Biology, ecology and therapies of puffer fish (Tetraodontiformes: Tetraodontidae).

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1. INTRODUCTION

The term Pufferfish reflects all species that can inflate their bodies when they feel threatened by a predator. The pufferfish is a fish in the phylum Chordata of the class Actinopterygii, which includes ray-finned fish. This unique creature is categorized in the order Tetraodontiformes and the four-toothed family Tetraodontidae, native to the western Atlantic and African coasts from Mauritania to Namibia. It reaches approximately 60 cm in length. They live in coastal regions, at shallow depths, and in estuaries, preferring substrates with sand and mud. Adults have pelagic habits (Figure 1) [1-2].

Figure 1. Pufferfish species - macaw *Lagocephalus laevigatus* (Linnaeus, 1766) (Tetraodontiformes: Tetraodontidae). Source: Photo: Reproduction/G1/João Luiz Gasparini.

With so much diversity among the group, these fish range in size anywhere from the dwarf or pygmy pufferfish to the giant freshwater pufferfish that grows up to 1 m in length. On the other hand, the body of these fish is elongated, with the head thicker and more voluminous than the rest of the body, and each species of pufferfish has assorted colors and designs. Furthermore, the pufferfish's eyes move independently of each other. The fish have a prominent beak with four teeth that allow them to eat algae, crustaceans, mollusks, and red worms (Figure 2) [2-3].

Figure 2. In addition to inflating their bodies, some species have spines and even poison to protect themselves from predators. Source: Photo: Luciana Soldi Bullara / Flickr /CC BY-NC 2.0).

The process of inflating its body begins with the fish swallowing a large amount of water, which is stored in a kind of bag at the end of the esophagus and in the stomach. As this organ is very elastic, it inflates like a balloon, leaving the puffer fish spherical in shape and up to three times its normal size. Pufferfish are oviparous: females lay their eggs among submerged leaves or trunks. Thus, this species needs temperatures that vary between 22 and 26 degrees to resemble the warm waters of tropical regions. As there is no sexual dimorphism, the male and female are very well differentiated [4-5].

The main defense mechanism is its ability to swell/inflate quickly. This means of defense can fool a potential predator; It is much more difficult to swallow a large fish than a small one. Some pufferfish, marine species, produce a powerful neurotoxin and have spines on their skin, conveying a threatening and intimidating appearance to potential predators. Their eyes can move independently and, in some species, can even change the color or intensity of their patterns according to environmental changes and needs (Figure 3) [4-6].

Figure 3. The pufferfish Southeast Asia, including Borneo, the Malay peninsula, Sumatra, and Thailand. Although this fish can be found in brackish water environments, it is still more common in freshwater habitats. Source: https://myaquarium.com.br/peixes/peixes-de-agua-doce/baiacu-figura-oito-tetraodon-biocellatus/

Some species: *Auriglobus modestus* (Bleeker, 1850), *Colomesus asellus* (Müller & Troschel, 1849) (Baiacu-de-água-doce), *Colomesus psittacus* (Bloch & Schneider, 1801) (baiacu-papagaio) *Lagocephalus laevigatus* (Linnaeus, 1758) (Baiacu-ará), *Takifugu ocellatus* (Linnaeus, 1758) and *Tetraodon erythrotaenia* Bleeker, 1853 [5-6].

Reproduction: Oviparous. They commonly spawn in the substrate among plants. The male will court the female to the designated spawning location, causing her to release the eggs. After the reproduction ritual, the male will expel the

female and take care of the eggs until they hatch. Individuals are ready to reproduce at around 3 years of age. Every year, couples meet in autumn, and after mating, pregnancy can take 5 to 8 months to produce 10 to 40 puppies in a single litter. It occurs during the flood season. Spawning takes place in numerous small eggs, which adhere to substrates such as rocks. The larvae are carried away by the current[5-6].

2. TETRODOTOXIN

The pufferfish or needlefish are poisonous fish common on the Brazilian coast. The most important in Brazil are the macaw or smooth pufferfish and the spotted pufferfish. Neurological complications manifest as numbness in the mouth and extremities, muscle weakness, visual disturbances, and other symptoms. Nausea, vomiting, abdominal pain, and diarrhea also occur, and convulsions and cardiorespiratory arrest may also occur in the first 24 hours [5-7].

The toxin blocks the nerves' ability to transmit orders to the muscles and ends up paralyzing the respiratory muscles. The toxin blocks the nerves' ability to transmit orders to the muscles and ends up paralyzing the respiratory muscles The pufferfish, also known as fugu in Japan, is a fish that intrigues due to its poisonous potential. The reputation of this fish is intrinsically linked to a deadly toxin called tetrodotoxin (anhydrotetrodoxin 4-epitetrodoxin, tetrodonic acid), which is present in its viscera, skin, and muscles. Is pufferfish considered dangerous, what are the risks associated with its consumption, and why, despite the dangers, it is appreciated in some specific cuisines (Figure 4) [7-9].

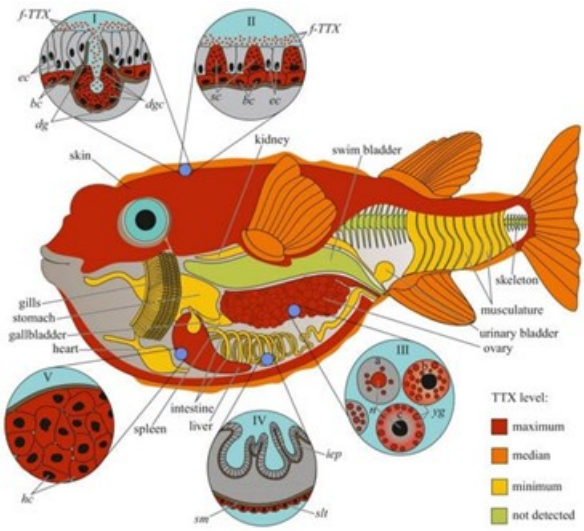

Figure 4. Schematic illustration of the levels and intra-organismal distribution of tetrodotoxin (TTX) in the adult pufferfish (family Tetraodontidae). The red color on the insects indicates TTX-positive cells. I—Skin with dermal gland (dg). II—Skin with singly scattered succiform cells (sc). III—Oocytes at different maturation stages: a—immature oocyte, b—newly mature oocyte, c—mature oocyte. IV—Intestinal epithelium (iep) and sac-like tissue (slt) outside the serous membrane (sm). V—Liver with hepatocytes (hc). Abbreviations: ec, epithelial cell; bc, basal cell; dg, dermal gland; dgc, dermal gland cell; f-TTX, free TTX; sc, succiform cell; n, nuclei; yg, yolk granules; iep, intestinal epithelium; slt, sac-like tissue; sm, serous membrane; hc, hepatocy. Source: Toxins 2022, 14(8), 576; https://doi.org/10.3390/toxins14080576.

This neurotoxin (TTx) is one of the most potent substances found in nature, capable of causing muscle paralysis and leading to death within a few hours. TTx is a heat-stable toxin that is not affected by cooking, washing, or freezing. Its level is seasonal and the highest concentrations are found in females during the reproductive season. Organic chemists love chemicals with complex structures. Tetrodotoxin is one of these. Tetrodotoxin has many oxygen and nitrogen atoms, making it soluble in water. The nitrogen atoms of tetrodotoxin form a functional group called guanidine, which is related to the sodium channel-blocking activity. Because it has such a complex structure, the chemical synthesis of tetrodotoxin was extremely difficult to conduct (Figure 5) [9-11].

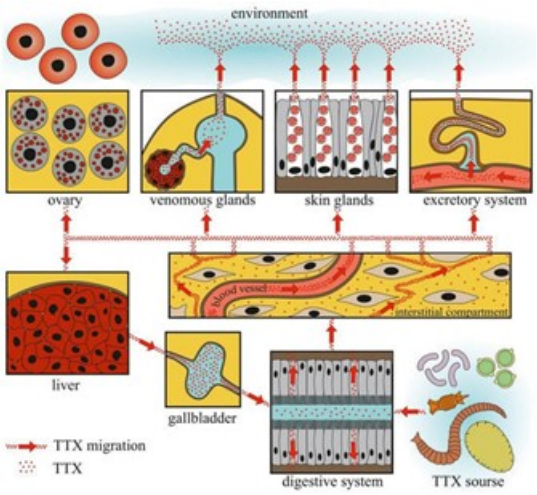

Figure 5. Schematic illustration of tetrodotoxin (TTX) migration in TTX-bearing animal. Source: Toxins 2022, 14(8), 576; https://doi.org/10.3390/toxins14080576.

Tetrodotoxin works by blocking sodium receptors depending on the voltage, preventing depolarization and the propagation of the action potential in nerve cells. This action occurs on the peripheral motor, sensory, and autonomic nerves and has a depressant action on the respiratory and vasomotor center of the brain stem. The production of tetrodoxin/anhydrotetrodoxin is carried out by certain bacteria, including strains of the family Vibrionaceae, Pseudomonas sp., and Photobacterium phosphoreum (Cohn) Beijerinck, which points to a bacterial origin. Tetrodotoxin: This toxin reversibly blocks the sodium channels of neurons and has been a widely used tool for mechanistic studies of neurons, skeletal muscle, and cardiac muscle cells [11-13].

It is considered the second most venomous vertebrate on the planet. Most pufferfish are marine, but there are a considerable number of species that live in freshwater. Two types of pufferfish are most common in Brazil: the macaw and the spotted. The macaw is less toxic and can be found in fish markets. The paint has extremely high doses of tetrodotoxin, being responsible for the poisoning in the country. At least two varieties of pufferfish species live exclusively in freshwater, in addition to some species that can also live in deep water, *Sphoeroides pachygaster* (Müller & Troschel, 1848), or even non-specific species that are found to live in completely polluted waters (Figure 6) [14-17].

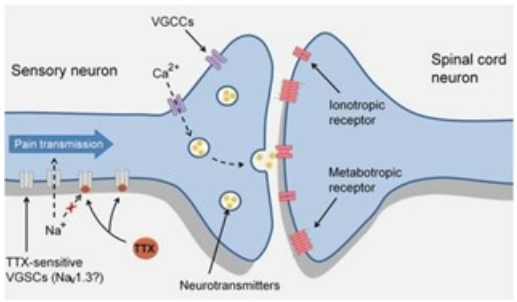

Figure 6. It is a sodium channel blocker-It blocks the firing action neurons. Sources: it is a sodium channel bloc kern-It blocks the firing of action neurons. TTX is a neurotoxin that acts to block voltage-dependent sodium channels present in the membranes of nerve cells, at peripheral and central levels. Due to this blockage, Na+ ions are unable to cross the channel and the cell does not depolarize. As a result, the generation of the action potential is affected and the propagation of the nerve impulse is impeded. Source: https://www.oswaldocruz.br/painel/painel.asp?id_painel=199.

3. THERAPEUTIC USE OF TETRODOTOXIN IN ANIMALS

Tetrodotoxin has shown its possible use in new therapies in several areas. The compiled studies showed that the pharmacological activity of TTX has been extensively studied and its use has been seen in therapies related to chronic and neuropathic pain, in reducing metastasis in cancer, and in therapies related to pathologies in the muscular, skeletal, and motor systems, in addition to its use in nervous system therapies. Ion channels are expressed in all cells, and their expression or activity changes may be involved in various pathologies from arrhythmias heart disease, epilepsy, and neuropathic pain (Figure 7) [18-20].

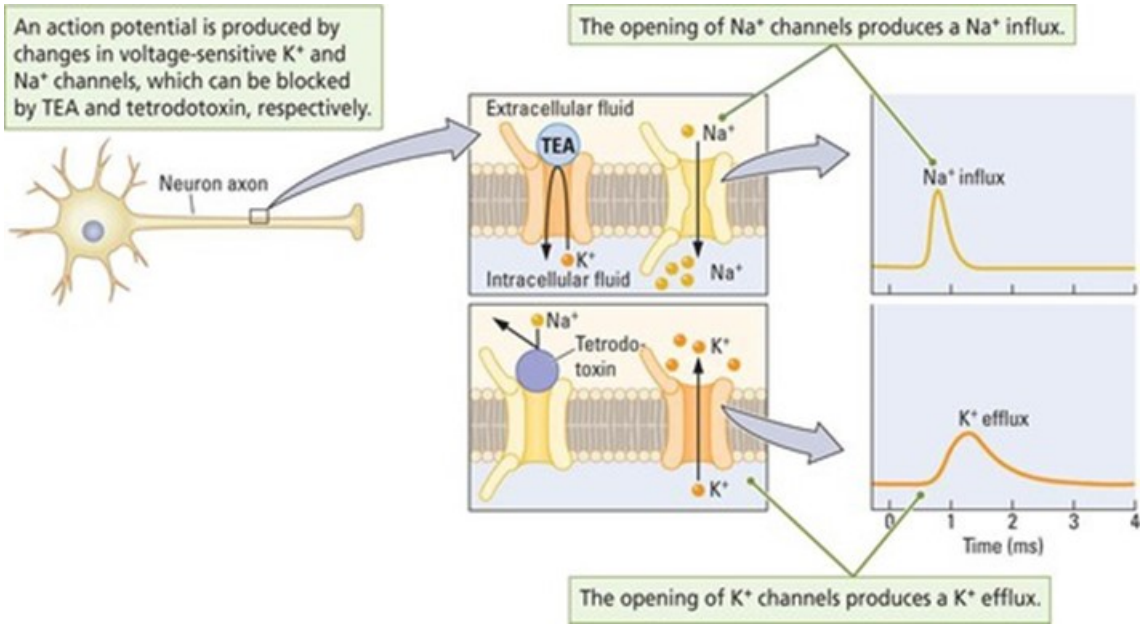

Figure 7. Toxin from puffer fish that blocks voltage-dependent sodium channels. Source: https://quizlet.com/558896283/aktionspotential-zug-flash-cards/.

1. Therapies for Cancer.

When studied by scientists, the toxin was announced as a powerful painkiller that helps with a series of problems involving cancer. In metastatic cancer cells, there is a change in the expression of voltage-dependent sodium channels in the cells and it is believed that these channels are intrinsically linked to the development of metastases, as they increase their aggressiveness. Insufficient Na+ for cancer cells causes changes in their integrity, resulting in the suppression of cell invasion and proliferation[20-21].

The toxin has no effects on non-human cells as they are unlikely to present significant amounts of voltage-dependent functional sodium when compared to cells with invasive potential. In cancer-related therapies, tetrodotoxin reaches cells with greater metastatic intensity, being less aggressive towards other cells in the body, as shown. In this way, treatment with this toxin becomes less invasive when compared to traditional methods such as chemotherapy [20-24].

2. The Muscular System, Cardiac and Motor

One of the changes observed in the heart after suffering from ischemia/hypoxia is the existence of a direct electrical current caused by the opening of sodium channels. However, this current can lead to arrhythmias and therefore failure cardiac. Tetrodotoxin proves effective when applied to myocardial cells, bringing a normalization of these beats uncontrolled. Furthermore, tetrodotoxin can be used as a hypotensive agent. This effect is caused by its direct action on the central nervous system since it can cross the blood-brain barrier [25-27].

4.1.Duchenne muscular dystrophy tetrodotoxin acts to reduce fiber loss muscles in genetically modified mice for this disease and this fact can bring an increase in the survival of infected patients, as well as an improvement in quality of life, since the loss of fibers is the main vector for the death of the individual [28-29].

4.2. In system-related therapies cardiac, this toxin was successful as a hypotensive and regulating heart rate after ischemia/hypoxia [30].

Are all pufferfish toxic?

The toxin is not a poison, which means that the fish does not inject poison through its spines or by biting, but the fish is extremely dangerous if ingested. The toxin is found in the fish's liver, intestines, and ovaries, and in some cases, the skin, but never in the meat itself. In addition, the toxin is produced by bacteria that live in the intestine and are found in some of the foods that pufferfish eat a pufferfish raised in an aquarium or commercially produced under human care will therefore not have the toxin [31]

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