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First Occurrence of a Food Poisoning Incident Due to Ingestion of *Takifugu oblongus*, Along with a Toxicological Report on Three Marine Puffer Species in Bangladesh

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On November 16, 1998 a food poisoning incident due to ingestion of roe of *Takifugu oblongus* occurred in Bangladesh, affecting 8 people inclusive of 5 deaths. Their symptoms resembled those caused by tetrodotoxin (TTX) or paralytic shellfish poison (PSP). Immediately after the incident, twenty-two specimens of *T. oblongus* were collected from the seashore adjacent to the concerned poisoning area and their anatomical distribution of toxicity was determined. They showed a high level of toxicity in the ovary (24.5–323.8 MU/g), though the toxicity levels of the other tissues, skin, muscle, liver, testis, and the viscera (except liver), were relatively low (< 2–21.3 MU/g). In contrast, a total of 336 specimens of three marine puffers, *T. oblongus*, *Lagocephalus wheeleri* and *L. lunaris*, which were collected on a regular sampling basis from Bangladesh, showed very low toxicity of less than 10 MU/g in all or most tissues including gonad. The toxin partially purified from the *T. oblongus* specimens, as well as from the other two species, was indistinguishable from TTX in HPLC analysis. From this result and the symptoms of the victims, the causative agent in the above incident was assumed to be TTX.

Key words: food poisoning; fatality; marine puffer; *Takifugu oblongus*; Bangladesh

Introduction

Puffer fish command a very low price or are even unsaleable in rural areas of Bangladesh. However, they are gaining popularity as a food item among the poor and tribal people, resulting in sporadic occurrence of food poisoning incidents. In Bangladesh, puffer poisoning is mainly caused by freshwater species *Chelonodon patoca* and *Tetraodon cutcutia*. Between 1994 and 1996, seven poisoning incidents due to consumption of freshwater puffer fish, involving 43 patients with 16 deaths, were reported¹⁾. However, there is no official record of food poisoning caused by marine puffer in Bangladesh. Recently, for the first time, a serious food poisoning incident involving 8 people with 5 deaths occurred due to ingestion of a marine puffer *Takifugu oblongus* in the coastal district of Cox's Bazar in Bangla-

desh. Immediately after the incident, some *T. oblongus* specimens were collected from a nearby place and assayed for toxicity and toxin composition.

This paper deals with the above results, along with epidemiological information on the aforesaid poisoning incident. In addition, the toxicity scores and toxin composition of three marine puffers, viz. *T. oblongus* (Japanese name 'takifugu'), *Lagocephalus wheeleri* ('shiro-sabafugu') and *L. lunaris* ('doku-sabafugu'), collected from Bangladesh on a regular sampling basis are described. To our knowledge, this is the first toxicological report on Bangladeshi marine puffers.

Materials and Methods

Poisoning information

Information on the poisoning was obtained through the newspapers, and by interviewing

the patients, their families and the physicians, or in some cases by means of questionnaires.

Puffer specimens

One week after the poisoning incident, twenty-two specimens of *T. oblongus* (body weight from 340–940 g; hereafter designated as 'poisoning-related samples') were collected from the seashore adjacent to the incident area of Kutubdia near Cox's Bazar. On the other hand, as a part of our regular study a total of three hundred and thirty-six specimens (hereafter designated as 'regular samples') of three marine puffer species, *T. oblongus* (body weight from 198.8–328.0 g), *L. wheeleri* (9.8–81.5 g) and *L. lunaris* (16.9–62.9 g), were collected from Khulna and Cox's Bazar regions of Bangladesh (Fig. 1) in 1997 and 1998. They were caught concomitantly with other commercially important fishes in seine nets from the offshore area of the Bay of Bengal. All the samples were immediately frozen at -20°C , transported by air to our laboratory at Nagasaki University, Japan and subsequently kept frozen at below -30°C until used.

Assay of toxicity

Puffer specimens were partially thawed and dissected into skin, muscle, liver, ovary/testis and viscera. Each tissue was minced and a small portion (3–5 g) was extracted with an equal volume of 0.1% acetic acid by heating in a boiling water bath for 5 min. The extract was, directly or after dilution with water, intraperitoneally injected into a male mouse of ddY strain (18–20 g). Lethal potency was determined by the Japanese standard method for tetrodotoxin (TTX)²⁾ and expressed as mouse units (MU). One MU is defined as the amount of toxin required to kill a mouse in 30 min after injection.

Purification of toxin

About 460 g of *T. oblongus* tissues (including some remaining parts of the 'poisoning-related samples'), 280 g of *L. wheeleri* ones and 710 g of *L. lunaris* ones were used as materials. Each sample was extracted with three volumes of 75% methanol containing 1% acetic acid. The operation was repeated twice more for the residue. The supernatants obtained were combined,

Fig. 1. A map of Bangladesh showing the sample collection locations of marine puffers

evaporated to dryness and defatted with dichloromethane. The resulting aqueous layer was concentrated to remove dichloromethane under reduced pressure and ultrafiltered through a YM-1 membrane (Amicon) whose cut-off limit was 1,000 daltons. The filtrate was concentrated, dissolved in a small amount of water adjusted to pH 5.5 with 1 mol/L NaOH, and applied to a column (5×90 cm) of Bio-Gel P-2 (Bio-Rad Laboratories) equilibrated with 1 L of water. Toxin adsorbed on the column was eluted with 0.1% acetic acid and the fractions were monitored by mouse assay. Toxin recovery was 87% for *T. oblongus*, 93% for *L. wheeleri* and 91% for *L. lunaris*. Toxins thus eluted, which will hereafter be referred to as OTX (*T. oblongus* toxin), WTX (*L. wheeleri* toxin) and LTX (*L. lunaris* toxin), were analyzed by HPLC.

High-performance liquid chromatography (HPLC)

HPLC was carried out on a Waters LC Module 1 system. A Lichro CART Superspher RP-18(e) column (4×250 mm, Merck) was used for analysis of both TTX and paralytic shellfish poison (PSP). For detection of TTXs, 2 mmol/L hep-

Table 1. Toxicity of "Poisoning-related Samples" from Bangladesh

Date of collection	Specimen number	Sex	Body weight (g)	Total length (cm)	Toxicity (MU/g)					
					Skin	Muscle	Liver	Testis	Ovary	Viscera
Nov. 24, 1998	1	♂	370.0	23.2	13.8	4.3	4.7	2.4	—	24.8
	2	♂	395.0	24.6	17.5	2.8	ND	ND	—	24.9
	3	♂	350.6	24.0	18.9	2.8	3.8	3.6	—	22.9
	4	♂	400.0	25.8	13.5	3.3	2.1	2.8	—	13.8
	5	♂	340.0	24.0	18.6	4.8	2.8	ND	—	24.7
	6	♂	400.0	24.2	12.4	3.9	2.6	ND	—	23.9
	7	♂	370.4	24.8	13.9	3.6	2.8	ND	—	34.9
	8	♂	540.0	26.5	21.3	3.2	ND	ND	—	30.8
	9	♀	480.6	26.6	15.5	3.8	ND	—	106.5	28.6
	10	♀	465.0	26.4	17.6	2.9	2.8	—	323.8	34.9
	11	♀	610.0	29.8	18.8	4.3	2.2	—	132.0	39.8
	12	♀	540.8	29.5	11.8	4.9	ND	—	122.9	29.7
	13	♀	550.0	27.2	13.9	2.8	2.4	—	64.8	12.8
	14	♀	710.0	28.8	18.5	3.4	2.9	—	96.8	15.9
	15	♀	500.5	27.6	12.9	3.7	ND	—	24.5	13.8
	16	♀	940.0	29.5	12.7	3.8	2.6	—	134.6	19.8
	17	♀	400.2	24.6	13.1	3.6	3.7	—	108.7	29.6
	18	♀	685.0	28.6	14.7	3.3	2.6	—	133.5	31.8
	19	♀	605.0	28.0	17.1	4.6	5.9	—	234.6	46.3
	20	♀	482.0	28.2	14.6	3.7	4.9	—	109.6	35.9
	21	♀	700.0	29.0	13.6	2.9	4.4	—	204.6	23.7
	22	♀	530.9	26.2	18.6	4.2	3.6	—	88.6	27.8
Mean±S.D.			516.5±148.1	26.7±2.1	15.6±2.7	3.7±0.6	3.3±1.9	2.9±0.6	134.7±75.1	26.9±8.7

ND: Not detected (<2 MU/g)

ND value is not included in the mean±S.D.

tanessulfonic acid in 10 mmol/L ammonium phosphate buffer (pH 7.0) was used as a mobile phase at a flow rate of 0.8 mL/min. The eluate from the column was mixed with 4 mol/L NaOH (flow rate 0.8 mL/min) and heated at 110°C for 1.5 min^{3, 4}. The monitoring of fluorescence was carried out at 505 nm with 381 nm excitation. For analysis of PSPs, two mobile phases were used: (I) 2 mmol/L heptanesulfonic acid in 10 mmol/L ammonium phosphate buffer (pH 7.3) for gonyautoxins (GTXs); and (II) 2 mmol/L heptanesulfonic acid in 4% acetonitrile–30 mmol/L ammonium phosphate buffer (pH 7.3) for saxitoxins (STXs)^{5, 6}. The eluate from the column was continuously mixed with 50 mmol/L periodic acid (flow rate 0.4 mL/min) and with 0.2 mol/L KOH containing 1 mol/L ammonium formate and 50% formamide (flow rate 0.4 mL/min), and heated at 65°C for 1.5 min⁷. The fluorophors formed were monitored at 392 nm with 336 nm excitation. Toxin was identified from

the retention times of the standard toxins purified from "hachinojifugu".

Results and Discussion

Epidemic investigation

A food poisoning incident due to ingestion of roe of a marine puffer *T. oblongus* occurred at Kutubdia, Cox's Bazar district, Bangladesh on November 16, 1998. A total of 8 victims including 5 deaths were reported. Symptoms of the victims were dyspnea, numbness of the lips, paralysis, and stomachache followed by vomiting. A villager bought some roe (roughly 120 g) of a *T. oblongus* puffer fish from a local market, cooked it and ingested most of it together with his family members. The symptoms appeared two hours after ingestion and developed. Two victims became unconscious within five to six hours. Since the victims had no previous experience of this problem, the incident was not immediately recognized as a poisoning. Later,

Table 2. Toxicity of *Takifugu oblongus* ("Regular Samples") from Bangladesh

Place of collection	Month of collection	Specimens tested	Average body weight (g)	Average body length (cm)	Toxicity (MU/g)				
					Skin	Muscle	Liver	Gonad	Viscera
Cox's Bazar	August '97	4	328.0	23.0	5.7	3.1	ND	7.2	49.3
		12	267.5	22.0	2.9	2.5	2.6	2.9	20.6
		7	297.8	22.5	4.8	2.9	2.8	3.4	14.7
		Mean $\pm$ S.D.	(297.8 $\pm$ 30.3)	(22.5 $\pm$ 0.5)	(4.5 $\pm$ 1.4)	(2.8 $\pm$ 0.3)	(2.7 $\pm$ 0.1)	(4.5 $\pm$ 2.4)	(28.2 $\pm$ 18.5)
	September '97	5	265.0	21.8	3.9	4.0	2.6	5.1	17.5
		8	298.7	22.5	5.4	5.1	2.6	2.6	19.2
		6	325.0	23.2	2.7	4.7	2.9	2.1	8.2
		Mean $\pm$ S.D.	(296.2 $\pm$ 30.1)	(22.5 $\pm$ 0.7)	(4.0 $\pm$ 1.4)	(4.6 $\pm$ 0.6)	(2.7 $\pm$ 0.2)	(3.3 $\pm$ 1.6)	(15.0 $\pm$ 5.9)
	February '98	9	224.8	21.8	5.3	4.8	2.0	3.8	7.9
		5	271.4	22.8	6.9	5.4	2.4	2.9	25.3
		3	210.8	20.3	5.9	6.7	3.4	2.8	6.8
		Mean $\pm$ S.D.	(235.1 $\pm$ 31.7)	(21.6 $\pm$ 1.3)	(6.0 $\pm$ 0.8)	(5.6 $\pm$ 1.0)	(2.6 $\pm$ 0.7)	(3.2 $\pm$ 0.6)	(13.3 $\pm$ 10.4)
	November '98	6	220.6	21.1	6.8	4.7	2.2	3.3	4.8
		8	234.9	22.7	5.2	3.9	2.7	2.8	6.6
		5	287.5	22.9	4.9	6.2	2.4	2.6	12.4
		Mean $\pm$ S.D.	(247.7 $\pm$ 35.2)	(22.2 $\pm$ 1.0)	(5.6 $\pm$ 1.0)	(4.9 $\pm$ 1.2)	(2.4 $\pm$ 0.3)	(2.9 $\pm$ 0.4)	(7.9 $\pm$ 4.0)
Khulna	August '97	5	225.7	22.8	5.3	4.9	3.9	3.9	6.9
		2	234.9	23.5	4.9	4.8	2.6	2.8	9.7
		6	217.9	21.8	6.5	4.2	2.5	2.9	19.8
		Mean $\pm$ S.D.	(226.2 $\pm$ 8.5)	(22.7 $\pm$ 0.9)	(5.6 $\pm$ 0.8)	(4.6 $\pm$ 0.4)	(3.0 $\pm$ 0.8)	(3.2 $\pm$ 0.6)	(12.1 $\pm$ 6.7)
	September '97	7	221.8	22.1	5.9	4.6	2.9	2.8	17.4
		2	198.8	20.1	5.4	4.2	2.5	2.5	22.9
		3	245.9	21.1	5.8	4.9	ND	2.1	17.2
		4	276.4	22.7	5.2	4.2	2.6	2.8	11.7
	February '98	Mean $\pm$ S.D.	(235.7 $\pm$ 33.2)	(21.5 $\pm$ 1.1)	(5.5 $\pm$ 0.3)	(4.5 $\pm$ 0.3)	(2.7 $\pm$ 0.3)	(2.6 $\pm$ 0.4)	(17.3 $\pm$ 4.6)
		8	239.7	22.3	5.4	4.1	3.9	2.0	6.9
		2	217.9	20.6	4.9	3.9	3.1	3.1	12.1
		5	256.8	23.2	6.4	4.1	2.9	2.9	11.8
		4	228.6	21.1	7.1	4.4	2.7	2.4	12.8
		Mean $\pm$ S.D.	(235.8 $\pm$ 16.6)	(21.8 $\pm$ 1.2)	(6.0 $\pm$ 1.0)	(4.1 $\pm$ 0.2)	(3.2 $\pm$ 0.5)	(2.6 $\pm$ 0.5)	(10.9 $\pm$ 2.7)

ND: Not detected (< 2 MU/g)

ND value is not included in the mean $\pm$ S.D.

Table 3. Toxicity of *Lagocephalus wheeleri* from Bangladesh

Place of collection	Month of collection	Specimens tested	Average body weight (g)	Average body length (cm)	Toxicity (MU/g)			
					Skin	Muscle	Liver	Viscera
Cox's Bazar	August '97	7	81.5	15.5	3.3	2.3	ND	ND
		3	25.7	11.0	2.5	2.0	ND	ND
		2	9.8	9.0	2.6	2.1	ND	ND
		Mean±S.D.	(39.0±37.7)	(11.8±3.3)	(2.8±0.4)	(2.1±0.2)		(2.3±0.4)
	September '97	4	78.7	15.0	2.7	2.0	ND	ND
		2	81.5	14.7	2.8	3.4	ND	ND
		5	19.6	9.2	2.9	2.8	ND	ND
		4	41.5	10.5	3.0	2.1	ND	ND
	February '98	Mean±S.D.	(55.3±30.0)	(12.4±3.0)	(2.9±0.1)	(2.6±0.7)		(2.3±0.3)
		4	45.8	10.7	2.7	2.1	2.0	2.1
		2	34.1	9.4	2.3	2.1	ND	ND
		3	39.6	10.1	2.1	2.7	ND	2.9
Khulna	August '97	Mean±S.D.	(39.8±5.6)	(10.1±0.7)	(2.4±0.3)	(2.3±0.3)		(2.5±0.6)
		4	26.8	12.7	2.8	2.4	ND	ND
		6	25.6	12.8	2.5	2.6	ND	ND
		3	23.9	11.2	3.8	2.4	ND	ND
	September '97	Mean±S.D.	(25.4±1.5)	(12.2±0.9)	(3.0±0.7)	(2.5±0.1)		
		4	23.9	10.4	2.8	2.8	ND	ND
		3	19.5	10.3	3.9	2.2	ND	ND
		2	43.1	11.8	3.2	2.4	ND	ND
	February '98	5	26.9	11.1	3.1	2.4	ND	2.2
		Mean±S.D.	(28.4±10.3)	(10.9±0.7)	(3.3±0.5)	(2.5±0.3)		(2.6±0.4)
		4	67.4	14.9	2.3	2.8	ND	ND
		2	23.7	10.2	2.7	2.5	ND	ND
	September '97	3	32.9	10.8	2.4	ND	ND	ND
		Mean±S.D.	(41.3±23.0)	(12.0±2.6)	(2.6±0.2)	(2.5±0.2)		(2.6±0.4)
		3	19.6	9.8	2.7	ND	ND	ND
		2	56.9	12.5	2.5	2.1	ND	ND
	February '98	3	34.8	11.2	2.5	2.2	ND	ND
		6	75.8	15.9	3.7	2.8	2.7	2.1
		Mean±S.D.	(46.7±24.7)	(12.0±2.6)	(2.5±0.2)	(2.4±0.4)		

ND: Not detected (<2 MU/g)

ND value is not included in the mean±S.D.

Table 4. Toxicity of *Lagocephalus lunaris* from Cox's Bazar, Bangladesh

Month of collection	Specimens tested	Average total length (cm)	Average body weight (g)	Toxicity (MU/g)				
				Skin	Muscle	Liver	Gonad	Viscera
August '97	11	11.8	62.9	2.9	3.2	ND	2.3	2.2
	9	10.1	41.3	2.4	2.4	ND	ND	ND
	5	9.3	28.2	2.1	2.1	ND	ND	ND
	4	9.0	30.3	2.9	2.9	ND	ND	ND
	5	10.7	17.4	2.6	3.2	ND	ND	ND
	Mean±S.D.	(10.2±1.1)	(36.0±17.3)	(2.6±0.3)	(2.8±0.5)			
September '97	8	8.1	18.0	3.1	3.1	ND	ND	ND
	4	8.6	29.9	2.8	3.4	ND	ND	ND
	9	9.3	35.3	2.9	2.9	ND	2.1	2.5
	4	10.1	51.3	3.0	3.6	ND	ND	ND
	10	9.4	35.3	3.2	3.1	ND	2.4	2.1
	Mean±S.D.	(9.1±0.8)	(34.0±12.0)	(3.0±0.2)	(3.2±0.3)		(2.2±0.2)	(2.3±0.3)
February '98	7	7.9	17.9	2.9	3.2	ND	ND	ND
	8	8.3	28.3	3.2	3.2	ND	2.7	ND
	11	8.0	18.1	2.6	2.9	ND	ND	ND
	5	9.1	29.8	3.5	2.8	ND	ND	ND
	Mean±S.D.	(8.3±0.5)	(23.5±6.4)	(3.1±0.4)	(3.0±0.2)			
November '98	8	6.6	17.0	2.5	2.2	ND	ND	ND
	5	5.9	16.9	3.2	2.8	ND	ND	2.2
	11	7.6	17.1	2.9	2.6	2.3	ND	ND
	Mean±S.D.	(6.7±0.9)	(17.0±0.1)	(3.0±0.2)	(2.5±0.3)			

ND: Not detected (<2 MU/g)

ND value is not included in the mean±S.D.

neighbours took them to a local hospital. On the way to hospital, two patients (age 6 and 10 years) died. The others six were hospitalized. On admission, the body temperature, heart beat, respiratory rate and blood pressure of the five patients were in the ranges of 36 to 37°C, 112 to 116/min, 20 to 22/min and 130 to 160/70 to 110 mmHg, respectively. Three (age 12–40 years) of the six patients became weaker due to continuous vomiting and died in the morning of the following day, while the remaining three (age 37–40 years) were treated with intravenous hydration and gastric lavage after a presumptive diagnosis of tetrodotoxin poisoning. Vomiting and weakness of the extremities of the three patients became less prominent and disappeared within 2 to 5 days after admission with supportive treatment. Five to six days later, they left hospital with no residual symptoms.

Despite the high mortality of tetrodotoxin poisoning, three persons survived probably because of the small amount of toxin ingested and effective medical treatment (stomach evacua-

tion, artificial respiration and so on).

Toxicity of 'poisoning-related samples'

The toxicity scores of 'poisoning-related samples' ranged from 11.8 to 21.3 MU/g in the skin, 2.8 to 4.9 MU/g in the muscle, <2 to 5.9 MU/g in the liver, <2 to 3.6 MU/g in the testis, 24.5 to 323.8 MU/g in the ovary and 12.8 to 46.3 MU/g in the viscera (except liver) (Table 1). The female specimens contained a considerably higher level of toxin in their ovary in comparison with that in the testis of male specimens. The victims of the incident ingested a total of about 120 g of roe, which would be equivalent to 3,000–39,000 MU, if the roe contained the same level of toxicity as that of ovary in the 'poisoning-related samples'. Since the minimum lethal dose (MLD) to humans is estimated to be 10,000 MU, it is likely that the poisoning due to ingestion of roe was caused by high levels of toxin accumulated in the ovary.

Fig. 2. HPLC patterns of toxin of (a) *Takifugu oblongus*, (b) *Lagocephalus wheeleri* and (c) *L. lunaris*, along with (d) authentic TTXs

Toxicity of 'regular samples'

In the case of *T. oblongus*, all the tissues of all batches except for the liver of one batch from Khulna showed toxicity, ranging from 2.0 to 49.3 MU/g (Table 2). Toxicity of the viscera was high among the 5 tissues examined. No clear regional or seasonal variation in toxicity was recognized. These results did not coincide with those of *T. oblongus* from Taiwan, which exhibited much higher toxicity scores in the liver and ovary, ranging from 640 to 1,700 MU/g and 180 to 2,700 MU/g, respectively⁸. *T. oblongus* found in Japan was also identified as a toxic species⁹. When comparing 'regular samples' with the 'poisoning-related samples', a large difference in toxicity, especially in the gonad (the ratio of testis to ovary in the former specimens was more or less similar to that of the

latter ones) was observed, although some of the former specimens were collected from a nearby location at almost the same period (November '98) as the latter specimens. This result might be due to a difference in the maturity of the ovaries, since both the body weight and gonads were much larger in the 'poisoning-related samples'.

As shown in Table 3, *L. wheeleri* collected from the Cox's Bazar and Khulna regions showed very low toxicity, the scores being always under 4.0 MU/g. A small anatomical but no regional or seasonal variation of toxicity was observed. In Japan, 10 MU per 1 g of edible part is regarded as the criterion of acceptability of a puffer fish as food. In this context, the Bangladeshi *L. wheeleri* are acceptable as a food item.

Bioassay results of *L. lunaris* are shown in Table 4. The toxicity scores of liver, gonad and viscera were below 3.0 MU/g. The toxicity appeared to be almost exclusively present in the skin and muscle ranging from 2.1 to 3.5 MU/g and 2.1 to 3.6 MU/g, respectively. These findings partially coincide with those in Taiwan specimens of *L. lunaris* as reported by Hwang *et al.*⁸. In Japan, *L. lunaris* was identified as a toxic species after a food poisoning incident including four fatalities in the Kyushu area in 1959. *L. lunaris* inhabiting the East China Sea was found to be a highly toxic species, some of them showing toxicity scores exceeding 1,000 MU/g in the ovary or liver. To ensure the edibility of Bangladeshi *L. lunaris*, a year-round investigation should be carried out from the viewpoint of public health.

In Bangladesh, poor people are the main consumers of puffer fish. Since they do not have any knowledge of puffer fish toxicity, a baseline survey should be carried out on marine puffers found in Bangladesh to identify toxic species for the safety of consumers.

Toxin composition

The HPLC patterns of the partially purified toxin from the three marine species are shown in Fig. 2. All samples showed a main peak and a small one whose retention times agreed well with those of authentic TTX and anhydro TTX, respectively. A shoulder of 4-*epi* TTX appeared between the peaks of TTX and anhydroTTX in

the case of OTX only. In PSP analysis, the toxin of the above three species did not exhibit any peak corresponding to authentic GTXs and STXs. Based on these results, it was established that the main toxic component of Bangladeshi marine puffers, *T. oblongus*, *L. wheeleri* and *L. lunaris* was TTX.

Thus, it can be concluded that the causative toxin of the poisoning was TTX. To our knowledge, this is the first toxicological report on Bangladeshi marine puffers.

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