



# CHANGES OF SEXUAL AND SEASONAL VARIATION OF TETRODOTOXIN (TTX) LEVELS IN PUFFER FISH (*Lagocephalus sceleratus*) CAUGHT FROM MERSIN GULF



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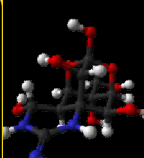
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## ABSTRACT

In this study, changes of sexual and seasonal variation of TTX levels of puffer fish (*Lagocephalus sceleratus*) caught from Mersin Gulf during the period of December 2012 and November 2013 were investigated. Changes in the levels of tetrodotoxin (TTX) of gonads, liver and muscle depending on season and sex were also investigated. The samples used seasonally were caught by bottom trawl, longline and fishing line. Acidic extracts from tissues of *L. sceleratus* specimens of different sizes were examined by means of the official mouse bioassay for tetrodotoxin. Toxicity in mice, with symptomatology indicative of tetrodotoxin, was confirmed in a number of samples and indicated a correlation with season and sex. The highest TTX levels in female fish were detected in their gonads in the winter season following fall seasons. In the male gonads, TTX levels were higher than 10 MU/g only in the autumn. TTX levels were higher than 10 MU/g in liver of winter and summer female individuals. Although muscle extract of puffer fish given mice has shown poisoning symptoms of TTX, death of mice was not observed and TTX level was less than 5 MU/g.

## INTRODUCTION

Alien marine species into the Mediterranean through the Suez Canal migration is observed. Some alien species have economic value. But some species are harmful on ecosystem, native species and human health especially in the eastern Mediterranean (Galil and Zenetos, 2002). Pufferfish regarded as one of the "worst alien fishes" of the entire Mediterranean Sea (Bilecenoglu, 2010). The puffer fishes are commonly known of all type of fish poisoning and has been recognized from ancient times (Aydın, 2010). Most members of the family include the Tetrodotoxin (TTX). However In the Far East, this fish is considered a delicate fish, especially in Japan where it is reared by experts (Kheifets et al., 2012).



Tetrodotoxin is considered the most lethal toxin in the marine toxins. TTX is responsible for human intoxications and fatalities. Its usual route of toxicity is via the ingestion of contaminated puffer fish which are a culinary delicacy, especially in Japan (Bane et al., 2014). TTX was believed to be confined to regions of South East Asia, but recent studies have demonstrated that the toxin has spread to regions in the Pacific and the Mediterranean. There is no known antidote to TTX which is a powerful sodium channel inhibitor. (Fernández-Ortega et al., 2010 ; Bane et al., 2014)

The Silverstripe Blaasop *Lagocephalus sceleratus* (Gmelin 1789) is a fish species of the Tetraodontidae family. *L. sceleratus* in terms of ecological, economic and social effects clearly a harmful in the Mediterranean (Kaligori, 2011).

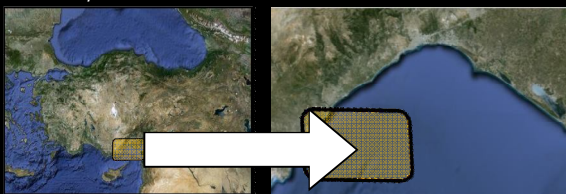
The present study is the first study on the toxicity of pufferfish in Turkey. Aim of this study, determined to changes of sexual and seasonal variation of TTX levels of puffer fish (*Lagocephalus sceleratus*) caught from Mersin Gulf.



## MATERIAL AND METHOD

### FISH COLLECTION, IDENTIFICATION AND MEASUREMENTS

Samples of the pufferfish *L. sceleratus* were collected by trawl fishing, longline and fishing line, in the Northeast Mediterranean, Gulf of Mersin, during December 2012–October 2013. All fish were submitted by fishermen to the relevant authorities for identification. Measurements of total length and weight. The weight and length was measured between 43-70 cm and between 947.26-4128.17 g, respectively. After identification and conduction of measurements, fish were frozen and transferred to the Cukurova University Faculty of Fisheries, Department of Seafood Processing Technology. Samples were kept frozen at -20 °C until analysed.



### MOUSE BIOASSAY

Mouse Bioassay (MBA) were performed at DETAUM, Cukurova University. Use 4-week old, 19-21 g, Swiss albino, male mice. In a preliminary assay, 1 ml of the test solution is injected i.p. into each of 2 mice. Determine the survival time to a second and calculate Mouse Unit (MU) from the Table for dose-survival time relationship (Kawabata, 1978). To produce good results, the survival time should be around 10 min. If the survival time is much shorter than 10 min, appropriate dilution of the test solution is necessary to bring down the survival time to around 10 min. Use the Table for calculating the dilution. Inject the diluted solution into two mice. If the survival time falls between 7 and 13 min, add 1-3 mice for assaying. Determine the mean survival time (not the average time) obtained on 3-5 mice, including mice that did not die. Read MU from the observed mean time. Make calibration from the second Table, if mice weighing below 19 g or over 21 g were used. One MU is equivalent to 0.22 µg of tetrodotoxin. Symptoms and signs produced in mice by tetrodotoxin is very similar to those by saxitoxin. Survival time is determined by the arrest of breathing, not by arrest of heart.



## RESULTS AND DISCUSSION

- Results of the toxicological analyses by MBA are presented in Table 1. The limit of detection of the MBA method was 5 MU TTX eq/g (or 1.10 µg TTX eq/g) for muscle, 10 MU TTX eq/g (or 2.20 µg TTX eq/g) for gonads and livers.
- The highest level of TTX in female fish gonads were detected in winter. This is followed by autumn, summer and winter respectively.
- In the male gonads, TTX levels were higher than 10 MU/g only in the autumn.
- TTX levels were higher than 10 MU/g in liver of winter and summer female individuals.
- Although muscle extract of puffer fish given mice has shown poisoning symptoms of TTX, death of mice was not observed and TTX level was less than 5 MU/g.
- Many scientists have reported that maximum TTX levels of puffer fish in the late spring and summer, reproduction season, however minimum toxicity level in autumn and winter seasons.
- Our study showed, maximum TTX levels of puffer fish in the between autumn and winter seasons. Similarly Katikou (2009) and Roriguez (2012) reported TTX levels were higher in autumn and winter seasons by using *L. sceleratus* caught in the Mediterranean.
- This case may be a situation typical of the Mediterranean and Aegean.

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