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MORTALITY OF FISH LARVAE FROM EATING TOXIC DINOFLAGELLATES OR ZOOPLANKTON CONTAINING DINOFLAGELLATE TOXINS

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ABSTRACT

First-feeding red sea bream (Pagrus major) and Japanese anchovy (Engraulis japonica) larvae were fed the toxic dinoflagellate Gonyaulax excavata. Older larvae were fed zooplankton (mostly copepods) that had eaten G. excavata. Despite low toxin content of the dinoflagellates relative to field conditions, effects of the toxins were apparent. The mortality rate of first-feeding red sea bream larvae feeding on Gonyaulax was about three times that of starved controls. First-feeding Japanese anchovy larvae fed poorly on Gonyaulax, and no difference in mortality between treatments and controls was observed. Older larvae of both species showed symptoms typical of "paralytic shellfish poisoning" within a few hours after eating zooplankton that contained Gonyaulax toxins; 20 to 30% of the larvae died. Results indicate that fish larvae, like adult fish, are sensitive to paralytic shellfish toxins and suggest that blooms and red tides of G. excavata and its toxic relatives cause kills of larval, as well as adult, fish.

INTRODUCTION

Blooms and red tides of the toxic dinoflagellate Gonyaulax excavata (tamarensis) have caused mass kills of adult fish in nature [1,2], resulting in losses to traditional fisheries and mariculture. Kills of Atlantic herring, sand lance, menhaden, and cage-cultured Atlantic salmon and rainbow trout have been reported [3,4,5]. Laboratory experiments have shown that Gonyaulax toxins are lethal to various marine fishes, including Atlantic herring, Atlantic salmon, winter flounder, American pollock, and cod and that the sensitivity of these fishes to the toxins is similar to that of warm-blooded animals [6]. Fish acquire the toxins either by direct ingestion of Gonyaulax or, as has been well documented, by ingestion of zooplankton that have fed on Gonyaulax and accumulated the toxins [4,6,7,8]. It is not known whether other routes of toxin acquisition are important for fish, such as ingestion of contaminated shellfish or direct uptake of toxins from solution.

Of even greater concern in terms of fish populations is whether larval fish are killed by Gonyaulax toxins. Year-class strength is determined in large part by the success of early life stages of fish. Fish larvae in the midst of a toxic bloom have little choice but to eat either the toxic dinoflagellates or toxin-containing zooplankton. The one previous study on this topic showed increased mortality of winter flounder larvae upon exposure to G. excavata [9].

We investigated the effects of G. excavata on first-feeding larvae of two commercially important fishes in Japan, red sea bream (Pagrus major) and Japanese anchovy (Engraulis japonica), and the effects of zooplankton containing G. excavata toxins on older larvae.

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MATERIALS AND METHODS

Gonyaulax excavata (clone 7 from eastern Canada) was grown bacteria-free in 1.5 L of enriched seawater medium "f/2" in 2.8-L Fernbach flasks at 18°C and 4,000 lux illumination on a 16:8-hr LD cycle. Seawater (32-33 ppt salinity) was obtained from Hiroshima Bay and glass-fiber filtered before use. Cells in mid-log phase growth were used for feeding fish larvae and zooplankton. Single cells were 25-40 µm in length, duplets were twice as long. Cells looked normal and maintained normal swimming behavior during the experiments; in fact, they doubled in number after several days in the tanks.

Wild zooplankton were collected from Hiroshima Bay, using either 300 µm net tows or continuous plankton traps. In the laboratory the material was screened through 600 µm netting to remove large animals. Screened material was split between two, 10-L plastic buckets, gently aerated, and fed either G. excavata or the non-toxic diatom Phaeodactylum tricornutum. Zooplankton composition varied between experiments, but generally the dominant organisms were the copepods Acartia clausii and Labidocera japonica. Fortunately, the fish larvae showed a clear feeding preference for copepods. Zooplankton were allowed to feed for 2-3 hours on G. excavata (when their intestines became full) and were then gently collected on a piece of netting and added to the experimental tanks containing fish larvae.

Toxin content of G. excavata and zooplankton which ate G. excavata was measured by modifications of the mouse bioassay described before [7].

Experiments were done in cylindrical, 9-L glass tanks (15 cm high, 28 cm diam.) covered externally, except on top, with black plastic to provide a good background for larval feeding. Tanks were filled with 7 L of filtered seawater (31.5 ppt salinity) and kept in a walk-in incubator at 18°C (for red sea bream) or 20°C. Tanks received light from cool-white fluorescent lamps at 3,000 lux at the surface for 16 hr/day and at 25 lux from an incandescent bulb at night. Water was gently aerated with glass pipettes.

In experiments with first-feeding larvae, 5 treatments were made in duplicate: starved controls; G. excavata; G. excavata in a cage made of 10-µm netting; rotifers (Brachionus plicatilis); and rotifers plus G. excavata. Gonyaulax was supplied at an initial concentration of 300 cells/mL. Rotifers (fed Chlorella and screened through 100-µm netting) were supplied at 5/mL.

The experiment with first-feeding red sea bream larvae was started by adding 100, 2-day-old larvae in yolk-sac stage to each tank after Gonyaulax and rotifers had been already been added. Since Japanese anchovy larvae were so delicate, eggs (100) were added to each tank and 3 days later (2 days after hatching), when larvae were ready to begin feeding, Gonyaulax and/or rotifers were added.

Twice daily, dead larvae were removed and counted and observations on larval behavior were made. Temperature, pH, and oxygen in the tanks were checked periodically; temperature remained constant, pH remained at 7.8-7.9 and oxygen remained at near saturation levels in all tanks throughout the tests.

RESULTS AND DISCUSSION

First-feeding red sea bream larvae began feeding behavior (S-posturing and striking) one day after the experiment was started (3-day-old larvae). They fed well on rotifers and moderately well on Gonyaulax. After 4 days about 95% of the larvae exposed directly to Gonyaulax alone had died, whereas only about 20-40% of the larvae in the other treatments, including starved controls, had died (Fig. 1). Larvae which died in the Gonyaulax treatments usually had Gonyaulax cells observable in their stomachs; most contained only several cells, but some contained a mass of yellow-brown material representing the remnants of about 12 or more cells. These larvae lay

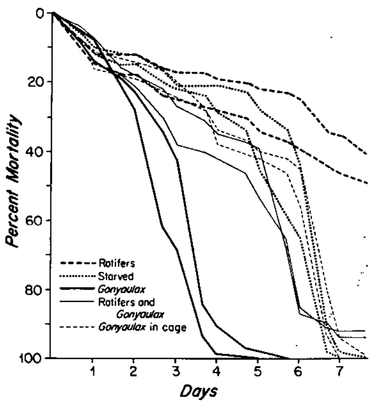

FIG. 1. Mortality of first-feeding red sea bream larvae

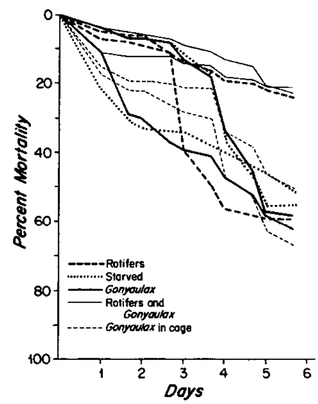

FIG. 2. Mortality of first-feeding Japanese anchovy larvae

paralyzed on the tank bottom, unable to move when prodded and with pumping of the heart being the only sign of life, until death ensued. Paralysis was not observed in larvae in the other treatments. There was a tendency for the larvae which were alone with Gonyaulax to have a more sluggish avoidance response (when prodded with a pipette) than larvae in the other treatments. Paralysis and reduced avoidance was also observed in winter flounder larvae fed G. excavata [9].

Survival was best in the rotifer treatments, although there was gradual mortality even in these treatments (Fig. 1). Effects of starvation on mortality were not clearly apparent until the fifth day when many larvae died. The pattern of mortality in the treatments with Gonyaulax separated from larvae by plankton netting was not different from that in the starved controls, indicating that excretion products from Gonyaulax were not responsible for the increased mortality in the treatments in which larvae were fed Gonyaulax. The data suggest that Gonyaulax caused a slight increase in mortality over starved controls even in the presence of rotifers. Indeed, some dead larvae in the tanks containing rotifers and Gonyaulax had Gonyaulax cells in their guts.

First-feeding Japanese anchovy larvae fed poorly on G. excavata. The rate of mortality of larvae exposed to Gonyaulax, either directly or indirectly, was similar to that for starved larvae (Fig. 2). Characteristic feeding behavior (searching, S-posturing, and striking) developed only in tanks containing rotifers, by the end of the first day. Intestines of larvae soon became packed with rotifers. After 5 days, at the termination of the experiment, 50-65% of the larvae in the Gonyaulax or starvation treatments had died, whereas only 20-25% of the larvae in the rotifer treatments (including rotifers plus Gonyaulax) had died, with the exception of one rotifer tank in which a sudden bacterial contamination caused rapid mortality (see Fig. 2).

On occasion in the Gonyaulax treatments one or two Gonyaulax cells were seen in the guts of dead anchovy larvae. An interesting exception was that one larva contained about 8 Gonyaulax cells, lined up in its intestine like beads on a string, and it was paralyzed except for the beating of its heart. Aside from this, however, there was no clear difference in avoidance response behavior among treatments.

Regarding older larvae (4-6 weeks), in several experiments in which either red sea bream or Japanese anchovy larvae were exposed to zooplankton which had fed on Gonyaulax, effects of the toxins were clearly apparent.

Within 1-2 hr after feeding on Gonyaulax-fed zooplankton had begun, some larvae of both species (22-38% of the fish tested) lost their equilibrium and swam on their sides or upside down or in circles, as was previously observed in adult fish of other species [6,8]. Symptoms progressed to include paralysis on the tank bottom, with little motion of the opercula. A few larvae recovered from this condition and resumed normal swimming, but most died within a few hours. In total, in the experiments in which pronounced symptoms of poisoning developed, 20% (11 of 55) red sea bream larvae and 33% (5 of 15) Japanese anchovy larvae died. Larvae of both species which ate Phaeodactylum-fed zooplankton showed no abnormal behavior and no deaths occurred.

Effects of G. excavata on first-feeding larvae and of zooplankton containing G. excavata on older larvae were observed despite low toxin content of the dinoflagellates and zooplankton relative to field conditions. In these experiments toxin content of G. excavata ranged from 1 to 2 x 10^{-5} µg STX equivalent/cell, and toxin content of zooplankton averaged 3.5 µg STX equivalent/g wet weight. Toxin levels 10 times greater than this often occur in G. excavata and zooplankton in nature [3,10].

At 2 x 10^{-5}µg STX equivalent/cell, and assuming the oral LD_{50} to be similar to that for adult fish [6], calculations suggest that a first-feeding fish larva would need to eat only 6-11 G. excavata cells to acquire a lethal dose.

This study shows that some fish larvae can be killed by eating G. excavata and zooplankton containing G. excavata and suggests that blooms of toxic dinoflagellates have impact on fish larvae, as well as adults, in nature.

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