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Sensitivity of Marine Fishes to Toxins from the Red-Tide Dinoflagellate *Gonyaulax excavata* and Implications for Fish Kills

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Abstract

Marine fishes (Atlantic herring, American pollock, winter flounder, Atlantic salmon, and cod) were dosed orally and intraperitoneally (i.p.) with "paralytic shellfish toxins" extracted from Bay of Fundy *Gonyaulax excavata* (*tamarensis*) cells. The toxins are lethal to these fishes in low oral doses, and in extremely low i.p. doses. Symptoms are the same among these fishes, both for oral and i.p. administrations, including loss of equilibrium within 5 to 15 min, followed by immobilization and shallow, arrhythmic breathing. Death generally occurs within 20 to 60 min of toxin administration. Dose responses are also similar among these fishes. Oral LD50 values are 400 to 750 μg saxitoxin (STX) equivalent kg^{-1} body weight. Intraperitoneal LD50 values are 4 to 12 μg STX equivalent kg^{-1}. Toxins are undetectable in fish muscle tissue following lethal oral doses. The similarity of symptoms and dose responses suggest that fish as a group are sensitive to *G. excavata* toxins. Results, in combination with reports implicating these toxins in herring, sand lance, and menhaden kills, show the plausibility that the nearly worldwide blooms and red tides of *G. excavata* and its relatives may cause kills of a variety of fishes.

Introduction

Annual blooms of the eastern Canadian-New England toxic dinoflagellate *Gonyaulax excavata* (formerly *G. tamarensis*, see Materials and Methods) have been recognized for many years to have significant impact on shellfish resources because of the problem of "paralytic shellfish poisoning", or "PSP" (Prakash *et al.*, 1971; Hurst, 1975). In 1976 the potential effects of these blooms on finfish were first realized when the toxins caused an extensive herring kill in the Bay of Fundy (White, 1977). Until then it was generally thought that fish, like other cold-blooded vertebrates, were relatively insensitive to these toxins (Prakash *et al.*, 1971). Additional toxin-caused herring kills occurred in the Bay of Fundy in 1979 (White, 1980a). Through a combination of field and laboratory studies the picture has now clearly developed that the kills result from toxin transfer through planktonic herbivores (White, 1977, 1979, 1980a, b, 1981), a mechanism suggested earlier in connection with a sand lance (*Ammodytes* sp.) kill in the North Sea (Adams *et al.*, 1968; Coulson *et al.*, 1968).

Blooms of *Gonyaulax excavata* and its close relatives, *G. tamarensis* and *G. catenella*, are associated with red tides and PSP in many areas of the world, and seem to be intensifying and spreading (see Taylor and Seliger, 1979). Recent studies show that strains of these organisms from around the world have similar toxin compositions, including saxitoxin (STX) and STX-like compounds (Shimizu, 1979). The few reports concerning the effects of these toxins on fish indicate that *G. excavata* toxins can kill herring (White, 1977) and *G. catenella* toxins can kill *Gambusia* sp. minnows (Bates *et al.*, 1978) and killifish (Jackim and Gentile, 1968; McLaughlin and Down, 1969). A number of other marine dinoflagellates are known to produce ichthyotoxins, but these compounds are not related chemically or pharmacologically to *G. excavata* toxins – for example, toxins from *Gymnodinium breve* (Baden *et al.*, 1979; Risk *et al.*, 1979), *G. veneficum* (Abbott and Ballantine, 1957), and several species of *Amphidinium* (McLaughlin and Provasoli, 1957; Thurberg and Sasner, 1973). Even *Gonyaulax monilata* toxins, which are known to kill fish and have been associated for many years with kills in the Gulf of Mexico (Gates and Wilson, 1960; Aldrich *et al.*, 1967; Sievers, 1969) appear dissimilar from other *Gonyaulax* spp. toxins (Clemons *et al.*, 1980a, b).

This report presents results of a study aimed at delineating the sensitivity of herring to *Gonyaulax excavata* toxins and investigating the effects of the toxins on, and sensitivities of, other marine fishes. Results are discussed in terms of the plausibility of blooms of *G. excavata*, and its relatives, causing kills of many fishes in addition to herring and sand lance.

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Materials and Methods

The taxonomic situation of the "*Gonyaulax tamarensis* complex" of dinoflagellates is confused. It is not clear whether the taxa *G. excavata* and *G. tamarensis* actually represent different species, or varieties within the same species (Loeblich and Loeblich, 1979; Taylor, 1979). The isolate used here is toxic, bioluminescent, and lacks a ventral pore (F. Taylor, personal communication), and is referred to here as *G. excavata.*

The clone of *Gonyaulax excavata* (No. 7) used for toxin extracts was isolated by the author from the Bay of Fundy in July, 1976. It was grown axenically in enriched seawater medium "f", described by Guillard and Ryther (1962), used at one-half strength and modified by adding soil extract and omitting silicates. The 2.8-l Fernbach flasks were kept at 11 °C under 4000 lux illumination from cool-white fluorescent lamps on a 14 h L : 10 h D cycle. Cells were harvested in mid to late log phase growth by gentle centrifugation (1000 × G). Qualitative and quantitative aspects of the toxin content of *G. excavata* have been described (White, 1978; White and Maranda, 1978).

Toxin extracts were prepared by boiling the cells in a small volume of 0.1 N HCl for 5 min (White, 1977). Extracts were centrifuged and the clear, yellowish supernatant was kept at 5 °C at pH 1.2. Several extracts were pooled before final toxicity assays were conducted so that the same stock solution could be used for many experiments. The same solution was used for all herring and pollock work, a second solution for flounder, and a third for salmon and cod. Periodic assays of the toxin solutions confirmed that toxicity levels remained constant under these conditions. Potency of extracts was determined using the standard mouse bioassay for paralytic shellfish toxins (AOAC, 1975). Duplicate samples of each pooled extract were adjusted to pH 3.5 with a few drops of NaOH and sent to the Department of National Health and Welfare in Ottawa where "10-mouse" tests were performed. It should be noted that toxin concentrations are expressed in terms of μg STX equivalents throughout this report, because the bioassay is standardized with pure STX only.

All fish were collected from Passamaquoddy Bay, New Brunswick (Bay of Fundy), except for salmon smolts which had been hatchery-reared from eggs. Fish were acclimated to holding tanks in the laboratory for at least several weeks before experimentation. The salmon smolts were fully seawater conditioned. Fish were fed commercial pellets (RFM-Ewos Salmon Grower) and were in good condition when experiments were initiated. Experiments were conducted between October, 1979 and September, 1980. Work on herring, pollock, and salmon was done when the temperature of the laboratory seawater supply was from 10° to 12 °C, on flounder from 3° to 7 °C, and on cod from 6° to 9 °C. Fish used in the experiments and their weight ranges were the following: Atlantic herring (*Clupea harengus harengus*), 14–81 g, mostly 20–45 g (14.5–18 cm fork length); American pollock (*Pollachius virens*), 16–71 g, mostly 20–50 g; winter flounder (*Pseudopleuronectes americanus*), 21–382 g, mostly 50–200 g; Atlantic salmon (*Salmo salar*) smolts, 14–163 g, mostly 25–100 g; and cod (*Gadus morhua*), 50–296 g.

For each experimental trial ten fish were dosed with toxin extract, either orally or intraperitoneally (i.p.), and were placed in a 100-l tank. Frequent observations were made and the percent mortality recorded after 6 and 24 h. Fish received no food for one day before experiments. Just prior to injection, fish were weighed in a bucket of water, and the volume of extract was calculated to give the desired dosage. Fish were then injected without use of anesthetic. Total handling time was only about 30 s per fish. For oral doses, toxin extracts were delivered directly to the fish's stomach by syringe and a 14-gauge, 10-cm, blunted needle passed down the throat (White, 1977). Toxin extracts of high potency (250–275 μg toxins ml^{-1}) were used in order to keep the volumes required at high dose levels small, minimizing regurgitation of the toxins after oral administration. For both oral and i.p. treatments, 0.15–0.30 ml toxin extract was administered per 100 g body weight. For the lower toxin doses, extracts were diluted to keep volumes injected within this range. Control trials, in which 0.1 N HCl (pH 1.2) was administered, were run for each set of experiments. LD50 values and fiducial limits were determined by probit analysis.

In the experiments on the fate of the toxins, fish were dosed orally with high levels of toxins (2000 μg kg^{-1} body weight). The amounts of toxins contained in the viscera and muscle were measured at time zero, after 10–20 min when all fish had displayed symptoms and were at the bottom of the tank, and after 45–60 min when all fish were dead. Samples from 3 to 5 fish were pooled for toxin assays. Viscera samples consisted of all viscera except gonads. Strips of muscle tissue were taken from along the lateral line. Material was homogenized in a blender and boiled for 5 min in 0.1 N HCl. Toxin amounts were determined according to the standard mouse bioassay (AOAC, 1975), using ten mice for each test.

Results

Toxin extracts from *Gonyaulax excavata* were lethal to all fishes tested, both by oral and by intraperitoneal routes. Symptoms were similar among the fishes and were apparent within 5 to 15 min after either oral or i.p. administration of the toxins. Symptoms included swimming in an irregular, jerky manner, leading to a loss of equilibrium and swimming on the side or upside down. Generally, after 10–20 min, fish became immobilized on the tank bottom, showing shallow and arrhythmic breathing. Occasionally, fish in this condition exhibited hyperactivity, swimming wildly, often in tight circles, for a short period before resuming a position on the bottom. Death occurred generally within 20–60 min of toxin administration, regardless of the route. Often, fish showing mild to moderate symptoms recovered completely and swam normally within a few hours. Even some fish showing acute symptoms managed to recover and resume erratic, then normal

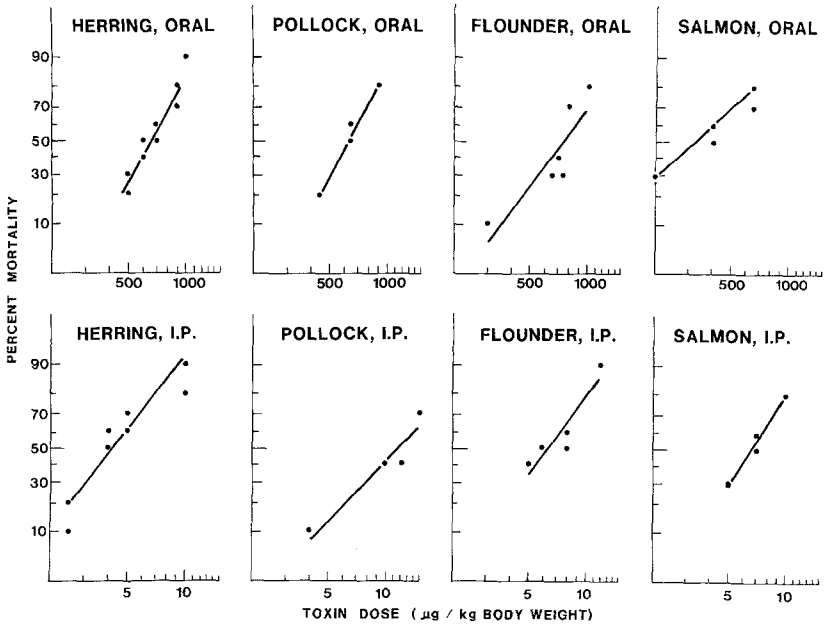

Fig. 1. *Gonyaulax excavata.* Dose responses of marine fishes to *G. excavata* toxins administered by oral and intraperitoneal routes. Dose is plotted on a log scale, percent mortality on a probability scale. Lines were fitted by eye and were drawn through the calculated LD50 values (see Table 1). Each point represents results from a group of ten fish 6 h after toxin injection

swimming within a few hours. Symptoms of disequilibrium in flounder were discernible upon prodding; when left alone they displayed arrhythmic undulations of their fins and shallow breathing. Unlike the other fishes, some herring floated while dying and some died with their mouths gaping and gills flared.

The percent mortalities recorded 24 h after toxin administrations were the same or slightly higher than the corresponding values after 6 h. No deaths of control fish were observed after 6 h and fish showed no abnormal behavior; a small percentage of mortality was observed in a few cases in controls after 24 h. Therefore, LD50 values were calculated using mortalities recorded after 6 h.

The oral dose responses of herring, pollock, flounder, and salmon were similar (Fig. 1). Calculated LD50 values ranged from 400 to 755 $\mu g\,kg^{-1}$ (Table 1). LD50's for herring, pollock, and flounder were not significantly different from one another ($P < 0.05$, Table 1). Salmon were slightly more sensitive to oral administrations of toxins than the other fishes ($P < 0.05$). The slopes of response lines (Fig. 1) were much the same, further indicating the similar sensitivities of these fishes to oral, toxin doses. Preliminary experiments (2 trials only) suggested that cod may be somewhat less sensitive to the toxins; the oral LD50 was about 1 000 $\mu g\,kg^{-1}$.

The i.p. dose responses of these fishes were also similar (Fig. 1). Calculated LD50 values were low, 4.2–12.0 $\mu g\,kg^{-1}$ (Table 1), only about 1% of the oral LD50 values. LD50's for herring and flounder, and for flounder and salmon were not significantly different ($P < 0.05$, Table 1). The LD50 value for pollock of 12.0 $\mu g\,kg^{-1}$ was slightly higher ($P < 0.05$) than the others. Again, the slopes of the response lines were not much different (Fig. 1), indicating similar sensitivities of these fishes to i.p., toxin doses. A preliminary experiment (1 trial only) suggested that the i.p. LD50 for cod is in this same vicinity; 60% mortality was recorded at 10 $\mu g\,kg^{-1}$.

The fate of a high, oral dose of toxins (2 000 $\mu g\,kg^{-1}$) in viscera and meats of the fishes was examined. Toxins were measurable in all viscera samples immediately after administration, after 10–20 min when all fish were immobilized on the tank bottom, and after 45–60 min when all fish were dead (Table 2). For herring and pollock the amounts of toxins in the viscera remained fairly constant

Table 1. LD50 values and 95% confidence limits, calculated by probit analysis, for *Gonyaulax excavata* toxins administered orally and intraperitoneally to marine fishes. See Fig. 1 for sample size

	LD50 ($\mu g\,kg^{-1}$ body weight)	
	Oral	I.P.
Herring	650 (722, 584)	4.2 (5.2, 3.4)
Pollock	626 (748, 532)	12.0 (17.0, 8.4)
Flounder	755 (856, 665)	6.4 (8.3, 4.9)
Salmon	400 (517, 309)	6.5 (7.8, 5.4)

Table 2. *Gonyaulax excavata.* Concentrations of toxins measured in viscera and muscle of fishes after oral administration of 2 000 μg toxins kg^{-1} body weight. Data represent results from pooled samples from three to five fish

	G. excavata toxins (μg 100 g^{-1} viscera or muscle)		
	Time zero	10 – 20 min	45 – 60 min
Viscera			
Herring	416	524	353
Pollock	343	247	275
Flounder	837	133	214
Salmon	408	520	941
Muscle	Undetectable (i.e., < 30) for all 12 assays		

throughout the experiment, indicating little, if any, regurgitation of the toxins. Flounder appear to have lost a substantial amount of toxins from the viscera within the first few minutes of exposure, suggesting toxin regurgitation. Inexplicably, salmon viscera appear to have increased in toxin content. Most important, however, the toxins did not accumulate to detectable levels in any of the muscle samples, supporting the earlier findings that only very low toxin levels are sufficient to kill fish via the i.p. route.

Discussion

Gonyaulax excavata toxins are lethal at low doses to herring, pollock, flounder, salmon, and cod via the oral route, and at extremely low doses via the i.p. route. The similarity of symptoms and sensitivities of these five fishes suggests that the responses to the toxins may well apply to marine fish as a group. Results represent a foundation for the possibility that many fishes in addition to herring and sand lance can be killed as a result of *G. excavata* blooms.

The detailed toxin composition of the strain of *Gonyaulax excavata* used in this study is not yet known. However, the toxin compositions of three other *G. excavata* strains have recently been elucidated. In addition to STX itself, which occurs as a minor toxin component, they all contain at least six other toxins (neoSTX plus five gonyautoxins) structurally similar to STX (Oshima *et al.,* 1977; Alam *et al.,* 1979; Shimizu, 1979), although the relative amounts of the different toxins vary from strain to strain. Thus it is highly probable that the effects on fish reported here are caused by this same complex of toxins.

There are only a few other reports on the effects of STX and STX-like compounds on fish. Jackim and Gentile (1968) showed that partially purified STX (from *Gonyaulax catenella*) and similar toxins from a blue-green bacterium (*Aphanizomenon flos-aquae*) are lethal to killifish in low, i.p. dosage. *Gambusia* sp. minnows die upon immersion in water containing *G. catenella* toxins (Bates *et al.,* 1978). Also, lysates from *G. monilata* kill mullet and guppies, with symptoms similar to the ones described here (Gates and Wilson, 1960; Aldrich *et al.,* 1967), and cyprinids (Sievers, 1969). Although the toxin composition of *G. monilata* is not known in detail, the symptomology suggests similarity between *G. monilata* and *G. excavata* toxin compositions. Yet there is evidence that *G. monilata* contains a lipid-soluble, hemolytic toxin (Clemons *et al.,* 1980 a, b) which therefore is quite different from the water-soluble neurotoxins of *G. excavata.*

Fish appear to be as sensitive as most warm-blooded animals to paralytic shellfish toxins. Exact comparisons using the data available are not possible because the present data for fish are based upon responses to extracts of *Gonyaulax excavata,* which contain a mixture of toxins, whereas data for warm-blooded animals are based largely upon responses to purified STX. This notwithstanding, the sensitivities of the two groups of animals are in the same ranges. The i.p. LD50 for white mice is about 10 μg STX kg^{-1} (E. Schantz, personal communication), which is within the range for fish of 4 to 12 μg STX equivalent kg^{-1} (Table 1). Evans (1972) reports the oral LD50 for mice, rats, rabbits, and cats to be from 200 to 600 μg STX kg^{-1}, which is comparable to the range for fish of 400–755 μg STX equivalent kg^{-1} (Table 1). It is not clear how human sensitivity compares. Rough estimates of the minimum lethal oral dose for humans (based on case histories from PSP incidents) are as low as 7–16 μg kg^{-1} (Evans, 1972; Schantz *et al.,* 1975). However a further indication that fish respond to the toxins much like homeotherms is that fish recover rapidly and completely from sublethal doses.

The i.p. dose responses show that fish are killed by extremely low, systemic levels of toxins. This explains why toxins are undetectable in muscle samples after exposure to very high oral doses (Table 2). Fish, unlike shellfish, are not able to accumulate toxins in their muscle tissues because even low systemic amounts cause death. Assays of herring muscle from toxin-caused Bay of Fundy kills support this. Toxins were also undetectable in these samples, with the exception of two samples from decayed fish which showed marginal levels of toxins, probably resulting from leakage from the viscera (White, 1980 a).

Toxins may be detectable in the viscera of dead fish, as shown here and in field studies (White, 1977, 1980 a; J. Hurst, personal communication). Although toxin levels in dead fish viscera are much lower than commonly found in shellfish, they can apparently be sufficient to cause seabird kills (Adams *et al.,* 1968; Coulson *et al.,* 1968; I. Nisbet, personal communication).

There is fair agreement between the oral levels of toxins shown here to be lethal to fish and the few estimated doses for fish from kills caused by *Gonyaulax excavata* toxins. For herring from the 1976 and 1979 Bay of Fundy kills estimated oral doses ranged from 23 to 200 μg toxins kg^{-1} (White, 1980 a). Estimates low in this range were derived either from fish showing symptoms, but still alive, or from decayed fish. Therefore they may reflect sublethal doses or toxin amounts after some degradation. On the other hand, estimates at the high end of this range probably reflect more closely the actual killing doses. The estimated 200 μg kg^{-1} dose is not much different from levels shown to kill fish in the laboratory. Furthermore, doses of about 100 μg kg^{-1} were estimated for sand lance from a kill in 1978 off Wellfleet, Massachusetts, USA (I. Nisbet, Codman Road, Lincoln, Massachusetts 01773, USA, personal communication). This figure is probably an underestimate of the actual, killing dosage because the fish assayed were dead and decayed – in fact, they were taken from the vomitus of terns. Also, doses as high as 80 μg kg^{-1} have been estimated for menhaden from a kill off the coast of Maine in 1979 (J. Hurst, personal communication).

Several routes exist through which *Gonyaulax excavata* toxins may lead to fish kills in nature (Fig. 2). Accumulation and retention of the toxins by many kinds of mol-

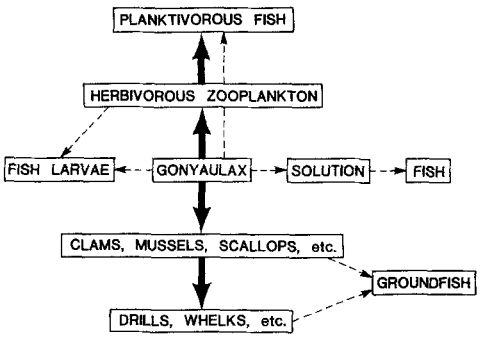

Fig. 2. *Gonyaulax excavata.* Some food web routes through which *G. excavata* toxins may cause finfish kills. Solid arrows represent known routes. Broken arrows represent possible routes

luscan shellfish are well recognized. We now know that many planktonic herbivores are able to accumulate the toxins too (White, 1979, 1981). Toxin transfer through the herbivorous zooplankton community appears to be a main route leading to fish kills, having caused herring kills in the Bay of Fundy (White, 1977, 1979, 1980a, 1981) and probably sand lance kills in the North Sea (Adams *et al.*, 1968) and off the coast of Massachusetts (I. Nisbet, personal communication). Simple calculations based on the oral LD50 values determined here (Table 1) and the amounts of toxins measured in zooplankton during *G. excavata* blooms underscore the practicality of the occurrence of fish kill events through this route. The toxin content of zooplankton reached as high as 60 μg STX equivalent g^{-1} animals (wet wt) each year during the 1977, 1978, and 1980. *G. excavata* blooms in the Bay of Fundy (White, 1979, and unpublished data). Calculations show that under these conditions a zooplanktivorous fish weighing 100 g could acquire a lethal dose of toxins upon ingestion of as little as 1 g or less (wet wt) of zooplankton.

Similar calculations show the plausibility of fish kills occurring through other food web routes (Fig. 2). Groundfish may be affected by ingestion of contaminated shellfish. Shellfish toxin levels frequently reach 60 μg toxins g^{-1} tissue during *Gonyaulax excavata* blooms, and occasionally much higher (from annual toxicity records compiled by the Dept. of Fisheries and Oceans Inspection Laboratory, Black's Harbour, New Brunswick). Thus as little as 1 g or so of shellfish tissue could contain a lethal dose of toxins to a 100-g groundfish.

Also, phytoplanktivorous fish such as menhaden may be affected by direct ingestion of the dinoflagellate. In culture, Bay of Fundy *Gonyaulax excavata* isolates contain 5×10^{-5} μg STX equivalent $cell^{-1}$ (White and Maranda, 1978). This means that by sieving just several hundred milliliters of red water (which often contains many millions of cells per liter) a 100-g filter-feeding fish could acquire a lethal dose. This possibility is supported by the detection of the toxins in the viscera of menhaden (up to 1.9 μg/g viscera) from a kill off the coast of Maine during the 1979 red tide season (J. Hurst, personal communication).

It is not clear if fish are affected by the presence of *Gonyaulax excavata* toxins in solution in seawater during blooms. Bates *et al.* (1978) report that *Gambusia* sp. minnows do not die in water containing *G. excavata* toxins, but do in water containing *G. catenella* toxins. Other dinoflagellates (*G. monilata, Gymnodinium breve,* and *G. veneficum*) exert their toxic action on fish by release of toxins into the water, with uptake by fish probably through the gills (Ray and Wilson, 1957; Gates and Wilson, 1960; Aldrich *et al.*, 1967; Sievers, 1969; Wilson *et al.*, 1975). It is not known whether these toxins also contribute to fish kills via the food web.

In summary, results suggest that repercussions of *Gonyaulax excavata* blooms and red tides may involve a variety of fishes in addition to herring and sand lance. The same may hold for blooms of *G. excavata's* close relatives which share similar toxin compositions and which occur nearly worldwide. In order to understand the full consequences of the toxic blooms to finfish, we need to know more about the fate of the toxins in the food web and in seawater. Furthermore, we need to extend studies of toxic blooms to include effects on fish larvae and juveniles – which may perhaps result in long-lasting impact on finfish resources (see Fig. 2; Mills and Klein-MacPhee, 1979; White, 1980b).

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