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First report on toxicity assessment of the Lessepsian migrant pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) from European waters (Aegean Sea, Greece)

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ABSTRACT

According to the current European Union legislative requirements (Regulation 853/2004/EC; Regulation 854/2004/EC, poisonous fish of the family Tetraodontidae and products derived from them must not be placed on the European markets. Following the increased publicity regarding the presence of the pufferfish species *Lagocephalus sceleratus* in Greek waters, this study was undertaken in order to confirm its toxicity and assess the risk of poisoning in case of accidental consumption. Acidic extracts from tissues of *L. sceleratus* specimens of different sizes were examined by means of the official mouse bioassay for tetrodotoxin, while some of the extracts were also tested for the presence of Paralytic Shellfish Poisoning (PSP) toxins with a commercial ELISA kit. Toxicity in mice, with symptomatology indicative of tetrodotoxin, was confirmed in a number of samples and indicated a correlation with fish size. Toxicity of certain tissues (liver, gonads, gastrointestinal tract) in larger individuals, expressed as μg/g tetrodotoxin equivalents, was largely above levels required to cause death in human adults. On the other hand, all tested extracts provided a positive reaction in the ELISA test for PSP toxins. This constitutes the first report for presence of toxicity in *L. sceleratus* caught in European coastal waters.

1. Introduction

With the opening of the Suez Canal in 1869, two markedly different zoogeographical areas were joined: the subtropical Mediterranean Sea, which connects with the Atlantic, and the tropical Red Sea, the most northern extension of the Indian Ocean. In order to pass between these areas, organisms must be able to bridge the difference in adaptive requirements, and also withstand the extreme conditions in the Canal itself (Papaconstantinou, 1990). In this context, the term "Lessepsian migration" has been introduced to characterize a new phenomenon of unidirectional and successful biotic advance from the Red Sea to the Eastern Mediterranean, while the term "Lessepsian migrant" refers to the Red Sea species that have passed through the Suez Canal and settled in the Eastern Mediterranean (Por, 1978).

The Lessepsian migrant *Lagocephalus sceleratus* (Gmelin, 1789), also known as silverstripe blaasop, is an Indo-Pacific

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originated pufferfish of the family Tetraodontidae. Similar to its congeneric tropical species, *L. sceleratus* maybe a source of food poisoning with a high associated risk of mortality, as it commonly contains tetrodotoxin (TTX), a toxin which can cause death by muscular paralysis, respiratory depression and circulatory failure (Field, 1998; Bilecenoglu et al., 2006). The minimum lethal dose and minimum acute dose of TTX to human (wt. 50 kg) are estimated to be around 2 mg and 0.2 mg, respectively. Depending upon the amount of the toxin ingested, symptoms usually appear within 10–45 min of exposure, though some cases have reported being asymptomatic until as much as 3–6 h after exposure. Oral paresthesia is usually the initial symptom, which gradually spreads to the extremities and trunk. Other early symptoms include taste disturbance, dizziness, headache, diaphoresis, and pupillary constriction. These may or may not be accompanied by gastrointestinal symptoms of salivation, hypersalivation, nausea, vomiting, hyperemesis, hematemesis, hypermotility, diarrhea, and abdominal pain (Noguchi and Ebesu, 2001). On the other hand, several species of pufferfish, mostly freshwater ones, have been reported to also contain Paralytic Shellfish Poisoning (PSP) toxins and mainly saxitoxin (Nakamura et al., 1984; Sato et al., 2000), which cause to humans a very similar symptomatology to that of TTX.

L. sceleratus was first collected in the Mediterranean Sea on February 2003 from Gökova Bay (southern Aegean Sea, Turkey) (Akyol et al., 2005), and on November 2004 from Jaffa along the Israeli coast (Golani and Levy, 2005). In Greek waters, *L. sceleratus* was first recorded from the Cretan Sea (Aegean Sea) in July 2005 (Kasapidis et al., 2007). Since then, *L. sceleratus* has been recorded with an increasing frequency, in many areas of Aegean Sea, Greece and for this reason it is considered as one of the faster expanding Lessepsian immigrants. This fast expansion rate indicates a better ability to adapt to different environmental conditions and may also affect diversity and/or abundance of native species in the near future (Peristeraki et al., 2006). On the other hand, this increased reporting could also be attributed to more public awareness and provision of relevant information by the competent authorities, as *L. sceleratus* occurrence has been a major issue in the Greek press since the beginning of 2007. The collection of numerous juvenile *L. sceleratus* fish, together with picarel, bogue and smelt in islands of the Southeast Aegean Sea, which resulted in confusion to both fishermen and consumers was one of the most important incidents reported in the press in October 2007. This increased publicity has resulted in familiarization of the fishermen with the species' characteristics, and subsequent contribution to over-reporting, since all specimens are being delivered to local authorities. It is also interesting to point out the fact that a wide range of sizes of *L. sceleratus* fish are caught, which is evidence that the species is now well established and reproducing in the Aegean Sea.

According to the current European legislative requirements (Regulation 853/2004/EC; Regulation 854/2004/EC), poisonous fish of the family Tetraodontidae and products derived from them must not be placed on the market. Despite this fact, however, one cannot exclude the possibility for accidental consumption of the species, as in a recent similar case in the Eastern Mediterranean (Bentur et al., 2008). The increased incidence of *L. sceleratus* in Greek waters together with the publicity and consumer concern related to this species, triggered off the investigation with regard to toxicity of the species, taking also into consideration the size of the fish, in order to assess the potential risk of poisoning in case the species was accidentally consumed. To the best of our knowledge, this is the first report of *L. sceleratus* toxicity investigation in European waters.

2. Materials and methods

2.1. Fish collection, identification and measurements

Specimens no. 1–4 and 6 (see Table 1) of the pufferfish *L. sceleratus* were collected by trawl fishing in the Southeast Aegean Sea, near the island of Rhodes, in depths between 27 and 36 m during October–December 2007. Specimen no. 5 was caught in the North West part of Aegean Sea in the area of Horeyto, Pelion in June 2007. All fish were submitted by fishermen to the relevant authorities for identification. Fish were identified as *L. sceleratus* by an expert marine biologist according to the characteristics described in the FAO Species Identification Sheets for Fishery Purposes (FAO, 1984), the morphological characteristics reported by Akyol et al. (2005) and with the aid of fish guide pictures. Measurements of total length and weight, or average weight in the case of group samples, were also conducted. After identification and conduction of measurements, fish were frozen and transferred to the National Reference Laboratory on Marine Biotoxins (NRLMB). Samples were kept frozen at −70 °C until analysed.

2.2. Sample preparation – toxin extraction

Toxicity of the different organs obtained from individual fish was determined according to the Japanese official method for tetrodotoxin in puffer (Kawabata, 1978b). Each specimen (individual or group of fish) was partially thawed, in order to avoid migration of toxin between tissues, and subsequently dissected into the muscle, liver, skin, gonads (when available) and gastrointestinal tract. A portion of 10 g from each organ was extracted individually with 25 ml of 0.1% acetic acid (Riedel de Haen, Sigma–Aldrich, Seelze, Germany) by heating for 10 min in a boiling water bath with occasional stirring. The mixture was cooled down and then filtered. The residue on filter paper was washed with portions of 0.1% acetic acid and the filtrate and washings were combined and made up to 50 ml with the same

Table 1
Sampling details and weight and length measurements of the experimental fish.

Specimen	Collection depth (m)	Collection date	No. of individuals	Average weight (g)	Length range (cm)
1	30	10/10/2007	15	5	5–10
2	36	3/12/2007	14	40	12–16.4
3	27	22/11/2007	11	78	16–20
4	27	22/11/2007	1	1060	43
5	nr	20/6/2007	1	1200	49.5
6	36	3/12/2007	1	3786	66

nr = Not reported.