



**Biotica
Research
Today**
Vol 3:9 ⁷²⁵
2021 ⁷²⁷

Stress Relieving Tetrodotoxin from Pufferfishes

R. Karthik^{1*}, S. Sudarshan¹ and
S. Aanand²

¹ICAR- Central Institute of Fisheries Education, Versova,
Mumbai, Maharashtra (400 061), India

²Erode Centre for Sustainable Aquaculture, Bhavanisagar,
Erode, Tamil Nadu (638 451), India



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Corresponding Author

Sibte Sayyeda

e-mail: karthik.fpbma802@cife.edu.in

Keywords

CRH-ACTH-cortisol axis, Pufferfishes, Stress, Tetrodotoxin

Article History

Received in 30th August 2021

Received in revised form 04th September 2021

Accepted in final form 05th September 2021

E-mail: bioticapublications@gmail.com

How to cite this article?

Karthik *et al.*, 2021. Stress Relieving Tetrodotoxin from Pufferfishes. *Biotica Research Today* 3(9): 725-727.

Abstract

Pufferfishes occupies its unique place in Japanese seafood restaurants because of its delicious nature. *Takifugu rubripes*, an IUCN near-threatened red-listed species is one of the most preferred species and widely consumed. Due to its high market demand, makes it a highly prized food fish which in turn leads to depletion of wild stocks by overfishing. Aquacultured Tiger pufferfishes meets out the demand to a small extent but still consumer's preference relies on those wild caught ones. Presently, Tiger pufferfish hatcheries faces severe stress related mortalities. This article deals with the aspects that how the Tetrodotoxin, a potent neurotoxin mitigates those stress-related mortalities in Tiger Pufferfishes hatcheries. And also, explains that how understanding on the interactive effects of tetrodotoxin on CRH-ACTH-cortisol axis and its mitigating stress effect can be exploited through further research.

Introduction

Aquaculture finds out to be a better option for the supply of Japanese pufferfish. *Takifugu rubripes*, is an IUCN near-threatened red-listed species. It is also known as the Tiger pufferfish and is one of the highly-prized food fish in Japan. Driven by huge market demand, it is cultured in Japan on a large scale. *Takifugu* culture has been practiced in East Asia since 1960's. In the 1990's, China first developed its puffer aquaculture program (Ma *et al.*, 2011) and by the mid-2000's, Japan surpassed as the primary producer of aquacultured puffer. In the early 2000's, when China first declared pufferfish commodity to the FAO, the volume of aquacultured puffer exceeded the volume of puffer landings declared. It is being commercially cultured in floating cages and on land-based facilities in Japan, China and Thailand. The supply of Tiger pufferfishes through aquaculture reduces the fishing effort and dependence on wild stock to a small extent. Even though farm-reared fishes meets out the demand, there still exists a preference for wild-caught pufferfishes. Presently, the culture of tiger pufferfishes is hindered by some unavoidable stress-related mortalities in the hatcheries. Much research is being undertaken to overcome these issues. This article deals with the aspects that try to mitigate those stress-related mortalities in the Tiger pufferfish juveniles reared in hatcheries.

Why *T. rubripes*, is Red Listed under Near-Threatened Species by IUCN

Previously *T. rubripes* was as an abundant predator of anchovies in the Seto Inland Sea. High fishing in 1980's and jelly fish blooms in 1990's led to the depletion *T. rubripes* stocks. Further, targeted fishing with improved fishing vessels, and fishing gears also carved out the stocks of *T. rubripes*.

According to IUCN, the catch of *Takifugu rubripes* was 4,62,000

million tonnes in 1982, which decreased to 2,65,000 million tonnes in 1993, a 43% decrease in twelve years. *Takifugu rubripes* stock continued to drop into the 2000's despite stabilization in the anchovy population.

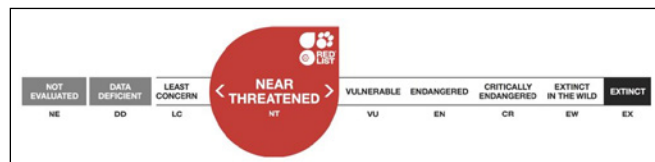


Figure 1: IUCN Red List Category Japanese Pufferfish *T. rubripes* -Near Threatened (Source: Shao et al.,2014)

Sea Ranching for Stock Improvement by the Japanese Government

The Japanese government took huge strides against Tiger pufferfish stock depletion by sea ranching programme. However, the fate of those released hatchery-reared tiger pufferfish juveniles into the sea is vulnerable to predation because of their swimming behaviour and non-toxic nature. The wild pufferfish juveniles escape predation due to their bottom-dwelling nature and tetrodotoxin (TTX) concentrations.



Figure 2: Farm Reared Pufferfish

TTX in Tiger Pufferfishes

Tiger Pufferfishes are well known for their potent neurotoxin, Tetrodotoxin. The degree of toxicity in pufferfishes differs by species, geographical area, and seasons. The wild-caught tiger pufferfishes are toxic as they bioaccumulate tetrodotoxin in their liver and ovaries.

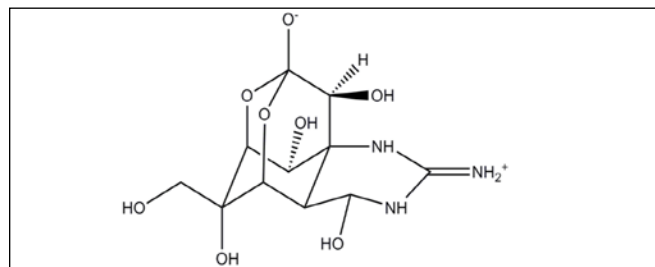


Figure 3: Chemical structure of tetrodotoxin (TTX)

The production of these TTX in the food chain, first starts with some marine bacteria (*Vibrio alginolyticus*, *Vibrio I*, *Vibrio VIII*, *Shewanella alga*, *S. putrefaciens*, *Alteromonas tetraodonis*, etc.) in minimal amounts. From these bacteria, it passes through some carnivores, omnivores, detritus feeders, and scavengers, where they are postulated to be intoxicated with TTX along with parasitism and symbiosis. This mechanism leads to a high accumulation of TTX in fishes like pufferfish occupying the upper level of the food chain.

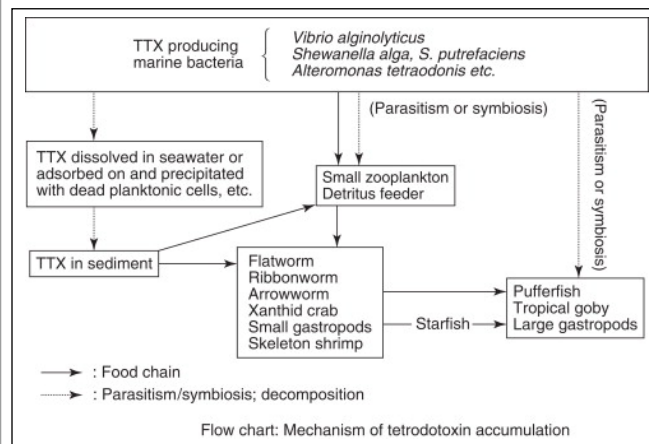


Figure 4: Mechanism of TTX accumulation in marine animals (Source: Noguchi et al., 2006)

Symptoms of TTX Toxicity

TTX is 1,200 times deadlier than cyanide. In humans, the onset and severity of the symptoms of TTX after ingestion is dose dependent (Islam et al., 2011). Initial symptoms include tingling (paresthesias) of the tongue and lips, followed by or concurrent with headache and vomiting, which may progress to muscle weakness and ataxia. In severe cases death may occur due to respiratory and/or heart failure.

Ingestion of TTX containing pufferfishes causes Paralytic shellfish poisoning. The mechanism behind this is that TTX blocks fast voltage-gated sodium channels between the body and brain, required for the standard transmission of signals. As a result, TTX causes loss of sensation and paralysis of voluntary muscles etc.

From Danger to Delicious

Pufferfishes have a long and rich culinary history in East Asian cultures, including China, Korea, and Japan. Out of the 120 species of pufferfishes, only 22 species are approved by the Japanese government for serving in restaurants. Among these approved species, Tiger pufferfish or torafugu is the highly priced ones. It is also a more poisonous one as it contains Tetrodotoxin (TTX) which is 1,200 deadlier than cyanide, and even a single fish has enough poison to kill 30 people. The liver and ovaries are highly toxic, the intestines slightly toxic, whereas flesh, skin, and testes are

innocuous. Because of its high risk, chefs must undergo two to three years of training to obtain a fugu-preparing license regulated through a national chef licensing program in Japan. Such expertise raises the price of a fugu dish to up to \$ 200. Even though, this risk factor has not stopped the Japanese from consuming pufferfishes, which stands at a whopping 10,000 tons of fish every year.

Wild-caught torafugu is often found at high-end restaurants in Japan, where it is served as ideally with paper-thinly sliced sashimi, deep-fried, and even used to make a hot sake called hirezake. Liver from cultured *T. rubripes* are usually non-toxic and can be used as a traditional Japanese food "Fugu-Kimo". *Takifugu rubripes* also finds use in Chinese medicine.

Tetradotoxin as a Stress Reliever

Usually, stress is observed frequently in Tiger pufferfish juveniles reared in hatcheries due to its high individual stocking densities. This stress results in aggressive behaviour of the juveniles, as nipping, causing caudal fin loss and mortalities. Thereby, it affects tiger pufferfish production. Amano *et al.* (2019) conducted an experiment with tiger puffer juveniles fed with a TTX-containing diet (2.35 mouse units (517 ng)/g diet) for 28 days under a recirculating system, and showed that relative CRH mRNA expression levels in the brain and cortisol levels in the plasma were significantly lower in the TTX-treated group when compared to the untreated ones. And concluded that TTX functions as a stress-relieving substance by affecting the CRH-ACTH-cortisol axis and thereby reduces agonistic interactions. Eventually, TTX-treated group showed a significant increase in their length and weight.

Tetrodotoxin Incorporation in Diet

Hatchery reared Tiger pufferfish juveniles fed with commercial feed are non-toxic or low toxic than wild-caught juveniles. This aspect is desirable since the edible part from the fishes is only the muscle portion and not the toxic liver or ovaries. Incorporation of tetradotoxin in the feed leads to accumulation of toxic content only liver and ovaries which are removed prior to cooking. Hence, the final yield will be same as that of the wild caught ones.

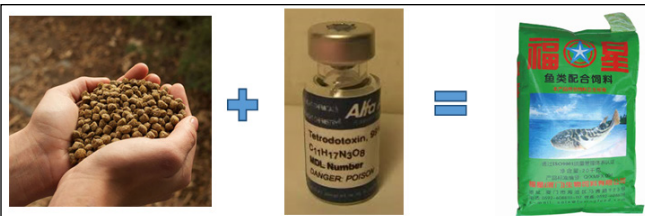


Figure 5: TTX administration in pufferfish feed

Conclusion

Incorporation of tetradotoxin in the diet of the tiger pufferfish juveniles is a desirable one. It relieves stress and reduces mortalities in hatcheries. Tetradotoxin also improves survival of juveniles during sea ranching as it enables them to escape predation. Understanding, the interactive effects of tetradotoxin on CRH-ACTH-cortisol axis and its mitigating stress effect has to be exploited through further research. This understanding can be used as an effective stress reliever not only in pufferfishes but can also be extended to other fishes.

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