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Nutritional Compositions in the Muscle of Yellow Puffer Fish, *Xenopterus naritus* (Richardson, 1848) from Kg. Manggut and Kabong, Sarawak, Malaysia

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Abstract: This study is to report the proximate compositions in the muscles of yellow puffer fish, *Xenopterus naritus* which was collected from Kg. Manggut and Kabong, Sarawak between February and July 2013. The internal organs of 20 specimens of *X. naritus* from Kg. Manggut and Kabong, respectively were removed by local residence that possess skills and experiences with the preparation of yellow puffer fish. In general, the moisture contents were ranging between 75.2% and 80.6%. In the present study, *X. naritus* from Kabong showed higher crude protein contents (88.2% dry weight) than the same species from Kg. Manggut (87.9% dry weight) but not significantly different ($p>0.05$). However, *X. naritus* from Kg. Manggut demonstrated a significantly higher ($p<0.05$) of crude fat contents (0.49% dry weight), crude fibre contents (0.44% dry weight) and ash contents (5.48% dry weight) compared to *X. naritus* from Kabong which were 0.47%, 0.25% and 5.08% dry weight, respectively. This study shows that the yellow puffer fish could be considered as an alternative source of protein for human consumption. Further, this is the first report on nutritional compositions in edible yellow puffer fish from Sarawak.

Keywords: Yellow puffer fish, Tetraodontidae, TTX food poisoning, proximate analysis, muscle, skills

Abtstrak: Kajian ini adalah untuk melaporkan komposisi proksimat dalam isi ikan buntal kuning *Xenopterus naritus* yang diambil dari Kg. Manggut dan Kabong, Sarawak di antara bulan Februari dan Julai 2013. Organ dalaman daripada 20 spesimen *X. naritus* dari Kg. Manggut dan Kabong masing-masing telah dikeluarkan oleh penduduk setempat yang mempunyai kemahiran dan pengalaman dalam penyediaan ikan buntal kuning. Secara amnya, kandungan lembapan adalah antara 75.2% dan 80.6%. Dalam kajian ini, *X. naritus* dari Kabong menunjukkan kandungan protein kasar yang lebih tinggi (88.2% berat kering) daripada spesies yang sama dari Kg. Manggut (87.9% berat kering) tetapi tiada perbezaan yang signifikan ($p> 0.05$). Walau bagaimanapun, *X. naritus* dari Kg. Manggut menunjukkan perbezaan yang lebih signifikan ($p <0.05$) pada kandungan lemak kasar (0.49% berat kering), kandungan fiber kasar (0.44% berat kering) dan kandungan abu (5.48% berat kering) berbanding *X. naritus* dari Kabong iaitu 0.47%, 0.25% dan 5.08% berat kering masing-masing. Kajian ini menunjukkan bahawa ikan buntal kuning boleh dipertimbangkan sebagai sumber alternatif protein untuk kegunaan manusia. Ini merupakan laporan pertama mengenai komposisi pemakanan dalam ikan buntal kuning yang boleh dimakan di Sarawak.

Introduction

Yellow puffer fish or *Xenopterus naritus* (Richardson, 1848) can be identified by the prominent yellowish or golden coloration particularly at the lower part of its body. It has a torpedo-shaped body and widely distributed in China, Thailand, Vietnam, Myanmar, India, Indonesia and Malaysia (Mohd Nor Azman *et al.,* 2015). In Malaysia, this species can be found abundant only in Sarawak especially in Southwest coast of Sarawak, particularly along the Batang (River) Saribas in Betong, Sarawak (Gambang and Lim, 2004). *X. naritus* is a migratory species that inhabited coastal waters and areas fringing the mangroves and return to the river to spawn at lower salinity environment. During non-spawning season, the juvenile inhabits coastal water out to the sea.

Figure 1: Yellow puffer fish, *X. naritus*

X. naritus is classified as trash fish in commercial fishery but this species has high demand in local markets and famous amongst the local people. Locally known as 'ikan buntal kuning', this species is considered as a delicacy by the local community, particularly in Kg. Manggut area. This area has become a tourist attraction to celebrate the 'Yellow puffer fish Festival' every year in August since 2003. During this festival, various dishes and products of yellow puffer fish are processed and prepared.

As a member of Tetraodontidae family, puffer fish is probably the most common fish that known to possess a neurotoxin or tetrodotoxin (TTX) which can cause a puffer fish poisoning. Members of this order are comprised of the spikefishes, triplespines, filefishes, triggerfishes, puffers, boxfishes, threetooth puffers, porcupinefishes or burrfishes and molas or sunfishes (Nelson, 2006). The toxins found in puffer fish are not actually produced by the fish themselves, but they are acquired through the food chain that starts from various species of bacteria living in symbiosis with these fishes. Puffer fish are believed to accumulate TTX mainly in its liver and ovary through the food chain by ingesting TTX-bearing organisms such as starfish, gastropods, crustacean, flatworms and ribbon worms (Noguchi *et al*., 2006; Ritchie *et al*., 2000).

Food poisoning has been reported from different geographical regions due to ingestion of puffer fish and the lethality depended on the amount of TTX present in the consumed fish tissues (Chou *et al.,* 1994). Outbreaks of TTX food poisoning have been reported in various countries in the world including Malaysia. Incidents of food poisoning due to ingestion of puffer fish in different parts of Malaysia such as Terengganu, Johor, Sabah and Sarawak have been reported in 1985 to 2009 (Lyn *et al.,* 1985; Kan *et al.,* 1987; Loke and Tan, 1997; Chua and Chew, 2009). Most of the cases were due to ingestion of contaminated puffer fish that was not properly prepared and the severity of poisoning depends on the amount of toxin ingested. All humans in all age groups are susceptible to TTX poisoning. TTX poisoning may be avoided by not consuming puffer fish or other animal species containing tetrodotoxin (Noguchi and Ebesu, 2001).

Even though the local people in Kg. Manggut area is aware of the poisonous effect of yellow puffer fish, this species and its products have high demand in local markets. According to Muliadi and Mohammad Raduan (2008), the price of dried salted roe of yellow puffer fish can reach up to RM 30-40 per kilogram (USD 7-10) and the price of the fish itself had doubled from RM 3 per kg (USD 1) in 2003 to RM 6 per kg (USD 2) in 2017 (pers. comm). Yellow puffer fish have a potential as a major source of income for fishing families in Sarawak.

The presence of toxin in this fish and its products could be more harmful because they are eaten whole as the toxin could not be removed easily. The preparation of this fish requires a skill and knowledge to ensure its safe to eat (Mohd Nor Azman *et al.,* 2015). If cleaned properly, the puffer fish flesh is fit for human consumption. According to Gambang and Lim (2004), some of the local residences living in the middle regions of Batang Saribas, Betong Division, Sarawak have experiences and skills with the preparation of yellow puffer fish as they have consumed the fish for generations. There are three methods to prepare the yellow puffer fish by the local people such as chopping, scissor cutting and traditional methods, but the concept remained the same (Parvaneh *et al.,* 2012).

Even though yellow puffer fish is served as a delicacy and have high demand for the locals in Sarawak, they are not aware of the nutritional aspects of this species. According to Osman *et al*. (2001), in general, fishes from different types contain the same nutritional value. Therefore, it is important to study the nutritional content of the edible part of yellow puffer fish as it is considered as a delicacy fish. In a previous study, we have focused on the detection of TTX with respect to safe consumption of yellow puffer fish (Mohd Nor Azman *et al.,* 2013). Several reports have been done to evaluate the nutritional quality of different puffer fish species. However, there is limited study in evaluating the nutritional content in puffer fish species from Malaysian waters. For this reason, this study was attempted to determine the nutritional content of the edible part of yellow puffer fish caught from Sarawak.

Materials and Methods

Specimen collection

A total of 40 specimens of the yellow puffer fish *X. naritus* were caught using trammel net or gill nets by local fishermen from Kg. Manggut (1˚31’55.90”N, 111˚20’9.60”E) and Kabong (1˚49’18.2”N, 111˚07’51.5”E), Sarawak (Fig. 2) between February and July 2013. The specimens were kept in plastic thermal insulated boxes with ice within 4 – 5 hrs and transported to the laboratory of the Fisheries Research Institute Sarawak, Bintawa, Sarawak, and subsequently kept frozen.

Sample preparation

The specimens were identified based on the morphological characteristics (Froese and Pauly, 2018). Then, the specimens were individually measured for their total body weight and length. The internal organs of 20 specimens from Kg. Manggut and Kabong, respectively, were removed by the local residence that had skills and experiences with the preparation of yellow puffer fish. The skin of each specimen was removed and the fish fillets were separated from both sides with a sharp knife without cutting the internal organs and weighed. Then the filleted specimens were minced and used for proximate analysis.

Proximate analysis

Moisture content analysis

Moisture content of yellow puffer fish minced fillets was determined according to the method described by AOAC (2000) with slight modifications. The samples (1.0 g) were weighed (MS304S Mettler Toledo, Switzerland) and dried in crucible in an oven (UFB400 Memmert, Germany) at 105˚C until constant weights were obtained. Meanwhile, approximately 100 g of the minced fillets was also dried in moisture dish as described for moisture content analysis. After constant weight was obtained, the samples were ground using a dry grinder (MX-800S Panasonic, Malaysia) and fine powders that obtained were used for ash, crude protein, crude fat and crude fibre content analysis.

Ash content analysis

Ash content of yellow puffer fish minced fillets was determined according to the method described by AOAC (2000) with slight modifications. Dried samples obtained from the moisture content analysis were ashed in a muffle furnace (Carbolite, UK) at 550˚C overnight.

Crude protein analysis

Crude protein content of yellow puffer fish minced fillets was determined according to the method described by AOAC (2000) with slight modifications as recommended by Kjeltec 2100 (Foss Analytical, Denmark). Briefly, 1.0 g dried sample was weighed into digestion tubes. Two Kjeltabs Cu 3.5 (catalyst salts) was added into each tube. Approximately 12 ml of concentrated sulphuric acid (H_2SO_4) was carefully added into the tube and then shaken gently. Digestion procedure was performed using pre-heated (420˚C) digestion block of 2066 Digestor (Foss Tecator, Sweden) for 60 min until clear blue/green solution was obtained. Digested samples were cooled for 20 min. Distillation procedure was then performed using distillation unit of 2100 (Foss Tecator, Sweden). Distillate was titrated using 776 Dosimat (Metrohm, Switzerland) with 0.5N hydrochloric acid (HCl) until the pinkish end point achieved.

Crude fat content analysis

Crude fat was extracted from the dried sample following the method described by AOAC (2000) using the Soxtec 2055 System (Foss, Sweden) with petroleum ether as the solvent. The content of crude fat was determined gravimetrically after the extract was dried by using an oven (105˚C) for overnight.

Crude fibre content analysis

Crude fibre was determined by hot extractor Fibertec 2010 (Foss Tecator, Sweden) with sequential extraction of dried samples with 1.25% H_2SO_4(1 L) and 1.25% NaOH (1L) using the Borosilicate 3.3 (Foss, Sweden) as a container. For drying and ashing, the crucible with sample was dried in an oven for 5hrs at 105˚C and ashed in the muffle furnace (Carbolite, UK) at 550˚C overnight. The weight of crucible with sample after drying and ashingwas recorded and the crude fibre content was calculated (AOAC, 2000).

Table 1: Size of *X.naritus* in length (cm) and weight (g)

Sampling location		**Kg. Manggut**	**Kabong**
Number of specimens		20	20
Body length (cm)	Range	13.4-28.0	17.8-28.8
	Mean±SD	18.1±3.93[a]	23.2±2.26[b]
Body weight (g)	Range	42.6-492.5	138.1-489.4
	Mean±SD	139.2±124.5[a]	294.1±74.5[b]

Data are shown as mean±standard deviation (SD)
Different alphabetical superscripts indicate difference among the measured values in each column ($p<0.05$)

Statistical analysis

Data were analysed by the statistical software of SPSS (Statistical Package for the Social Sciences) version 16.0 for Windows. The data were transformed to a normal distribution prior to analysis. One way analysis of variance (ANOVA) was used to compare differences in the means of the moisture content, ash content, crude protein content, crude fat content and crude fibre content of puffer fish. This was followed by Duncan multiple range test analysis to determine the differences between samples. All proximate compositions were analysed in triplicate and reported as mean on % dry weight basis. Means±SD of triplicate determinations were considered significantly different when $p<0.05$.

Results and Discussion

Length and weight comparison

In this study, 40 specimens of *X. naritus* were collected from Kg. Manggut and Kabong respectively between February and July 2013. The mean of total length of Kg. Manggut specimens and Kabong specimens was 18.1±3.93 cm and 23.2±2.26 cm respectively, and significantly different ($p<0.05$). The mean total length and body weight of Kabong specimens were higher than Kg. Manggut specimens and significantly different ($p<0.05$) (Table 1).

Proximate composition

The proximate composition of the muscle of *X. naritus* from Kg. Manggut and Kabong, Sarawak including the moisture, crude protein, crude fat, crude fibre and ash contents are presented in Table 2. The carbohydrate content was not estimated in this study, as the fish flesh contains virtually no carbohydrate and practically considered zero (Payne *et al*., 1999; Anthony *et al*., 2000). The mean of moisture contents of *X. naritus* from Kabong, Sarawak were measured as 79.97±0.56% and significantly higher than *X. naritus* collected from Kg. Manggut, Sarawak which measured as 77.36±1.13% ($p<0.05$).

The crude protein content was detected to be relatively high and ranged between 83.5% and 93.1% (dry weight basis), which was similar and not significantly different between the samples collected from Kabong and Kg. Manggut, Sarawak ($p>0.05$) (Table 2). The muscle of *X. naritus* collected from Kg. Manggut showed higher levels of crude fat content than from Kabong, Sarawak. However, the crude fat content of the muscle of *X. naritus* between Kg. Manggut (0.51%) and Kabong (0.43%) was not significantly different ($p>0.05$). There was a significant difference ($p<0.05$) of crude fibre and ash content between the samples from Kabong and Kg. Manggut. The crude fibre and ash contents in the muscle of *X. naritus* from Kg. Manggut were higher than Kabong (Table 2).

Table 2: Proximate composition of the muscle of *X. naritus* from Sarawak

Sampling location	**Kg. Manggut**	**Kabong**
Number of specimens	20	20
	Mean±SD (% dw)[b]	Mean±SD (% dw)[b]
Moisture (% ww)	77.36±1.13[a]	79.97±0.56[b]
Crude protein	87.91±2.94[a]	88.22±2.58[a]
Crude fat	0.49±0.26[a]	0.47±0.17[a]
Crude fibre	0.44±0.16[a]	0.25±0.16[b]
Ash	5.48±0.05[a]	5.08±0.19[b]

[a]ww – wet weight
[b]dw – dry weight
Data are shown as mean±standard deviation (SD)
Different alphabetical superscripts indicate difference between the measured values in each row ($p<0.05$)

In this study, the specimens of yellow puffer fish *X. naritus* that collected from Kabong and Kg. Manggut, Sarawak was not separated according to their sex. The yellow puffer fish that collected from Kabong, Sarawak were larger and heavier than the specimens from Kg. Manggut, Sarawak. In the previous study, the size of female specimens was larger than male specimens (Mohd Nor Azman *et al.,* 2013). Imelda *et al*. (2012) reported that the total length ranged between 21.6 cm and 33.9 cm in the female yellow puffer fish compared to the male's size ranged between 11.3 cm and 19.8 cm. While, Mohamad *et al*. (2008) observed the range total length in six samples of yellow puffer fish was between 19.6 cm and 26.9 cm. Nevertheless, the maximum body weight of puffer fish observed in this study (492.5 g and 489.4 g; Kg. Manggut and Kabong respectively) was higher than that of 190.7 g and 206 g as reported by Imelda *et al*. (2012) and Mohd Nor Azman *et al*., (2014) respectively, but lower than reported by Mohamad *et al*. (2008) (533.8 g) and Mohd Nor Azman *et al.* (2013) (711 g). According to Aydin (2011), length and weight are regarded as important growth criteria in the ecology of fish.

In this study, the mean moisture content of the muscles from Kg. Manggut and Kabong were 77.36±1.13% and 79.97±0.56%, respectively (Table 2). These values were in the range of values that usually found in other commercial fish from the West coast of Peninsular Malaysia (74.76±5.85% – 82.12±5.19%) (Nurnadia *et al.,* 2011). The values were slightly higher to that of farmed puffer fish muscle of *Fugu obscurus* (76.9%), *Fugu flavidus* (78.0%) and *Fugu rubripes* (77.3%) (Tao *et al.,* 2012), wild *Lagocephalus sceleratus* (78.5%) (Aydin *et al.,* 2013) and marine puffer fishes from Gulf of Mannar region (Karunanidhi *et al.,* 2017) but slightly lower than wild *L. inermis* (86.05%) (Eswar *et al.,* 2014) (Table 3).

The crude protein content from both locations was also in the range of values in other commercial fish of Peninsular Malaysia (17.46-20.86%) (Nurnadia *et al.,* 2011). The crude protein contents (wet weight) of the muscles from both locations were much higher than that muscle of farmed *F. obscurus* (18.4% ww), *F. flavidus* (18.1% ww), *F. rubripes* (17.8% ww) and *Takifugu obscurus* (18.44% ww) but slightly lower than wild *L. sceleratus* (21.62% ww) (Tao *et al.,* 2012; Aydin *et al.,* 2013; Yuqi *et al.,* 2014). While Saito and Kunisaki (1998) found lower protein (16.5% ww) for wild and cultured puffer fish *Takifugu rubripes*. However, Eswar *et al*. (2014) observed very low protein content in the tissues of wild puffer fish *Lagocephalus lunaris* (9.22%) and *L. inermis* (8.92%) (Table 3). These variations might be due to the different environmental condition and consumption pattern of the fish (Eswar *et al.,* 2014).

In general, the muscle of *X. naritus* collected from Kg. Manggut showed higher level of crude fat, crude fibre and ash content than from Kabong, Sarawak (Table 2). However, the crude fat content from both locations was lower from other puffer species. The difference might be due to the specimens were obtained from different locations and habitats. The feeds that consumed by the yellow puffer fish also might be different for both locations. As for the examination of stomach content, the yellow puffer fish was found to feed on varieties of food items ranging from gastropod, polychaetes and crustaceans with crab and fish being the highest food category. Other diets include bivalve, detritus, shrimp and sea urchin. Some diets were categorized as digested food. In the present study, the specimens from Kabong were caught in estuaries of Sungai Krian, while the specimens from Kg. Manggut were collected from the river, Batang Saribas (Fig. 2). Other puffer fish species showed higher fat contents for both wild and cultured samples. Puffer fish *L. lunaris* and *L. inermis* showed very high of fat content in the tissues with 11.25% and 11.98%, respectively (Eswar *et al.,* 2014) (Table 3). While Tao *et al*. (2012) showed higher fat contents in farmed puffer fish muscles of *Fugu obscurus* (0.83%), *Fugu flavidus* (0.81%) and *Fugu rubripes* (0.73%). Saito and Kunisaki (1998) found 0.7% and 0.9% fat composition for wild and cultured *T. rupribes* respectively. While Koizumi and Hiratsuka (2009) reported, fat content for cultured and wild *T. rubripes*, as 0.84-0.96% and 0.87-1.01% respectively. On the contrary, the crude fat content of cultured *T. rubripes* from two different areas of Taiwan was 0.2-0.4% and no difference was observed throughout the year (Hwang *et al.,* 2000).

However, high crude fat content was found in the 20 species of commercial fish from the West coast of Peninsular Malaysia (1.05-23.15%) (Nurnadia *et al.,* 2011). The differences and variations in proximate compositions might be due to geographical location, species type and seasonal effects (Aydin *et al.,* 2013). The feeds that consumed by the fish have also affected the muscle nutritional composition among the different puffer species (Saito and Kunisaki, 1998). Table 3 shows a summarized comparison of proximate composition from different puffer fish species.

Conclusion

This study showed that muscle of *X. naritus* collected from Kg. Manggut and Kabong, Sarawak contained high amounts of moisture and crude protein contents as compared with other commercial fish. While the crude fat, crude fibre and ash contents were low for both locations. The proximate values obtained from this study shows that the yellow puffer fish *X. naritus* are good protein resources and can be considered for human consumption in countries where it is accepted as a delicacy. Nevertheless, it is safe for human consumption if the poisonous parts were removed thoroughly. The fish had to be prepared properly and completely cooked. It is essential to advice and educates the local population of Sarawak of the potential health risk of puffer fish consumption if not carefully prepared. Thus, the local people should be trained thoroughly in the preparation of yellow puffer fish. To our knowledge, this is the first report on nutritional compositions in edible part of yellow puffer fish from Sarawak and this finding may lead new insight for further research.

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Table 3: Proximate composition from different puffer fish species

Samples	Location	Moisture	Crude protein	Crude fat	Crude fibre	Ash	References
		Mean percentage (% ww)					
Fugu obscurus	Cultured	76.9	18.4	0.83	NA	1.52	(Tao *et al.,* 2012)
Fugu flavidus	Cultured	78.0	18.1	0.81		1.47	
Fugu rubripes	Cultured	77.3	17.8	0.73		1.54	
Takifugu rubripes	Cultured	78.7±0.5	16.5±0.4	0.9±0.1	NA	1.3±0.1	(Saito & Kunisaki, 1998)
	Wild	78.9±0.8	16.5±1.1	0.7±0.1		1.4±0.1	
Takifugu rubripes	Cultured	79.1-80.6	17.5-18.9	0.2-0.4	NA	1.1-1.4	(Hwang *et al.,* 2000)
Lagocephalus sceleratus	Wild	78.5	21.62	NA	NA	NA	(Aydın *et al.,* 2013)
L. lunaris	Wild	80.32	9.22	11.25	NA	0.96	(Eswar *et al.,* 2014)
L. inermis	Wild	86.05	8.92	11.98		1.27	
Takifugu obscurus	Cultured	79.73±0.52	18.44±0.11	1.31±0.21	NA	1.42±0.04	(Yuqi *et al.,* 2014)
Takifugu obscurus	Cultured	NA	18.7	1.79	NA	NA	(Gu & Zhao, 2008)
	Wild	NA	20.2	2.38			
L. wheeleri		NA	19.3	1.94	NA	NA	(Zhang *et al.,* 2004)
Arothron hispidus	Wild	70.4±2.5	20.1±0.4	7.1±0.9	NA	1.1±0.1	(Karunanidhi *et al.,* 2017)
Takifugu oblongus	Wild	70.5±2.6	20.6±0.6	6.3±0.7	NA	1.3±0.1	
Chelonodon patoca	Wild	73.5±2.0	17.9±0.3	6.9±0.7	NA NA	0.8±0.2	
Arothron immaculatus	Wild	75.0±3.0	18.3±0.3	4.9±0.2	NA	1.1±0.2	
Lagocephalus guentheri	Wild	71.1±0.4	18.8±0.4	7.7±0.4		1.1±0.1	
X. naritus	**Kg. Manggut**	**77.36±1.13**	**19.9±1.58**	**0.11±0.06**	**0.10±0.04**	**1.24±0.06**	**This study**
	Kabong	**79.97±0.56**	**17.67±0.91**	**0.09±0.03**	**0.05±0.03**	**1.02±0.06**	

NA – Not Available, ww – wet weight

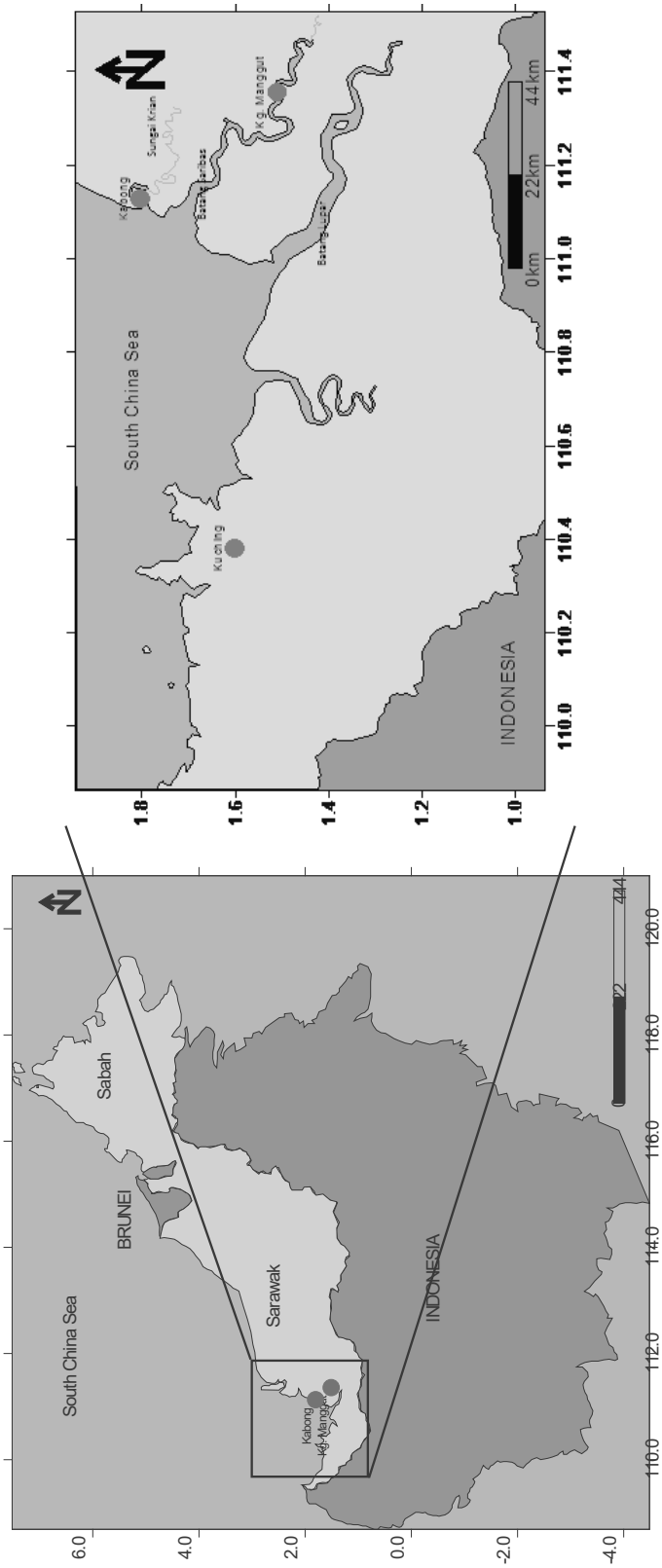

Figure 2: Location of yellow puffer fish *X. naritus* obtained in the present study