity in a population of P. kofoidii originally fed G. catenatum and then starved as a function of elapsed starvation time using the mouse bioassay. Similarly, we also measured the toxicities of G. catenatum only and P. kofoidii originally fed a non-toxic strain of Scrippsiella trochoidea as controls. Dense cultures (density = 145 cells ml$^{-1}$) of Polykrikos kofoidii growing on a non-toxic strain of Lingulodinium polyedrum were starved for 2 d and then transferred into two 220 l tanks containing each culture of Gymnodinium catenatum (density = 446 cells ml$^{-1}$) (Tank PG) and Scrippsiella trochoidea (density = 1410 cells ml$^{-1}$) (Tank PS). A tank of a G. catenatum culture without grazers (density = 415 cells ml$^{-1}$) (Tank GC) and a tank of S. trochoidea without grazers (density = 1320 cells ml$^{-1}$) (Tank ST) were also set up as controls.

Hours 0, 12, 24, 36, 48, 72, 81, and Hour 96 indicate the number of hours after this experiment started, and Hours –24 and –48 the number before the experiment started (pre-incubation). In Tank PG Polykrikos kofoidii eliminated the Gymnodinium catenatum cells within 48 h and there were no free G. catenatum cells remaining at Hour 0 (P. kofoidii starved for 0 to 48 h after almost satiation with G. catenatum), and the experiment started. Most P. kofoidii cells were observed to contain 2 to 5 ingested G. catenatum cells at this start time. At each sampling time, 16 l aliquots were taken from each tank to measure the toxicity, and additional 10 ml aliquots were taken and fixed with 5% acid Lugol's solution to determine the density of P. kofoidii, G. catenatum, or Scrippsiella trochoidea in each tank.

To extract toxic materials, the 16 l aliquots taken from each tank at each sampling time were harvested by filtration with glass-fibred filters (Whatman GF/C, 90 mm in diameter). Each filter paper contained 2 l aliquots. Toxicity was measured according to the Official Methods of Analysis of the Association of Official...
Analytical Chemists (AOAC) International (2000) with some modification; a filter paper was soaked with 1.0 ml of 0.1 N HCl, well minced, and then transferred to specially designed centrifuge tube units to retrieve the HCl solution. The units were centrifuged at 5000 × g for 30 min at 3°C. The solutions were injected intraperitoneally into 4 wk old ICR strain male mice (weight range: 19 to 22 g each). Dilution was made with 0.1 N HCl if necessary. Each solution was injected in triplicate. The injected mice were observed in a box in a calm room for 1 h. Toxicity described as Mouse Unit (MU) was corrected by using Sommer’s Table considering time of death and the weight of mice (AOAC Official Methods of Analysis 2000). Median MU for a group (triplicate mice) was determined.

Toxicities were calculated in terms of MU per 10^5 Polykrikos kofoidii cells and MU per all organisms (Gymnodinium catenatum + P. kofoidii between Hours –48 and 0 and only P. kofoidii after Hour 0) in 1 l water. Theoretically, the toxicity per P. kofoidii cell could be reduced if P. kofoidii cells divide (dilution effect) even if there were no detoxification. Therefore, change in the toxicity in all organisms in a certain volume as elapsed starvation time was considered.

Toxicity data were fitted to an exponential equation in these starvation experiments:

\[ T_t = T_0 e^{(-kt)} \]

where \( T_0 \) = the initial toxicity and \( T_t \) = the toxicity after time \( t \); \( k \) = decay constant.

Ingestion and clearance rates of Polykrikos kofoidii on Gymnodinium catenatum between Hours –48 and 0 were calculated using the equations of Frost (1972) and Heinbokel (1978).

Polykrikos kofoidii may expel toxin materials as fecal pellets. To test whether P. kofoidii produced fecal pellets after feeding on Gymnodinium catenatum, approximately 30 P. kofoidii cells were transferred into a well of a 6 well-plate chamber containing a dense culture of the prey. At 1, 2, 3, 6, 12, and 24 h, the bottom of the well was scrutinized using an inverted microscope.

**Results.** **Toxicity of Gymnodinium catenatum:** The toxicity of G. catenatum in Tank GC ranged from 2.0 to 3.9 MU/10^5 G. catenatum cells, while the density of G. catenatum ranged from 415 to 554 cells ml\(^{-1}\) (Fig. 1). Between Hours –48 and –24 the toxicity of G. catenatum in Tank GC decreased from 3.9 to 3.0 MU/10^5 G. catenatum cells, while the density of G. catenatum increased from 415 to 521 cells ml\(^{-1}\). However, there was no marked trend in the toxicity and cell density of G. catenatum between Hours –24 and 96.

**Toxicity retained in a population of Polykrikos kofoidii feeding on Gymnodinium catenatum:** The toxicities of P. kofoidii fed S. trochoidea in Tank PS were all zero (Fig. 2).

**Cell densities and ingestion rates of Polykrikos kofoidii feeding on Gymnodinium catenatum:** The cell density of P. kofoidii feeding on G. catenatum in Tank PG increased from 31 to 62 cells ml\(^{-1}\) between Hours –48 and 0, while that of G. catenatum decreased from 446 cells ml\(^{-1}\) to an undetectable level (Fig. 3). With increasing time of starvation, cell density of P. kofoidii increased from 62 to 98 cells ml\(^{-1}\) (Hours 0 to 36) and then gradually decreased to 15 cells ml\(^{-1}\) by Hour 96.

Ingestion and clearance rates of Polykrikos kofoidii feeding on Gymnodinium catenatum between Hours
Toxicity retained in a population of *Polykrikos kofoidii* fed *Gymnodinium catenatum*: 

The toxicity (MU/10^5 *P. kofoidii* cells) of *P. kofoidii* fed *G. catenatum* and then starved changed over time (Fig. 4). The equation of the regression line is as follows: toxicity (MU/10^5 *P. kofoidii* cells) = 16.6 e^(-0.0591x), r^2 = 0.907, where x = elapsed starvation time.

Fig. 5. Toxicity (MU) of *Gymnodinium catenatum* plus *Polykrikos kofoidii* fed *G. catenatum* in 1 l water between Hours –48 and 0 and of only *P. kofoidii* fed *G. catenatum* and then starved between Hours 0 and 96 as a function of the elapsed starvation time. The equation of the regression line is as follows: between Hours –48 and 0, toxicity (MU) = –0.11x + 10.6, r^2 = 0.983, where x = elapsed starvation time; between Hours 0 and 96, toxicity (MU) = 10.0 e^(-0.036x), r^2 = 0.945.

–48 and 0 were 5.6 cells grazer^-1 d^-1 and 3.3 µl grazer^-1 h^-1, respectively.

**Toxicity retained in a population of *Polykrikos kofoidii* fed *Gymnodinium catenatum***: The toxicity (MU/10^5 *P. kofoidii* cells) of *P. kofoidii* fed *G. catenatum* and then starved changed over time (Fig. 4). The toxicity of *P. kofoidii* drastically decreased from an Hour 0 value of 17.3 MU/10^5 *P. kofoidii* cells to 6.2 MU/10^5 *P. kofoidii* cells at Hour 12. The toxicity between Hours 24 and 81 was 2.6 to 3.9 MU/10^5 *P. kofoidii* cells, but became undetectable at Hour 96. The curve fitted by Eq. (1) gave a decay constant of 0.059.

The toxicity of *Gymnodinium catenatum* and/or *Polykrikos kofoidii* fed *G. catenatum* between Hours –48 and 0 decreased linearly from 16.0 to 10.8 MU per all organisms in 1 l water (Fig. 5). With increasing elapsed starvation time, the toxicity of *P. kofoidii* on *G. catenatum* and then starved also markedly decreased from 10.8 at Hour 0 to undetectable at Hour 96. The curve fitted by the Eq. (1) gave a decay constant of 0.036.

Careful observations using an inverted microscope revealed that no fecal pellets of *Polykrikos kofoidii* were produced during starvation experiments.

**Discussion. Toxicity of *Polykrikos kofoidii* fed *Gymnodinium catenatum***: The toxicity of *P. kofoidii* fed *G. catenatum* and then starved declined by 64% MU per *P. kofoidii* cell and 49% MU in 1 l water between Hours 0 and 12, and became undetectable by Hour 96. By contrast, there was no marked change in the toxicity of *G. catenatum* culture between Hours 0 and 96. This evidence suggests that *P. kofoidii* has the ability to reduce the toxicity of *G. catenatum*. *P. kofoidii* did not appear expel the toxin of *G. catenatum* via fecal pellets, but excretion of the toxins into the ambient water could not be excluded.

There have been several studies on the accumulation and/or transfer of phytotoxins through marine food webs (White 1977, Teegarden & Cembella 1996, Turner & Tester 1997, Turner et al. 2000), but few studies on a potential dissipation of the toxin in the food
webs (Jeong et al. 2001a). The present study represents the first report providing evidence for reduction in phytotoxicity by the feeding of heterotrophic protists in marine food webs and measuring the decay rate of the toxicity retained in the body of the grazers.

The decay constant (0.059) in the exponential equation of the regression line for the toxicity in 10^4 Polykrikos kofoidii cells between Hours 0 and 96 was greater than that for the toxicity retained in a population of P. kofoidii in 1 l water (0.036) (see Figs. 4 & 5). This difference might be caused by a possible dilution effect due to the binary division of a P. kofoidii cell containing a given toxicity.

The overall toxicity produced by Gymnodinium catenatun in 1 l water at Hour –48, 16.0 MU, decreased to 10.8 MU at Hour 0, when the toxicity was attributable to Polykrikos kofoidii containing prey cells. The retention value (ratio of the toxicity retained in the body of a grazer to the total toxicity of the prey ingested) when intensive feeding by P. kofoidii on G. catenatun occurred, ca. 66%, was much higher than that for the copepod Eurytemora herdmani or Acartia tonsa continuously fed Alexandrium spp., i.e. <10% (Teegarden & Cembella 1996). However, during the starvation of P. kofoidii fed G. catenatun, the retained toxicity became undetectable.

Metabolic transformation of toxins has been known to occur in several species of metazoa such as sea scallops (Cembella et al. 1994), mussels (Anderson et al. 1989), and copepods (Robineau et al. 1991, Teegarden & Cembella 1996). It is worthwhile to explore changes or destruction of toxins inside Polykrikos kofoidii and to examine the activity of enzymes involved in these processes.

Ecological importance: The interactions revealed in the present study may be important in marine ecosystems in the following ways: (1) A population of Polykrikos kofoidii feeding on Gymnodinium catenatun may provide a dissipation of the toxicity produced by the prey in marine food webs. Intensive grazing by P. kofoidii on G. catenatun has been known to cause the cessation of massive blooms dominated by this prey (Matsuyama et al. 1999). If P. kofoidii cells that eat G. catenatun and then starve after the termination of the bloom are ingested by copepods, accumulation and transfer of the toxins in the food web may not occur. Thus, this reduction in toxicity may prevent large-scale mortalities of finfish, marine mammals, and sea birds and poisoning of shellfish, and eventually imbalance in marine ecosystems. (2) However, if copepods ingest P. kofoidii cells that eat G. catenatun but have not starved for a while, toxins may be accumulated and transferred marine food webs.

So far, the heterotrophic dinoflagellates Oxyrrhis marina and Gyrodinium dominans have been known to feed on toxic dinoflagellates Amphidinium carterea (Jeong et al. 2001a) and Gymnodinium mikimotoi (Nakamura et al. 1995), respectively. In order to gain a further understanding of marine plankton food webs related to toxic dinoflagellates, it would be worthwhile to investigate the interactions among other toxic dinoflagellates and heterotrophic protists and toxin dynamics inside the body of the grazers.

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