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Vectorial transport of toxins from the dinoflagellate *Gymnodinium breve* through copepods to fish

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Abstract. Toxins from the dinoflagellate *Gymnodinium breve* were traced through experimental food chains from dinoflagellates, through copepod grazers, to juvenile fish. The generality of this food web transfer was demonstrated using three different combinations of copepods and juvenile fish during different seasons. Fish were not exposed directly to the toxic dinoflagellates but were fed toxin-laden copepods in order to examine sublethal vectorial intoxication. Toxins were shown to move from fish viscera to muscle tissue within periods of 2–6 h to 25 h. A new toxin detection method was used in this first stepwise demonstration of multi-trophic-level intoxication of a planktonic food chain by *G.breve*. Micellar electrokinetic capillary electrophoresis with laser-induced fluorescence allowed measurements of toxins at trace levels and nanoliter-sized volumes critical for planktonic food web transfer studies.

Introduction

Marine phytoplankton toxins can cause finfish mortality and shellfish toxicity, as well as poisoning or death of humans, marine mammals, seabirds and fish (Anderson and White, 1992; Steidinger, 1993; Work *et al*., 1993). Accumulation and transport of these toxins through marine food webs can also cause vectorial intoxication of higher trophic level consumers that do not feed directly on toxic phytoplankton. The mechanisms by which filter-feeding bivalves accumulate phycotoxins are better understood (Shumway, 1990) than toxin accumulation and transport through pelagic food webs leading to fish. Most studies of fish intoxication have focused on mortality rather than toxin accumulation, transvectoring or chronic sublethal effects (Kelly *et al*., 1992).

The pioneering work on accumulation of toxin from *Gonyaulax excavata* [= *Alexandrium tamarense*—see (Moestrup and Larsen, 1990)] in various compartments of zooplankton communities demonstrated the likelihood that food-chain transport of these toxins could cause fish kills in the Bay of Fundy (White, 1977, 1979, 1980, 1981a,b; White *et al.*, 1989). Except for a few studies in coral reef ecosystems focused on ciguatoxin accumulation in fish (Helfrich and Banner, 1963; Davin *et al.,* 1988; Kelly *et al*., 1992), there have been few subsequent investigations of vectorial transport of marine phytoplankton toxins to upper-level pelagic consumers via zooplankton.

The extent to which zooplankton graze upon, or are affected by, toxic phytoplankters appears quite variable [reviewed by (Turner and Tester, 1997; Turner *et al.,* 1998)]. Some copepods graze upon certain toxic phytoplankton with no

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apparent ill effects (Turner and Anderson, 1983; Uye, 1986; Turner and Tester, 1989), whereas others are repelled or otherwise adversely affected (Tomas and Deason, 1981; Ives, 1985, 1987; Huntley *et al*., 1986; Sykes and Huntley, 1987; Durbin and Durbin, 1989; Uye and Takamatsu, 1990). This is in contrast to larval or juvenile fish which, almost universally, appear to be poisoned by exposure to high densities [$>2.5 \times 10^5$ cells l^{-1} (Tester *et al*., 1998)] of toxic dinoflagellates (White, 1981b; White *et al.,* 1989; Gosselin *et al.,* 1989; Robineau *et al.,* 1991a, b).

A major obstacle to studies of vectorial intoxication has been the difficulty of measuring small concentrations of toxins in planktonic food webs. For instance, White measured *G.excavata* toxins using a mouse bioassay that required about 10 000 zooplankters per assay for reliable toxin measurements (White, 1991a). Recent advances in analytical techniques, such as high performance liquid chromatography or capillary electrophoresis, allow detection of phycotoxins in the picogram to femtogram range, making possible the measurement of toxins through multiple levels of marine food webs. Such studies are particularly important for regions where toxic algal blooms occur frequently, or where blooms persist.

One coastal ecosystem that experiences annual toxic algal blooms is the eastern Gulf of Mexico. Although the toxic dinoflagellate *Gymnodinium breve* is found throughout the Gulf of Mexico in background concentrations ($<10^3$ cells l^{-1}) (Dragovich and Kelly, 1966; Steidinger, 1975; Geesey and Tester, 1993), the region between Tarpon Springs/Clearwater and Sanibel Island, Florida (28°N, 82°50′W to 26°30′N, 82°15′W) has experienced blooms ($>5 \times 10^3$ cells l^{-1}) in 21 of the past 22 years (Steidinger *et al*., 1998). Since the toxins produced by *G.breve* (brevetoxins) are both lipid soluble and stable (Baden and Trainer, 1993), transvectoring and bioconcentration of these toxins in the west Florida coastal ecosystem is likely.

Our objective was to investigate the possibility of stepwise trophic transfer of brevetoxins from *G.breve* through copepod grazers to zooplanktivorous larval or juvenile fish. Accordingly, we performed a series of experiments using different species of copepods and fish to address the following questions:

(i) Can brevetoxins be detected in copepods fed toxic *G.breve*?
(ii) Can brevetoxins be detected in the gut or muscle tissue of fish fed toxin-laden copepods?

and if so, what is the time course for detection in the viscera and muscle tissue?

Methods

August, 1993—validation of the methodology

Inocula of *Gymnodinium breve* cultures (clone 78P5) were maintained at the Beaufort Laboratory in filtered sea water (34 p.p.t., 25°C), enriched with f/2 medium (Guillard and Ryther, 1962). Cultures were kept on a natural photoperiod (13.5L:10.5D) in front of a window and harvested for these experiments during log-phase growth. Copepods were collected on 18 August 1993 with a

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333 μm mesh plankton net towed at the surface in Beaufort Inlet, North Carolina, USA (34°41.8′N, 76°40.3′W) (Figure 1) (salinity = 34 p.p.t., 25°C). Zooplankton were returned to the laboratory within 1 h and sorted immediately. Actively-swimming adult female *Temora turbinata* were removed with a micropipette under a dissecting microscope and held in filtered sea water for 4 h. Replicate samples of *T.turbinata* were frozen to serve as controls for toxin exposure prior to capture, and 25 copepods each were added to *G.breve* concentrations of 8, 20 and 600 × 10^3 cells l^{-1}. Initial aliquots of 20 ml of *G.breve* culture from each of the three concentrations were frozen for toxin analyses. At each *G.breve* concentration, 25 *T.turbinata* were allowed to graze in 400 ml suspensions for 16.5–17.4 h for toxin accumulation. Copepods were separated from cell suspensions by screening with 210 μm mesh, placed in filtered sea water for 2 h to clear their guts, and frozen for toxin analyses. Note that these *G.breve* concentrations were lower than the maximum natural concentrations of 3.6–19.6 × 10^6 cells l^{-1} from the 1987 North Carolina bloom of *G.breve* (Tester *et al.*, 1989, 1991) used in the grazing experiments of Turner and Tester (Turner and Tester, 1989).

Concurrent with the conditioning described above, 25 *T.turbinata* each were fed upon two concentrations of *G.breve* (8 and 600 × 10^3 cells l^{-1}) for accumulation of toxins prior to being fed to fish. All copepods survived an overnight (16 h) feeding period. Subsequently, the copepods were sieved from the *G.breve* cell suspensions and held in filtered sea water for 2 h for gut clearance. *Temora turbinata* from each *G.breve* concentration were then placed in each of two 2 l jars with filtered sea water. Two juvenile fish (one *Eucinostomus argenteus*—spotted majarra, 22 mm standard length; and one *Fundulus majalis*—striped killifish, 26 mm standard length) were added and allowed to feed on the 25 copepods.

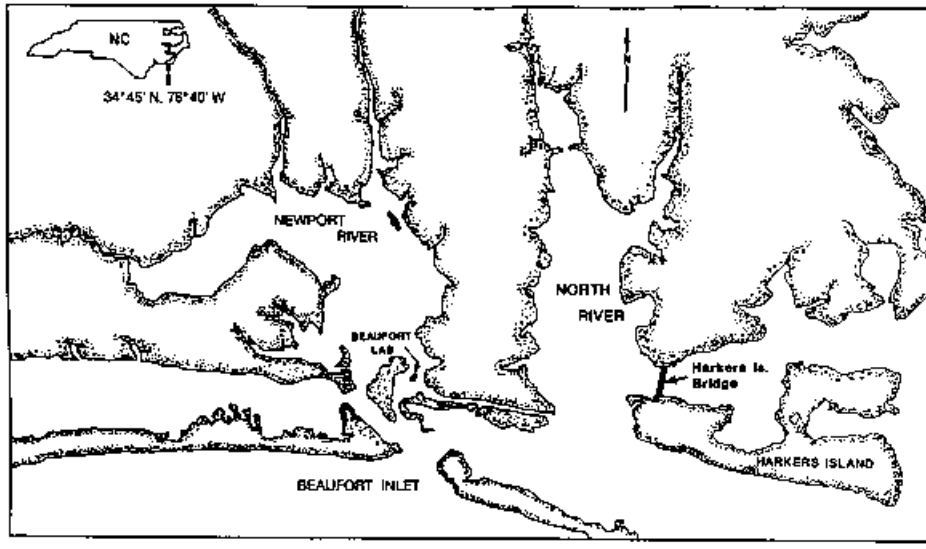

Fig. 1. Sampling locations near Beaufort, North Carolina (34°40′N, 76°40′W).

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A 2 h feeding period was allowed but the fish appeared to eat all the copepods in less than 60 s. After 2 h, the contents of the jar were screened through a 210 μm mesh for any remaining copepods, but none were found. The fish were then frozen for toxin analyses. Fecal material from the fish was also frozen in vials for toxin analyses. All samples were kept at –80°C until analyzed.

May, 1994—time course of brevetoxin detection in viscera

Gymnodinium breve cultures (clone 78P5) were shipped overnight from the USDA Agricultural Research Service in New Orleans, Louisiana, and maintained as described above. During log-phase, growth samples were frozen for toxin analyses. In May, a large pontellid copepod, *Labidocera aestiva*, was abundant off Beaufort, and adult females were sorted from mixed plankton in tows made on 13 May using a 333 μm mesh net. Adult female *L.aestiva* were held for 4 h in filtered sea water (35 p.p.t., 21°C) for laboratory acclimation. Samples of *L.aestiva* were frozen for controls (n = 20) and 190 adult females were fed on concentrations of 200×10^3 *G.breve* cells l^{-1} for 2 h in the dark. Each copepod consumed a mean of 53 *G.breve* cells. Copepod survival was >98%. *Gymnodinium breve*-fed *L.aestiva* were carefully rinsed, and two vials of 10 and a single vial of seven copepods were frozen for toxin analyses.

Juvenile pinfish (*Lagodon rhomboides*, 35 mm standard length) were seined along the beach and grass beds adjacent to the Harkers Island Bridge (Figure 1) on 11 May 1994. They were held in aerated, filtered sea water (35 p.p.t., 22°C) and remained unfed for 24 h. Three of these juvenile pinfish were frozen as controls for exposure to toxins prior to capture. At the start of the fish feeding period, nine *L.aestiva* were sieved from the *G.breve* suspension, rinsed, and placed in each of 18 containers containing 100 ml of filtered sea water each. A single juvenile pinfish was added to each container and allowed to feed on the copepods from 40–50 min in reduced light. During this time, all fish survived except for one that jumped out of the feeding container. Four of nine copepods in that treatment were unconsumed. In the other 17 containers, all but one copepod was consumed (152 of 153 copepods). At the conclusion of feeding, fish were placed in a common 4 l tank of filtered sea water; they were removed at 2, 4 and 8 h and frozen in triplicate for brevetoxin analyses.

February 1995—toxin transfer from gut to muscle tissue

Gymnodinium breve (clone 78P5) were grown in f/2 (33 p.p.t., 20°C, 12:12 L:D) to concentrations of $1–2 \times 10^6$ cells l^{-1}. Adult female *Acartia tonsa* were sorted from plankton sampled with 333 μm mesh net tows from the pier of the Beaufort Laboratory during outgoing tide on 17 February. Ambient water was 10°C and 31 p.p.t. *Acartia tonsa* were held in filtered sea water overnight (20 h) to both starve and temperature acclimate the copepods (to 20°C) prior to feeding upon *G.breve* suspensions of 1.6×10^6 cells l^{-1} for 23.75 h. All *G.breve* cells were eaten and grazing rates averaged 145 cells copepod^{-1} h^{-1} for copepods fed to juvenile spot (*Leiostomous xanthurus*) during the first 6 h of fish feeding. The copepods

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that were not fed to the juvenile spot during the first feeding cycle (0–6 h) were provided with excess *G.breve* cells so that a high toxin content was maintained for the final fish feeding time.

Juvenile spot (30 mm standard length) were caught using a 0.94 mm mesh net hung from the bridge leading to the Beaufort Laboratory during outgoing tide and placed in each of 15 containers with filtered sea water. Initially, they were fed six brevetoxin-laden *A.tonsa*. Three fish were frozen at $t = 0$ as controls. Three more fish were frozen at $t = 2$ h. Fishes 1 and 3 ate six copepods each immediately, and fish 2 ate five copepods by $t = 2$ h. Groups of three fish were analyzed for toxin content as one pooled sample. At $t = 6$ h, fishes 4, 5 and 6 were frozen. These three fish had each immediately eaten six copepods. The remaining fish (fish 7–12) were offered, and all ate, an additional five copepods. Fish 7, 8 and 9 were frozen at $t = 12$ h, and the final three fish (fish 10, 11 and 12) and the three control fish (not fed) were frozen at $t = 25$ h.

One other experiment was attempted, using larval menhaden (*Brevoortia tyrannus*, 30 mm standard length). These larvae were fed *G.breve*-laden *A.tonsa*. Adult female copepods were collected from the pier of the Beaufort Laboratory on 23 January 1995 (11°C, 32 p.p.t.), sorted into filtered water to acclimate for 20 h, and then fed on 127 *G.breve* cells ml^{-1} for 19 h. The grazing rate was 26–35 *G.breve* copepod^{-1} h^{-1}. Menhaden larvae from the fish rearing facility of the National Marine Fisheries Service Beaufort Laboratory were placed singly into 15 containers of filtered sea water. Six *G.breve*-fed *A. tonsa* were added to each of 12 containers. The remaining three fish served as controls and were fed *A.tonsa* that had been maintained on an *Isochrysis* sp. diet. Great care was taken in transferring the small menhaden larvae because they succumb readily to handling.

Brevetoxin analysis

The two major brevetoxins (Baden and Tomas, 1989), an aldehyde (PbTx-2) and an alcohol (PbTx-3), were detected and quantified using capillary electrophoresis with laser-induced fluorescence detection (Beckman P/ACE 2200 equipped with Liconix Embosser II HeCd laser). A brief summary of this method follows; for a detailed analytical description see Shea (Shea, 1997). *Gymnodinium breve* cultures and copepods were filtered using a 47 mm glass fiber filter (Whatman). After sonication (VirTis Tempest), the dissolved toxins were isolated from the filtrate using a C-18 bonded phase extraction disk and eluted with acetonitrile. Particulate toxins retained on the filter were extracted by sonication in methanol.

Fish viscera and muscle tissue were analyzed separately. The measurements made on muscle used the gutted fish minus the head but included fins and skin. Tissue samples were mechanically homogenized in methanol and then sonicated to complete the extraction.

Since we had no preliminary data on how much copepod or fish tissue was required for reliable toxin measurements, samples from triplicate treatments were pooled in order to maximize sensitivity. For example, in the February 1995 experiments, three fish at $t = 12$ h (fish 7, 8 and 9) and three fish at $t = 25$ h (fish

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10, 11 and 12) each ate five of six offered copepods, so each toxin measurement for each of those times represents the total amount of toxin contained in all 15 copepods ingested by all three fish.

Methanol extracts were partitioned with hexane to remove biogenic lipids, and the methanol fraction was blown to dryness with nitrogen, taken up in 1 ml dichloromethane (DCM), and charged to a gravity-flow alumina-silica column. The column was eluted with hexane to remove non-polar biogenic interferences and then eluted with DCM to obtain the toxins. The DCM fraction was reduced in volume to 2 ml using nitrogen and then injected into an automated high performance gel permeation chromatography (GPC) system using a Waters WISP 710B autosampler and M-45 pump, a Phenomenex Phenogel 5 mm, 100Å, 300 × 21.2 mm GPC column and an Isco Foxy Jr fraction collector. The GPC column was eluted with DCM and the fraction corresponding to the molecular weight range of 850–950 Da was collected. This fraction was blown to dryness, taken up on 100 µl acetonitrile, and then the PbTx-2 and PbTx-3 toxins were isolated using reversed phase HPLC according to the methods of Pierce *et al.* (Pierce *et al.*, 1985). The PbTx-3 toxin was exchanged into toluene, derivatized with an acyl azide coumarin (7-methoxycoumarin-3-carbonyl axide, M-1445, Molecular Probes, Inc.) at 80°C according to the method of Shea (Shea, 1997). The PbTx-2 toxin was reduced to PbTx-3 with sodium borohydride according to the method of Schulman *et al.* (Schulman *et al.*, 1990) and then derivitized as above.

The derivatized PbTx-2 (now converted to PbTx-3) and PbTx-3 fractions were exchanged into an acetonitrile-buffer mixture and separately analyzed by micellar electrokinetic capillary chromatography (MEKC). A Beckman P/ACE 2200 capillary electrophoresis instrument was used with a laser-induced fluorescence detection module coupled to an Omnichrome 3056–8M He/Cd laser. Laser excitation was at 325 µm; emission was monitored at 415 µm. Separations were performed using a 50 cm × 75 µm (i.d.) capillary (Polymicro Technologies). Sample injections were made using pressure injection (3.45 kPa) for 10–15 s which corresponds to 60–90 nl injected. The separation voltage ranged from 20 to 25kV. The running buffer was composed of 10 mmol sodium borate and 100 mmol cholate (pH = 9.3). Recoveries of PbTx-2 and PbTx-3 fortified tissue samples ranged from 62 to 81%. Results were not corrected for these recoveries. The limit of detection (LOD) for authentic standards was ~25 fg injected. The LOD for fortified tissue samples was higher due to background fluorescence of the matrix and was about 200 fg injected, which corresponds to about 200 pg in the original tissue samples.

Results

August, 1993—validation of the method

The toxin concentrations in *G.breve* cells, copepods and fish were measured (Table I) and the brevetoxin concentrations per cell were within the range reported by Pierce *et al.* (Pierce *et al.*, 1985). The mean aldehyde (PbTx-2) concentration was ~17 pg cell^{-1}, compared with a reported range of 6–30 pg cell^{-1}; the mean alcohol (PbTx-3) concentration was 4.4 pg cell^{-1}, compared with a reported range of 1–6 pg cell^{-1}. The resulting aldehyde:alcohol ratio was 3.84, in

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Table I. Brevetoxins PbTx-2 (aldehyde) and PbTx-3 (alcohol) concentrations in the August, 1993 culture water (aqueous), *Gymnodinium breve* cells, copepods fed upon *G. breve*, and fish fed the *G.breve*-laden copepods

G.breve (cells l^{-1})	Culture		Copepod		Fish	
	PbTx-2 (μg l^{-1})	PbTx-3 (μg l^{-1})	PbTx-2 (ng)	PbTx-2 (ng)	PbTx-2 (ng)	PbTx-3 (ng)
8000	0.16	0.04	<0.5	<2.0	1.2[a]	<2.0[a]
20 000	0.38	0.08	0.9	<2.0	–	–
600 000	8.40	2.38	22.0	4.3	33.0	6.0

[a]Control fish or background toxin levels.

good agreement with that of Baden (3.7±1.3) reported in Table 1 of Pierce *et al.* (Pierce *et al.*, 1985).

The toxin concentration in the copepods indicated that they ate ~195 *G.breve* cells each over the 16–17 h feeding period, or an average of 72 cells copepod^{-1} h^{-1}. There was no toxin detected in the control fish or in those fed the copepods from the suspensions with 8×10^3 cells l^{-1}. Only the fish fed copepods from the 600×10^3 cells l^{-1} treatment contained measurable toxin. Since the two fish consumed all 25 copepods there was ~658 ng of toxin available to them, but only 78 ng toxin was detected in the fish viscera, roughly a 10% transfer over a 2 h digestion time. Toxins were found only in the viscera of the fish; any toxins present in fish muscle tissue or fecal material were below the detection limit. However, toxins in both copepods and fish were higher in experiments with higher *G.breve* cell concentrations.

May, 1994—time course of brevetoxin detection in viscera

Measurements of both PbTx-2 and PbTx-3 were made on the *G.breve* food suspension before and after the *L.aestiva* grazed for 2 h. Total amounts of both toxins were reduced in the cell suspensions after the copepods fed, and the relative amounts of aqueous toxins differed after grazing (Figure 2). While brevetoxins are considered lipid soluble, there were measurable changes in the amount of toxins in the aqueous feeding suspension before and after the copeods fed. Here, the term aqueous is defined as the fraction that passed through a 0.7 μm filter. Initially, the PbTx-2 concentration in the *G.breve* suspension was 22%. Consistent with its greater polarity, the PbTx-3 concentration was 33%. The total toxin in the feeding suspension was 32 pg cell^{-1}, of which 7.88 pg was in the sea water or aqueous fraction. Therefore, the cells contained 24.1 pg of toxin cell^{-1}, consistent with the toxin content of the *G.breve* used in the August 1993 experiment (21.4 pg toxin cell^{-1}). After the *L.aestiva* grazed, there was an increase in the aqueous toxin concentration to 29% for PbTx-2 and 49% for PbTx-3, and the total toxin content was 18.66 pg cell^{-1}. This is probably due to cell breakage during feeding or, possibly, toxins excreted from copepods. No toxin was detected in the copepod fecal pellets (uncounted) or in eggs (n = 300), but it is not likely that a

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toxin signal could have become incorporated in the eggs in only 2 h [see (Tester and Turner, 1990)]. The *L.aestiva* controls had no detectable toxin but, after grazing for 2 h, copepods contained 2.02 ± 0.6 ng PbTx-2 and 0.8 ± 0.4 ng PbTx-3 copepod^{-1} (Figure 3) or about 100% of the toxin available to them from eating 53 cells each. The toxin transfer from copepods to fish was also efficient. Each fish ate nine *L.aestiva* (2.82 ± 1.0 ng total toxin each), or between 16.4 and

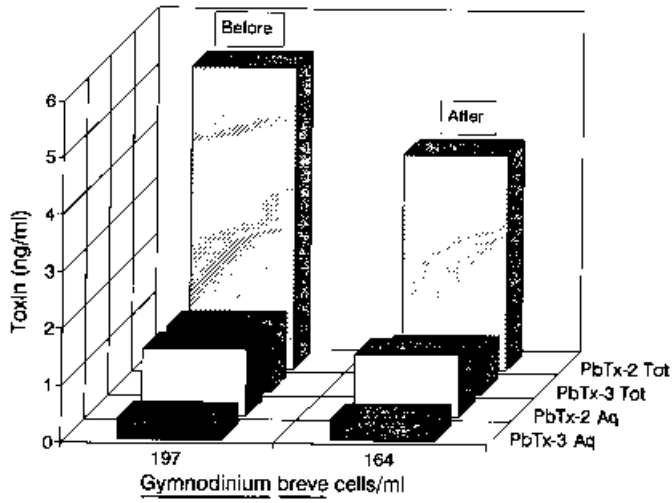

Fig. 2. Brevetoxin content of *Gymnodinium breve* food suspension before and after copepods had grazed for 2 h (May, 1994). Total toxin = Tot and aqueous toxin = Aq.

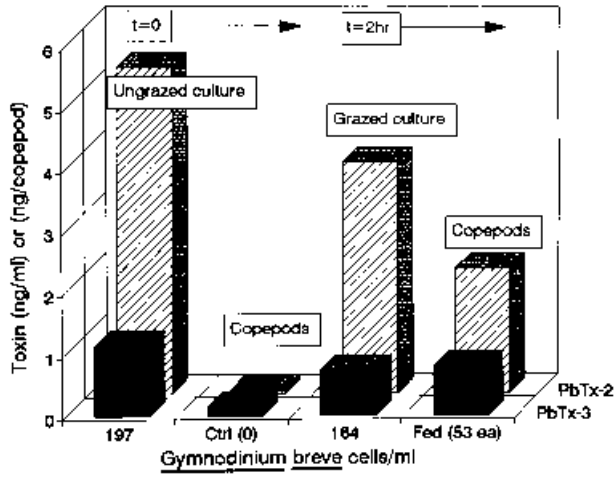

Fig. 3. Amounts of brevetoxins (PbTx-2 and PbTx-3) in the culture and in the copepods at $t = 0$ and after the copepods had grazed for 2 h (May, 1994).

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34.4 ng toxin. The amount measured in the viscera within 2 h of feeding was 22 ng fish^{-1}. From t = 2 h through t = 8 h, the PbTx-2 in fish viscera decreased, but the amounts in muscle tissue were not above the detection limits of the method until t = 8 h (Figure 4).

February 1995—toxin transfer from gut to muscle tissue

The measured toxin per *G.breve* cell (PbTx-2 and PbTx-3) was 23.3 pg cell^{-1}. Despite different handling and culturing methods over these three experiments, the total cell toxin content remained nearly the same throughout. There was also coherence in the toxin content of the copepods. During a >23.75 h grazing period used in this experiment, *A.tonsa* could have consumed up to 97 ng of toxin (145 cells h^{-1}), but the measured amount was only 34% of that, or 33.2 ng copepod^{-1}. This value was similar to the 26.3 ng copepod^{-1} for *T.turbinata* after they had grazed for 16–17 h. *Acartia tonsa* is, however, a smaller copepod and has a short gut retention time (~30 min), so it is possible that an upper limit of toxin content per copepod was reached during the >23 h grazing period.

The time course for toxin transfer from fish viscera to muscle tissue was observed during a 25 h experiment, wherein juvenile spot were fed toxin-laden copepods, and the toxin content of both viscera and muscle tissue was measured at t = 2, 6, 12 and 25 h (Figures 5 and 6). There was also an increase in PbTx-2 after feeding five additional copepods to the fish at t = 12. The highest toxin (both PbTx-2 and PbTx-3) levels were measured at t = 2 h in the viscera and at t = 25 h in the muscle, as the toxin was translocated within the fish. The total toxin measured in the fish decreased from 25% of the total ingested to 10% over the experimental period.

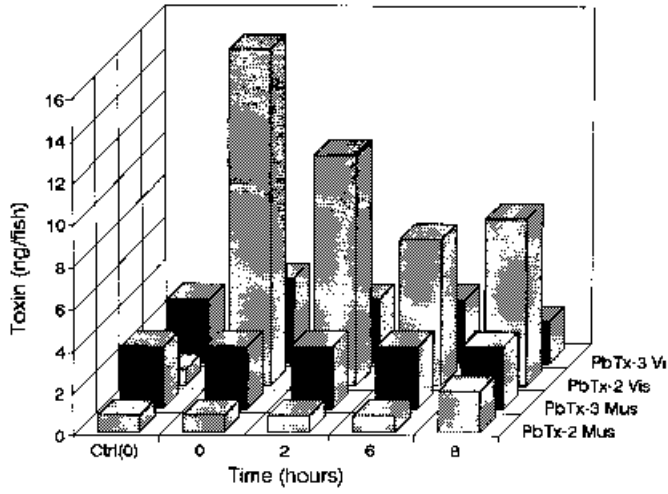

Fig. 4. Amounts of brevetoxins (PbTx-2 and PbTx-3) in the viscera and muscle tissues of juvenile pinfish, *Lagodon rhomboides,* after feeding upon brevetoxin-laden copepods (May, 1994).

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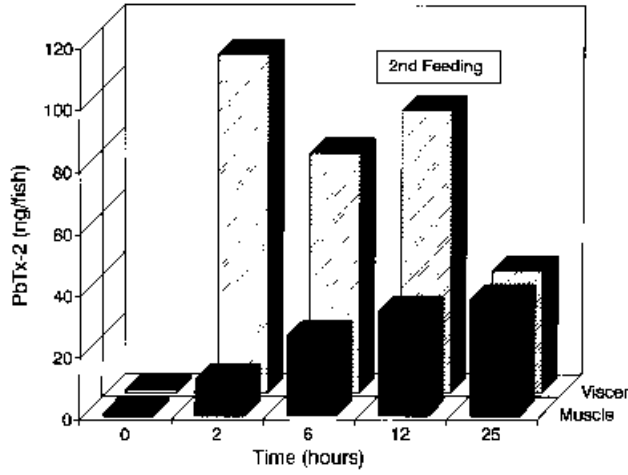

Fig. 5. Brevetoxin (PbTx-2) content of the muscle and viscera tissues of juvenile spot, *Leiostomus xanthurus*, fed upon brevetoxin-laden copepods at $t = 0$ h and $t = 12$ h (February, 1995).

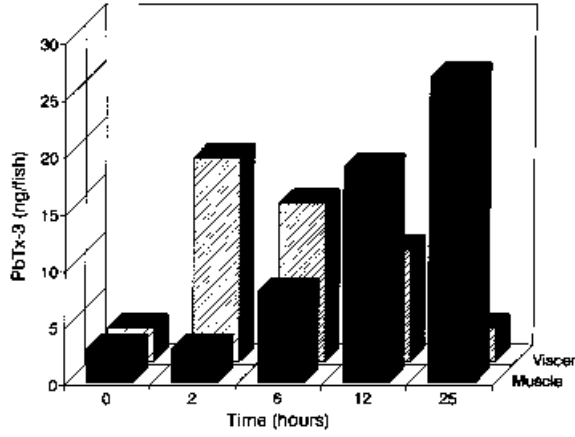

Fig. 6. Brevetoxin (PbTx-3) content of the muscle and viscera tissues of juvenile spot, *Leiostomus xanthurus*, fed upon brevetoxin-laden copepods at $t = 0$ h and $t = 12$ h (February, 1995).

During the first three experiments, none of the four species of juvenile fish died after eating copepods fed on *G.breve*. Larval menhaden differed in this respect. Apparently, there was enough brevetoxin in one copepod to cause death in these small larvae, but our results are not conclusive. At $t = 2$ h and $t = 4$, h all the larval menhaden appeared well but none had eaten any copepods. At $t = 22$ h, two larvae were dead and in each case, there was one copepod missing and we presume it was eaten. One of three control fish was also dead by $t = 22$ h. By $t = 28.5$ h, three more larvae had died after eating a single copepod. Observations of

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young-of-the-year menhaden (120 mm) indicate that they succumb to *G.breve* concentrations of 175–500 × 10^3 cells l^{-1} (Tester and Haugen, unpublished data). During the 1987–88 *G.breve* bloom off North Carolina [see (Tester *et al*., 1989, 1991)], Warlen *et al*. (Warlen *et al*., 1998) noted that menhaden larvae recruited to the estuary both later in the season and at a significantly larger size when compared with average times and sizes of eight seasons (1985–1992). If this species can avoid toxic phytoplankton in a natural setting, then the sensitivity displayed during laboratory experiments could be misleading.

Discussion

The vectorial trophic transport of toxins from *G.breve* to herbivorous zooplankton and zooplanktivorous fish has been confirmed using three different combinations of copepods and species of juvenile fish. Included were: the copepod *T.turbinata* and spotted majarra, *Euchinostomus argenteus* and striped killifish, *Fundulus majalis* (August, 1993); the copepod *L.aestiva* and pinfish, *Lagodon rhomboides* (May, 1994); and the copepod *A.tonsa* and spot, *Leiostomus xanthurus* (February, 1995). In all three cases, fish were not exposed directly to *G.breve,* but they accumulated toxins by feeding upon copepods that had grazed upon this dinoflagellate. In the February, 1995 experiments with *A.tonsa* and *L.xanthurus*, we were able to further track toxin movement from the viscera into the muscle tissues of the fish.

The vectorial intoxication of fish and other upper-level consumers in pelagic food webs is evidenced by accumulation of phytoplankton toxins in numerous apex predators (Anderson and White, 1992). Included are brevetoxins from *G.breve* in dead bottlenose dolphins, *Tursiops truncatus* (Anderson and White, 1989; Geraci, 1989), saxitoxins from *Alexandrium tamarense* in dead humpback whales (*Megaptera novaeangliae*) whose stomachs contained toxin-laden Atlantic mackerel (*Scomber scombrus*) (Geraci *et al*., 1989), and terns (*Sterna hirundo*) apparently poisoned by eating toxin-laden sand launce (*Ammodytes americanus*) (Nisbet, 1983). The toxin domoic acid from the diatom *Pseudo-nitzschia australis* caused mass mortality of pelicans (*Pelicanus occidentalis*) and cormorants (*Phalacrocorax penicillatus*) after they fed on domoic acid-containing phytoplanktivorous anchovies (*Engraulis mordax*) (Fritz *et al*., 1992; Work *et al*., 1993).

Brevetoxins have also been implicated in mass mortalities of manatees (*Trichechus manatus latirostris*) in Florida (O'Shea *et al*., 1991; Landsberg and Steidinger, 1998). However, since manatees are grazers on aquatic macrophytes, it is unlikely that intoxication was vectorial in the same way as for other carnivorous marine animals such as whales, dolphins, birds or fish. However, O'Shea *et al*. presented circumstantial evidence that manatees may have been poisoned by incidental ingestion of benthic filter-feeding ascidians attached to seagrasses upon which the manatees grazed (O'Shea *et al*., 1991).

The present investigation was made possible by new methodology devised for these studies (Shea, 1997), using capillary electrophoresis for high sensitivity measurements of small amounts of brevetoxins. Instrumental detection limits for brevetoxins were ~0.10 fg, or about 10^3-fold more sensitive than existing liquid

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chromatographic methods. Detection limits for brevetoxins in fish tissue were ~4 pg g^{-1}. We have verified the utility of this methodology for measuring brevetoxins in suspensions of *G.breve* cells in cultures, in copepods, and in fish viscera and muscle tissue.

The greater analytical sensitivity of capillary electrophoresis substantially expands the potential for similar studies with brevetoxins and possibly other phycotoxins. In view of the limited sensitivity of the mouse bioassay for PSP (paralytic shellfish poisoning) toxins in common usage two decades ago, White (White, 1981a) had to use about 10 000 zooplankters per assay for reliable toxin measurements. In the present study, capillary electrophoresis detected brevetoxins in juvenile spot (*L.xanthurus*) that had eaten as few as 15 of the smallest copepods used (*A.tonsa*).

This is, to our knowledge, the first demonstration of accumulation of phycotoxins in zooplankton and subsequent trophic transvectoring to fish since the studies of White (White, 1981a) and Robineau *et al.* (Robineau *et al.*, 1991a) with saxitoxins from *Alexandrium tamarense*. Previous attempts to quantify transvectoring of brevetoxins into molluscs and crabs (Roberts *et al.*, 1979) and ciguatoxins into brine shrimp and fish (Kelly *et al.*, 1992) failed to demonstrate toxin accumulation. A likely contributing factor was that these studies attempted to quantify toxins with mouse bioassays that are not as sensitive for low toxin concentrations as the capillary electrophoresis method used in this study.

Results of the present study raise additional questions about vectorial intoxication with phycotoxins in pelagic food webs. For instance, none of the juvenile fish of four different species examined died from ingesting brevetoxins, whereas preliminary results suggested that larval menhaden (*Brevoortia tyrannus*) were killed by eating a single toxin-laden copepod (*A.tonsa*). There is considerable variability for various species and stages of fish (Warlen *et al.*, 1998), and probably, for the toxin burden of zooplankters in terms of how much zooplankton-associated toxin is lethal to fish. Since total toxin levels in juvenile spot were declining even while toxin levels in muscle tissues were increasing, we clearly need to know more about the time course for depuration (excretion and/or metabolism) of brevetoxins by fish once the dietary source of toxin has been withdrawn. There may be variations in these parameters depending on the lipid composition of various stages and species of fish. Our experiments were conducted for a maximum of 25 h. Thus, future studies should extend this period to determine how long it takes for toxins to be completely depurated. Brevetoxins detected in dolphins were from ingested menhaden that are filter-feeders, but whether brevetoxins killed the dolphins was uncertain. Studies of vectorial transport of brevetoxins and other phytoplankton toxins should be extended to higher trophic levels in pelagic food webs. Finally, since humans who eat fish are feeding at the same trophic level as other predators, such as marine mammals and seabirds vulnerable to fatal vectorial intoxication from phytoplankton toxins, the question arises as to whether toxin trophic pathways such as those demonstrated here pose a human health risk. These and other questions will form the basis for future investigations.

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