Faculty of Resource Science and Technology

STUDY OF MORPHOLOGY AND SCREENING THEIR TOXIN OF *Xenopterus naritus* FROM ASAJAYA, SARAWAK

Nur Diana Binti Sarizan
(57185)

Bachelor of Science with Honours
(Aquatic Resource Science and Management)
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Study of Morphology and Screening their Toxin of *Xenopterus naritus* from Asajaya, Sarawak

Nur Diana Binti Sarizan

(57185)

A thesis submitted
in fulfillment of the Final Year Project (STF 3015) course

Aquatic Science and Resource Management

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2019

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NUR DIANA BINTI SARIZAN (57185)

Aquatic Resource Science and Management

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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Toxicity Study of Yellow Puffer Fish, *Xenopterus naritus*, in Asajaya water

Nur Diana Sarizan

Aquatic Resource Science and Management
Faculty of Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Puffer fish from Tetraodontidae family is the common puffer fish to possess tetrodotoxin (TTX) which can cause puffer fish poisoning and effect human health. In this study, a total of 15 yellow puffer fish or *Xenopterus naritus* (9 males, 6 females) purchased from Asajaya, Sarawak were analysed for the content of Tetrodotoxin (TTX) in different tissues (stomach, liver, testis, gonad) by using the Thin Layer Chromatography (TLC) screening method. The morphological measurements were measured by standard measuring using DAN electronic balance and Wildco Fish measuring board. The mean of body weight, total length and standard length of the males (n=9) were ranges from 52 – 94 g, 11.6 – 21.7 cm and 10.7 – 14.5 cm respectively. While, the female (n=6) were ranges from 76.8 – 424 g, 16.7 – 26.5 cm and 14.2- 23.0 cm respectively. Results show that all the tissues in this study contain TTX that range between 0.71 and 0.50 in TLC analysis. The highest Rf value recorded for TLC analysis was in gonad (0.80 ± 0.13). The information in this study indicates the importance of the proper removal of the toxin tissues to guarantee safe consumption of puffer fish.

Keywords:Puffer fish, Asajaya, Tetrodotoxin, *Xenopterus naritus*, Thin Layer Chromatography

ABSTRAK

Ikan buntal dari keluarga Tetraodontidae adalah jenis ikan buntal yang biasa memiliki tetrodotoksin yang boleh menyebabkan keracunan ikan buntal dan membahayakan kesihatan manusia. Dalam kajian ini, sebanyak 15 ekor ikan buntal kuning atau *X. naritus* (9 jantan, 6 betina) yang diperolehi dari Asajaya, Sarawak telah di analisis untuk kandungan tetrodotoksin di dalam organ yang berbeza (perut, hati, testis dan gonad) dengan menggunakan kaedah TLC. Pengukuran morfologi diukur dengan menggunakan DAN penimbang eletronik dan papan pengukur Wilco Fish. Julat berat badan, jumlah panjang dan panjang piawai jantan (n=9) adalah 52 – 94 g, 11.6 – 21.7 cm dan 10.7 – 14.5 cm masing-masing. Sementara itu, bagi betina (n=6) pula adalah 76.8 – 424 g, 16.7 – 26.5 cm dan 14.2- 23.0 cm masing-masing. Keputusan menunjukkan bahawa semua tisu dalam kajian inimengandungi Tetrodotoksin yang berkisar antara 0.71 dan 0.50 dalam analisis TLC. Nilai Rf tertinggi yang direkodkan untuk analisis TLC adalah dalam gonad (0.80 ± 0.13). Maklumat dalam kajian ini menunjukkan pentingnya penyingkiran tisu toksin yang betul untuk menjamin kesihatan setelah memakan ikan buntal.

Kata Kunci: Ikan buntal, Asajaya Tetrodotoksin, *Xenoterus naritus,* TLC

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List of Abbreviations

TTX	Tetrodotoxin
MLD	Minimum lethal dose
MU	Mouse Unit
mg	Milligram
TLC	Thin Layer Chromatogrraphy
°C	Degree Celcius
g	Gram
cm	Centimeter
BW	Body weight
TL	Total length
SL	Standard length
mL	Milliliter
nm	Nanometer
ANOVA	One way analysis of variance
UV	Ultraviolet

1.0 INTRODUCTION

Puffer fish also known as fugu, toadfish, balloon fish, and globefish is one of the many diverse animal species that contains neurotoxin or tetrodotoxin (TTX). The other animal species that also contain TTX are some species of newts, frogs, gobies, flat worms, ribbon worms, starfish, crab, blue-ringed octopus and carnivorous gastropods (Miyazawa & Noguchi, 2001). Puffer fish from the Tetraodontidae family is the most common puffer fish to possess TTX (Hwang *et al.,* 1992). There are 28 genera and at least 185 species of puffer fish in the family of Tetraodontidae (Oliveira, *et al,* 2006). Tetraodontiform puffer fishes scattered in tropical to temperate seas and freshwater of the world. Tetrodontiform puffer fishes are identified by a small mouth with a few enlarged teeth or massive beak-like tooth, a small gill opening at the side of the body, spines scales, enlarged plates, or a carapace (Tyler, 1980: Nelson, 2006).

The common species of puffer fishes in Malaysian waters are *Lagocephalus lunaris* (Bloch & Schneider, 1801), *L. sceleratus* (Gmelin, 1789), and *L. spadiceus* (Richardson, 1845). However, among the local people in Sarawak, yellow puffer fish or *Xenopterus naritus* is the popular one. This Tetraodontiform puffer is a migratory species and widely distributed in China, Thailand, Vietnam, Indonesia and Malaysia. In Malaysia, this species is the most abundant in Sarawak and generally inhabited coastal waters and areas fringing the mangrove. Locally, the puffer fish is known as 'ikan buntal kuning' as the lower part of their body is prominent yellowish or golden coloration. It is considered as delicacy among the local people and tourist attraction in Sarawak. This fish and their products (salted/eggs) are high demand in the local market even though the locals are aware of their poisonous effects. The severity and lethality of the cases are due to the ingestion of the puffer fish and depend on the concentration of TTX present in the tissues of the consumed fish. It requires a skill and knowledge on the preparation of the puffer fish to remove the internal organ thoroughly to ensure that it is safe to consume it.

However, studies on the toxin analysis of yellow puffer fish, *X. naritus* in Sarawak is still limited. To our knowledge, no data of toxin analysis collected from Asajaya, Sarawak. The previous studies were done in Betong and Kuching (Mohd Nor Azman *et al.*, 2013). The toxin analysis of *X. naritus* in Kg. Manggut, Betong, Sarawak was analysed by using liquid chromatography-mass spectrometry mass spectrometry (LC-MS/MS) and also involved the assessment of gonadosomatic index (GSI). While, the study in Kuching involved the toxin analysis of dried yellow puffer fish and their salted eggs by using mouse bioassay method.Hence, the objective of this study is to identify the toxin properties in different tissues of *X. naritus* in Asajaya, Sarawak by using the Thin Layer Chromatography (TLC) method.

2.0 LITERATURE REVIEW

2.1 *Xenopterus narituus*, Yellow puffer fish

Xenopterus naritus species is commonly known as yellow puffer fish or bronze puffer fish. In Sarawak, it is known as *buntal kuning* or *buntal pisang* among the local people. The first name recorded for *X. naritus* is *Chonerhinos naritus* (Richardson, 1848) and *C. naritus* is the only species in the genus *Chonerhinos*. However, *Xenopterus naritus* is the current valid name used for this thesis and there are several reasons to justify it.

One of the reason is because of the confusion of the species habitat. The available information stated that *X. naritus* is a freshwater species (Froese & Pauly, 2017). However, this statement contradicted with Roberts (1982), which stated this species is an estuarine species. This contradiction happened because of the similarities of this species and *Chonerhinos modestus*. However, *Chonerhinos modestus* is much more smaller than *X. naritus* and this species is categorized into new genus *Auriglobus* (Kottelat, 1999). Roberts (1982) confirmed that the *Auriglobus* indeed is a freshwater species.

Another reason to keep using the name of *X. naritus* is that many literatures were using the name (Atack, 2006 ; Mohd Nor Azman *et al.*, 2013; 2014; 2015). The local government bodies such as Malaysian Fisheries Department and Fisheries Research Institute also used the current valid name for identification of this species. Furthermore, the latest publication from the neighboring countries also using *X. naritus* name in their literature (Randall & Lim, 2000; Chulanetra *et al.*, 2011; Santini *et al.*, 2013).

2.1.1 Morphology

Most of the species in Tetrodontiformes generally contain seven to 15 dorsal rays (Maatsura, 1997). However, *Xenopterus naritus* differ from the other puffer fish in Tetraodontidae family in terms of the number of dorsal and anal fin rays and their sizes. *X. naritus* possess almost double the number of rays in the dorsal and anal (Richardson, 1848). The ranges of the dorsal and anal fin rays for *X. naritus* are 32-38 and 28-29 respectively (Roberts, 1982). Furthermore, the size of the dorsal and anal fins of *X. naritus* are almost proportioned compared to the other species (Atack, 2006). The shape is considerably arched and both dorsal and anal are rather longer than high (Richardson, 1848). *Xenopterus naritus* possess torpedo-shaped body compared to other species with the golden coloration on the body, darker coloration at the above part of the body while the lower part is in lighter coloration (Atack, 2006).

2.2 Tetrodotoxin (TTX)

2.2.1 Properties of TTX

Tetrodotoxin (TTX) is one of the marine toxin that can cause food poisoning. This potent neurotoxin with low molecular weight was first isolated from toxic puffer fish in the form of crystalline prism in 1950 (Noguchi *et al,* 2011). This neurotoxin inhibits the action potential in nerve cells and muscle system by the blocking action of sodium channels (Narahashi, 2001).This can result in the failure of respiratory system that can cause death. The fatal potency is 5000 to 6000 MU/mg, and the minimum lethal dose (MLD) for humans is approximately 10000 MU (=2 mg) of TTX.

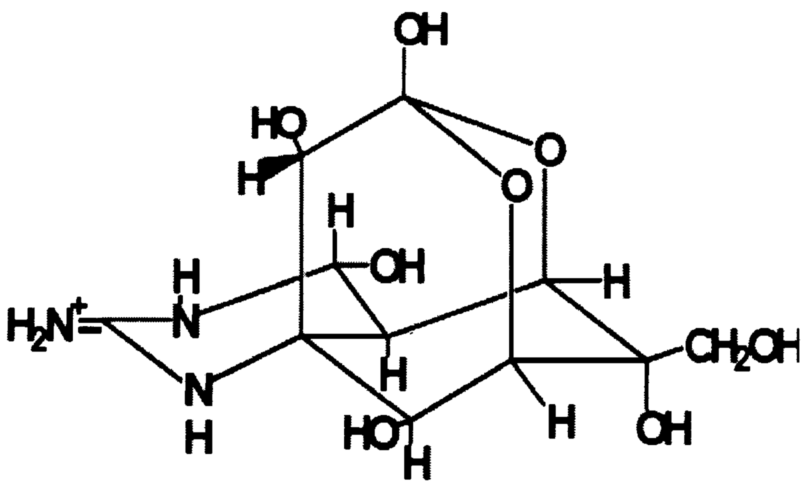

Figure 1: The structure of TTX (Taken from Noguchi *et al,* 2008)

TTX was assumed to be found exclusively in puffer fish. However, since 1964, the distribution of TTX has spread to other aquatic and terrestrial animals, such as newts, gobies, frogs, blue-ringed octopus, gastropods, starfish, crabs, flatworms and ribbon worms (Miyazawa & Noguchi, 2001 ; Noguchi & Arakawa, 2008). Hypotheses were made to discuss the origin of TTX in the puffer fish whether it was endogenous or exogenous. In the exogenous hypothesis, TTX assumed to form in the environment and spread by the bearers after ingestion. Feeding experiment with artificial bred larvae put forward this hypothesis, which suggesting the presence of the symbiotic relationship between the TTX bearing organisms and TTX-producing bacteria (Matsui *et al.,* 1981), Noguchi proposed the idea of the mechanism of TTX accumulation in puffer fish through food chain (Noguchi *et al.*, 2006). Meanwhile, in the endogenous hypothesis, it was proposed that TTX as the accidental product of physiological functions in the animals that produce it. It might occur because of the survival instinct of these animals. For example, the increase level of TTX in newts after they were kept in captivity in a year (Hanifin *et al.*, 2002). Many favors this hypothesis compared to the exogenous hypothesis.

2.2.2 Distribution of TTX in Puffer Fish

The distribution of TTX in puffer fish bodies based on their species-specific (Table 1). For the marine species, the liver and ovary have the highest concentration of TTX, followed by intestines and skin. Meanwhile, the muscle or testis are non-toxic or weakly toxic, exclude for in *Lagocephalus lunaris* and *Chelonodon patoca*. The liver shows the highest concentration of TTX throughout the year in the marine puffer fish. However, during the spawning season, the ovary recorded the highest concentration of TTX by the transfer of the accumulated TTX from the liver to the ovary (Kanoh, 1988). The presence of TTX in the eggs spawned can be the mechanical defense of the eggs from the predator. On the other hand, for the adult puffer fish, they will swell their bodies two or three times of their original size and excreted the TTX from their skin when encountered with the enemies. These suggests that TTX can act as the biologic defense mechanism for puffer fish.

Table 1: Toxicity of puffer fish (Taken from: Noguchi and Arakawa, 2008)

Family	Habitat	Species	Maximal toxicity*						
			Ovary	Testis	Liver	Skin	Intestine	Muscle	Blood
Tetraodontidae	Marine	Japanese pufferfish							
		Takifugu niphobles	●	○	●	◎	●	○	–
		T. poecilonotus	●	◎	●	◎	◎	○	–
		T. pardalis	●	○	●	◎	◎	×	×
		T. snyderi	●	×	●	◎	◎	○	–
		T. porphyreus	●	×	●	◎	◎	×	–
		T. chinensis	●	–	●	–	–	–	–
		T. obscurus	●	×	◎	◎	◎	×	–
		T. exascurus	●	×	◎	◎	–	×	–
		T. pseudommus	●	×	○	○	○	×	–
		T. chrysops	◎	×	◎	◎	○	×	×
		T. vermicularis	◎	×	◎	◎	○	×	–
		T. rubripes	◎	×	◎	×	○	×	×
		T. xanthopterus	◎	×	◎	×	○	×	–
		T. stictonotus	◎	×	◎	○	×	×	–
		Tetraodon alboreticulatus	●	–	○	○	◎	○	–
		Pleuranacanthus sceleratus	●	–	○	○	◎	○	–
		*Chelonodon patoca***	◎	◎	◎	●	–	◎	–
		Arothron firmamentum	◎	×	×	○	×	×	–
		Canthigaster rivulata	×	–	○	◎	○	×	–
		Lagocephalus lunaris	×	×	×	◎	×	●	–
		L. inermis	×	×	◎	×	×	×	–
		L. wheeleri	×	×	×	×	×	×	–
		L. gloveri	×	×	×	×	×	×	–
		Sphoeroides pachygaster	×	×	×	×	×	×	–
	Marine	Chinese pufferfish							
		Takifugu flavidus	●	◎	●	◎	◎	○	–
	Brackish	Thai pufferfish							
		Tetraodon nigroviridis	–	–	×	◎	○	○	–
		T. steindachneri	–	–	×	◎	×	×	–
Diodontidae	Marine	Japanese pufferfish							
		Diodon holocanthus	×	–	×	×	×	×	–
		Chilomycterus affinis	×	–	×	×	×	×	–
Ostraciidae	Marine	Japanese pufferfish							
		Ostracion immaculatum	×	×	×	×	×	×	–
		Lactoria diaphana	×	×	×	×	×	×	–
		Aracana aculeata	×	×	×	×	×	×	–

2.2.3 Distribution of TTX in Other Animals

Other than puffer fish, TTX also detected in other aquatic or terrestrial animals. TTX was detected in the eggs of the California newt *Taricha torosa* in 1964 (Mosher *et al,* 1965). The glandular skin possess by newt was investigated for the origin of TTX in newt. It is reported that the glandular skin play the important role in the production of TTX (Hanifin *et al.*, 2004). Besides that, TTX also detected in goby *Yongeichthys criniger*, atelopid frogs, the blue-ringed octopus *Hapalochlaena maculosa* and the carnivorous gastropod (Miyazawa and Noguchi, 2001; Hwang and Noguchi, 2007; Noguchi and Arakawa, 2008). **Figure 2** shows the distribution of TTX in other animals besides puffer fish. These TTX- bearers are unlikely to possess a common gene that codes the production of TTX with the particular species of different phyla.

Table 2: The distribution of TTX in other animals (Taken from: Noguchi & Arakawa, 2008)

Animals		Toxic parts	Maximal toxicity*	Ref
Platyhelminthes				
Turbellaria				
Flatworms	*Planocera* spp.	Whole body	●	[44]
Nemertinea				
Ribbonworms	*Lineus fuscoviridis*	Whole body	●	[45]
	Tubulanus punctatus	Whole body	◎	[45]
	Cephalothrix linearis	Whole body	●	[46]
Mollusca				
Gastropoda	*Charonia sauliae*	Digestive gland	●	[47]
	Babylonia japonica	Digestive gland	○	[48]
	Tutufa lissostoma	Digestive gland	◎	[49]
	Zeuxis siquijorensis	Whole body	●	[50]
	Niotha clathrata	Whole body	●	[51]
	Niotha lineata	Whole body	●	[52]
	Cymatium echo	Digestive gland	○	[53]
	Pugilina ternotoma	Digestive gland	○	[53]
Cephalopoda	*Hapalochlaena maculosa*	Posterior salivary gland (adult)	●	[54]
		Whole body (semi-adult)	○	[55]
Annelida:				
Polychaeta	*Pseudopolamilla occelata*	Whole body	○	[56]
Arthropoda:				
Xanthidae crabs	*Atergatis floridus*	Whole body	○	[57]
	Zosimus aeneus	Whole body	○	[58]
Horseshoe crab	*Carcinoscorpius rotundicauda*	Egg	○	[59]
Chaetognatha:				
Arrowworms	*Parasagitta* spp.	Head	△	[60]
	Flaccisagitta spp.	Head	△	[60]
Echinodermata:				
Starfish	*Astropecten* spp.	Whole body	◎	[61,62]
Vertebrata:				
Pisces				
Goby	*Yongeichthys criniger*	Skin, viscera, gonad	◎	[63]

2.2.4 Tetrodotoxin (TTX) Symptoms

Tetrodotoxin is responsible for 30-50 cases of fish poisoning occurred every year and the minimum lethal dose (MLD) for human to get poison is estimated to be approximately 10000 MU (Noguchi & Ebesu, 2001). TTX will give effect to the humans by inhibiting the action potential by blocking the sodium channels on the nerve and muscle membrane at low concentration. The severity of the poisoning depends on the dose of TTX (Homaira *et al.*, 2010). The symptoms of the puffer fish poisoning are categorize into four degrees as shown in **Table 3**. The common symptoms of the puffer fish poisoning are numbness of lips, headache, vomiting, muscle weakness, ataxia and heart failure that can caused death (Noguchi & Ebesu, 2001). At the present, there are still no antidote for TTX and the best treatment are the elimination of the toxin from the body and management of the respiratory system using an artificial respiration.

Table 3: Symptoms of TTX poisoning (Taken from: Noguchi *et al*, 2011)

Degree	Characteristic symptoms
First	Neuromuscular symptoms (paresthesia of lips, tongue, and pharynx; taste disturbance; dizziness; headache; diaphoresis; pupillary constriction); gastrointestinal symptoms (salivation, hypersalivation, nausea, vomiting, hyperemesis, hematenesis, hypermotility, diarrhea, abdominal pain)
Second	Additional neuromuscular symptoms (advanced general paresthesia; paralysis of phalanges and extremities; pupillary dilatation, reflex changes)
Third	Increased neuromuscular symptoms (dysarthria; dysphagia, aphagia; lethargy; incoordination, ataxia; floating sensation; cranial nerve palsies; muscular fasciculations); cardiovascular/pulmonary symptoms (hypotension or hypertension; vasomotor blockade; cardiac arrhythmias including sinus bradycardia, asystole, tachycardia, and atrioventricular node conduction abnormalities; cyanosis; pallor; dyspnea); dermatologic symptoms (exfoliative dermatitis, betechiae, blistering)
Fourth	Respiratory failure, impaired mental faculties, extreme hypotension, seizures, loss of deep tendon and spinal reflexes

2.3 Toxin Analysis

2.3.1 Thin Layer Chromatography (TLC)

In 1940s, Thin Layer Chromatography was known as paper chromatography before being replaced and become one of the most commonly used chromatography techniques (Ettre & Kalasz, 2001). Thin layer chromatography or TLC is a common liquid-solid adsorption technique to separate the compounds in the mixture to analyze the mixture. This method helps to determine the number of components, identify the compound and determine their purity. TTX can be spotted onto a coated Silica gel 60 plate in TLC analysis. TLC analysis is divided into three processes, which are spotting, development and visualization.

Development process consists of the placement of the plate in the solvent. The solvents travel up the plate by capillary action and move over the original spot. The movement of the spotted materials depend on the competition between the silica gel and the solvent. The spotted materials remain on the original place if used very polar silica gel but it will move some distance if the solvents are very polar. The results depend on the balance among the plate, the solvent and silica gel polarities. When the solvent almost reached the top of the plate, removed the plate and allowed it to evaporate. Mark the front solvent with a pencil.