



POSTER

FOOD SAFETY CONCERNS ASSOCIATED WITH THE INVASIVE PUFFERFISH (LAGOCEPHALUS SCLERATUS): MULTI-ANALOGUE TETRODOTOXIN PROFILING AND RISK ASSESSMENT

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Aim. Considering the increasing spread of *Lagocephalus scleratus* in the Mediterranean Sea and the associated food safety concerns related to tetrodotoxin (TTX), this study aimed to characterize the tissue distribution of TTX and its analogues in Mediterranean populations of *L. scleratus*, evaluate differences related to tissue, sex and sampling period, compare the obtained results with previous Mediterranean studies (Greece, Spain, Turkey) and perform a risk assessment based on TTX and selected analogues using the relative potency factors proposed by EFSA.

Methods. Samples were collected from offshore fishing grounds and coastal waters around Lampedusa Island by ISPRA and CNR-IRBIM, in collaboration with ALIENFish. Liver, gonads, skin and muscle tissues were dissected and analyzed for the determination of TTX and 4-epiTTX, while the naturally occurring analogues 11-norTTX-6(S)-ol, 4,9-anhydroTTX, 5-/11-deoxyTTX, 5,11-/6,11-dideoxyTTX, 5,6,11-trideoxyTTX, 4,9-/4,4a-anhydro-5,6,11-trideoxyTTX were relatively quantified using the TTX calibration curve. Toxins were extracted using a simple acidified methanol extraction procedure followed by a defatting step prior to High Performance Liquid Chromatography-High Resolution Mass Spectrometry (HPLC-HRMS) analysis.

Results. TTX and its analogues were detected in all analyzed samples, showing a clear tissue-dependent distribution. Gonads exhibited the highest contamination, followed by liver, skin and muscle, confirming the accumulation pat-

tern previously reported for Mediterranean populations. Using the middle-bound (MB) approach, mean concentrations of TTX + 4-epiTTX ranged from $0.51 \pm 0.61 \mu\text{g/g}$ in skin to $7.66 \pm 13.15 \mu\text{g/g}$ in gonads. The most abundant analogues were 4,9-/4,4a-anhydro-5,6,11-trideoxyTTX, predominant in all tissues especially in gonads. Female samples exhibited a higher total toxin burden than males across all tissues, with significantly higher concentrations observed only in the gonads. This trend is consistent with previous studies suggesting that toxin accumulation in females increases during the reproductive period, probably reflecting maternal transfer of TTX to developing oocytes as a chemical defense mechanism for eggs and larvae. Seasonal analysis suggested higher toxin concentrations during winter and early spring than during summer and autumn; however, no statistically significant differences were detected, due to the limited sample size and the marked inter-individual variability. Using the EFSA relative potency factors and the MB approach, the estimated exposure from a 100 g portion of muscle for a 70 kg adult was $1.06 \mu\text{g}$ TTX equivalents/kg body weight, corresponding to a 4.2-fold exceedance of the EFSA group acute reference dose.

Conclusions. These findings indicate that the consumption of *L. scleratus* muscle may pose a health risk under realistic exposure scenarios and reinforce the need to maintain strict control measures, improve species identification, and increase awareness among fishermen and consumers to minimize the risk of accidental intoxication.