

## THE TOXICITY OF FLORIDA GULF PUFFERS, GENUS *SPHOEROIDES*\*

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(Accepted for publication 24 August 1970)

**Abstract**—A survey of toxicity of puffers from the eastern Gulf of Mexico showed *Sphoeroides spengleri* extremely toxic in all parts, *S. nephelus* completely nontoxic, and *S. dorsalis* variably toxic. Separation of the three species may be based on bathymetric occurrence.

### INTRODUCTION

PUFFER poisoning is a well-known form of ichthyosarcotoxism whose effects have been recorded throughout history. It is caused by eating the flesh, viscera, or skin of a toxic puffer. Depending on the species and other factors, the endogenous toxin may be concentrated in the liver, ovaries, musculature, skin, or throughout the entire fish. In general, the occurrence and amount of toxin are related to the reproductive cycle (RUSSELL, 1965).

The chemical formula of puffer poison is probably  $C_{11}H_{17}O_8N_3$  (TSUDA and KAWAMURA, 1952; TSUDA *et al.*, 1964). An identical substance, tarichatoxin, was isolated from the California newt by MOSHER *et al.* (1964). Toxic extracts of puffer can be prepared by aqueous extraction (LALONE *et al.*, 1963) and further purified by an acid-alcohol separation, followed by chromatography (MURTHA, 1960). The pure toxin dissolves only in acidic solutions, and crystals can be isolated by alcohol-water extraction, silicic acid chromatography, electrophoresis, and evaporation (TSUDA and KAWAMURA, 1952; MURTHA, 1960). Tetrodotoxin is a potent neurotoxin and has some anesthetic-like properties (RUSSELL, 1967; MOSHER *et al.*, 1964).

Puffers are mainly tropical fishes, although some species extend into temperate zones. Toxicity has been demonstrated for *Sphoeroides* from Florida east coast waters (LARSON *et al.*, 1959, 1960; LALONE *et al.*, 1963). The skin and viscera of *S. maculatus* (Bloch and Schneider) from Chesapeake Bay, New Jersey, and Long Island Sound were toxic, but the musculature was nontoxic. This puffer can be used commercially after proper removal of toxic portions (YUDKIN, 1944; LYNCH *et al.*, 1967; ROBINSON and SCHWARTZ, 1968). In contrast, the California Gulf puffer, *S. annulatus*, is toxic in all tissues and should never be used for food (GOE and HALSTEAD, 1953).

The three species of *Sphoeroides* encountered in this study can be distinguished as follows: *S. spengleri* (Bloch) is characterized by two distinct caudal bars, one basal and one distal; *S. dorsalis* (Longley) is characterized by a pair of small black fleshy lappets anterior to the dorsal fins; and *S. nephelus* (Goode and Bean), the most distinctively colored of the three, lacks both caudal bars and lappets.

\*Contribution No. 147.

*S. parvus* (Shipp and Yerger) is similar to *S. nephelus* but much smaller (<100 mm standard length) and has a shorter snout. *S. nephelus* and *S. parvus* are generally separated geographically; however, sympatric populations occur in the extreme northern Gulf of Mexico (SHIPP and YERGER, 1969a, b).

#### METHODS AND MATERIALS

Puffers were collected at random by the crew of the R/V *Hernan Cortez* from locations inshore and offshore in the Gulf of Mexico, extending from Panama City to Key West and the Tortugas shrimp grounds (Fig. 1). Additional specimens were provided by shrimp and

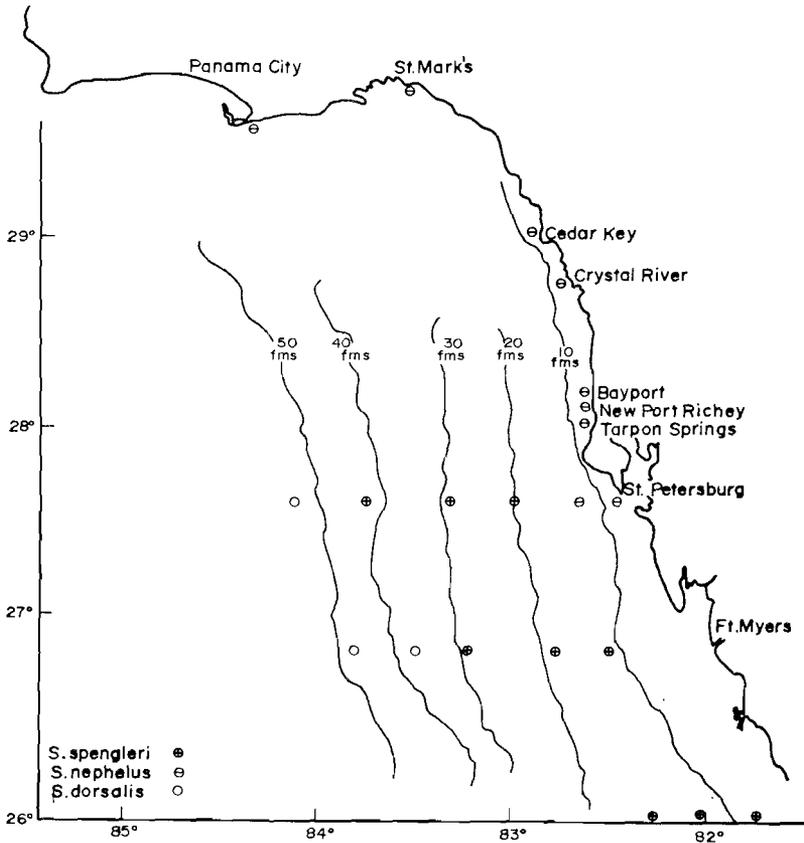


FIG. 1. DISTRIBUTION OF *Spherooides* IN GULF OF MEXICO.

bait fishermen. All fishes were frozen as soon as possible and transported to the Marine Research Laboratory for processing. When the catch was large, only representative samples were analyzed. All fishes were weighed and measured prior to analysis.

A modification of the GOE and HALSTEAD (1953) method was used in the preparation of whole fish or separate tissue extracts. Two ml of distilled water were added for each g of tissue and the mixture was homogenized in a blender at low speed for 5 min. The homogenate was filtered or centrifuged to obtain a clear extract. Skin was cut into small pieces, macerated

in distilled water, and the extract filtered. Toxicity was determined by a mouse test. A white (Swiss-Webster strain) mouse weighing approximately 20 g was injected i.p. with 1 ml of extract and carefully observed for 1 hr for signs and death time. Toxicity was based on a dose-death time relationship for a 20 g mouse. Toxicity was expressed in mouse units (MCFARREN and BARTSCH, 1960) and by a toxicity scale rating developed by this laboratory: TX=4 Death in 30 min or less; TX=3 Death in more than 30 min; TX=2 No death, moderate signs; TX=1 No death, mild signs; TX=0 No signs.

Puffer toxin usually kills mice within 30 min (MCFARREN and BARTSCH, 1960). With high concentrations of toxin, mice may die within 30 sec. Signs develop rapidly and consist of muscle weakness, paralysis, respiratory failure, and convulsions (KAO and FUHRMAN, 1963).

## RESULTS

Three species of puffers were analyzed: *Sphoeroides spengleri*, *S. nephelus*, and *S. dorsalis*.

### *Sphoeroides spengleri*

A total of 38 fish were processed from catches of this species. Tissue extracts of skin, muscle, and viscera were prepared from 29 specimens. Whole fish extracts were prepared from the remaining nine.

Three of the 29 fish used for skin, muscle, and viscera extracts were collected in June 1967 (27°42'N, 84°10'W). One skin and one viscera extract were slightly toxic and one viscera extract was extremely toxic. All three muscle extracts were nontoxic. Of the nine whole fish extracts from the same vicinity (July 1967), two were extremely toxic, five produced mild to moderate signs followed by recovery, and two extracts from very small fish (less than 5 cm) were nontoxic (Table 1). In August 1967, 20 fish from Key West were analyzed. All extracts were extremely toxic (TX=4) with mouse units ranging from 0.86 to 6.28 (Table 1). Six fish were analyzed from catches at Tortugas shrimp grounds in July 1968. Five skin, muscle and viscera extracts were extremely toxic (TX=4) with mouse units ranging from 1.86 to 1.91. One small fish was nontoxic in all three extracts (Table 1).

TABLE 1. TOXICITY OF *S. spengleri* EXTRACTS TO MICE

Location	Date	No. fish	Extract	No. toxic	Toxicity level	Mouse units (per 100 g)
27°42'N, 84°10'W	6-67	3	3 Skin	1	1	*
			3 Muscle	None	0	None
			3 Viscera	2	4,1	1.45*
27°42'N, 84°09'W	7-67	9	9 Whole fish	7†	4, 2, 1	1.12, 1.18
Off Key West	8-67	20	20 Skin	20	4	0.86-6.28
			20 Muscle	20	4	
			20 Viscera	20	4	
Tortugas shrimp grounds	7-68	6	6 Skin	5†	4	1.01-1.86
			6 Muscle	5†	4	
			6 Viscera	5†	4	

\*Undetermined.

†Negative extracts from extremely small fish.

*Sphoeroides nephelus*

Tissue extracts of skin, muscle, and viscera were prepared from 45 of the 61 fish analyzed. Whole fish extracts were prepared from the remaining 16 fish. Collection sites were predominantly inshore from Panama City to off St. Petersburg Beach, Florida (Fig. 1). No tissue extracts were toxic (Table 2).

TABLE 2. TOXICITY OF *S. nephelus* EXTRACTS TO MICE

Location	Date	No. fish	Extract	Toxicity
27°52'N, 83°11'W	5.67	1	1 Whole fish	None
	8.67	4	4 Skin, muscle and viscera	None
Ozona	8.67	7	6 Whole fish, 1 skin, muscle and viscera	None
Skyway Br. channel	8.67	1	1 Whole fish	None
New Port Richey	3.68	12	12 Skin, muscle and viscera	None
Tarpon Springs	4.68	18	4 Whole fish, 14 skin, muscle and viscera	None
St. Marks	4.68	6	3 Whole fish, 3 skin, muscle and viscera	None
Panama City	4.68	3	3 Skin, muscle and viscera	None
Cedar Key	4.68	3	3 Skin, muscle and viscera	None
Crystal River	5.68	1	1 Whole fish	None
	6.68	5	5 Skin, muscle and viscera	None

*Sphoeroides dorsalis*

Tissue extracts of skin, muscle, and viscera were prepared from three of the 12 fish collected. Whole fish extracts were prepared from the remaining nine (Table 3). Two skin, muscle, and viscera extracts (June 1967) produced no signs of poisoning. However, one specimen analyzed from a nearby location in August 1967 showed toxicity in skin and viscera portions (TX=4 toxicity rating and 2.08 mu from the skin, 3.04 mu from the viscera). Of the nine whole fish extracts (May 1967) eight showed no toxicity, while one was rated at TX=4 with 4.44 mu.

TABLE 3. TOXICITY OF *S. dorsalis* EXTRACTS TO MICE

Location	Date	No. fish	Extract	No. toxic	Toxicity level	Mouse units (per 100 g)
27°42'N, 84°10'W	6.67	2	2 Skin	None	0	None
			2 Muscle	None	0	None
			2 Viscera	None	0	None
27°48'N, 84°09'W	5.67	9	9 Whole fish	1	4	4.44
84°09'W	7.67	1	1 Skin	1	4	2.08
			1 Muscle	None	0	None
			1 Viscera	1	4	3.04

## DISCUSSION

*Distribution*

SHIPP and YERGER (1969b) report *S. nephelus* as occurring along the Florida east coast and throughout the Caribbean and eastern Gulf of Mexico. Because of their morphological similarities, the southern puffer, *S. nephelus*, and the northern puffer, *S. maculatus*, have often been confused. SHIPP and YERGER (1969b) attempted to clarify their taxonomic and distributional separation; however, the identification of these species in areas of overlap is still open to question.

*S. spengleri* is reported from the tropical Atlantic Ocean, Gulf of Mexico, west coast of Africa and the West Indies (HALSTEAD, 1967). Usually, *S. spengleri* and *S. nephelus* are allopatric but occasionally these species may be found in the same area, in which case the two can be readily separated morphologically.

*S. dorsalis* is reported from the Bahamas to Surinam and the Gulf of Mexico (BÖHLKE and CHAPLIN, 1968).

Our data and supplemental Laboratory data (JOYCE and WILLIAMS, 1969) indicate that the three species of *Spherooides* can be separated bathymetrically: *S. nephelus*, 10 fathoms or less; *S. spengleri*, 10–30 fathoms; and *S. dorsalis*, 30–50 fathoms.

*Toxicity*

Our data indicates that *S. nephelus* should be considered a nontoxic species; however, toxicity has been reported for *S. nephelus* collected on the east coast of Florida (Edward Larson, personal communication). This disagreement in species toxicity may arise from geographical differences, or from taxonomic uncertainties within the genus. Prior to SHIPP and YERGER's (1969b) work, the east coast species in question was identified as *S. maculatus*, a toxic species (HALSTEAD, 1967). Consequently, Larson's work on the toxicity of this species in Florida was in agreement with that of previous workers. Shipp and Yerger, however, felt that the species had been misidentified and was in reality *S. nephelus*, with *S. maculatus* extending only as far south as Jacksonville, Florida. The apparent disagreement in toxicity between east coast and Gulf coast populations therefore suggests a differential toxicity between populations but further consideration should be given, both from the standpoint of systematics and toxicology.

All parts of *S. spengleri* tested were toxic. No problem thus far exists in the identification of this species nor is its toxicity questionable.

The third species, *S. dorsalis*, yielded diverse results. Some parts were toxic at different times while some fish showed variations in levels of toxicity.

Since a correlation between toxicity and reproductive cycle has been established for some puffers (YUDKIN, 1944), maturation studies of males and females might prove valuable. A species may be toxic in one area and not another, even in the same season, and the toxin can be transvectored from one fish to the next (HALSTEAD, 1967). Factors other than reproduction, such as food source, seasonality of food supply, and feeding habitat may play a role in the uptake and production of the poison. Further studies are needed to fully understand the biogenesis of tetrodotoxin.

*Acknowledgements*—We wish to acknowledge the assistance of the staff editorial board in reviewing this paper. In addition, we sincerely thank Mr. R. M. INGLE, Chief, Bureau of Marine Science and Technology, for his encouragement and direction.

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