Faculty of Resource Science and Technology

MORPHOLOGY AND STOMACH CONTENT ANALYSIS OF GREEN ROUGH-BACKED PUFFER FISH, *LAGOCEPHALUS LUNARIS* FROM KUCHING, SARAWAK

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Bachelor of Science with Honours
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Morphology and stomach content analysis of green rough-backed puffer fish, *Lagocephalus lunaris* from Kuching, Sarawak

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DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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Table of Contents

List of Abbreviations

&	and
BW	body weight
^oC	degree Celcius
cm	centimeter
g	gram
GA	Group a
GB	Group b
GC	Group c
GD	Group d
GPS	Global Positioning System
IRI	Index Relative Importance
mL	milliliter
mm	millimeter
MU/g	Mouse unit per gram
N	number of prey item
O	frequency of occurrence
%	percentage
psu	per salinity unit
S1	Station 1
S2	Station 2
SE	standard error
SL	standard length
TL	total length
W	weight of prey item

List of Tables

List of Figures

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ABSTRACT

A study of morphology and stomach content analysis of *Lagocephalus lunaris*, the Tetraodontidae puffer fish had been carried out. Total of 101 individuals were used for morphological identification and stomach content analysis. These samples were collected in two months; August and October, 2010 at two stations from Muara Tebas, Kuching, Sarawak. The samples were injected by using 1mL of 10% formalin on their anus to prevent the stomach content being ruined. The physical characteristic was observed and body weight (BW), total length (TL), standard length (SL) and number of fins also had been measured. The average of TL was 8.7 ± 0.2cm, and SL was 7.1 ± 0.1 cm. There were no significant different ($p > 0.05$) in term of TL, SL and BW at Station 1, but the samples collected in Aug were slightly larger than samples collected in Oct at Station 2. Ranges of caudal, anal, pectoral and dorsal fins were 7 to 17, 9 to 12, 7 to 11 and 5 to 12. *L. lunaris* also was having small and short prinkles distributed at its upper site of body extended to dorsal fins. Percent Index of Relative Importance (%IRI) was carried out to determine the important of prey item in the diet. The most important prey item was crab with %IRI value of 51.61% (S1) and 53.13% (S2). It was followed by prawn with 34.57% (S1) and 37.30% (S2) and fish with the value %IRI was 12.70% (S1) and 6.66% (S2). The fewer prey items consumed by *L. lunaris* were squid (%IRI = 0.19% (S1), 1.17% (S2)) and bivalve with value of %IRI was 0.94% (S1) and 0.64% (S2). Furthermore, the ranking of most prey items were crab, prawn, fish, and the less important were squid and bivalve.

Keywords: *Lagocephalus lunaris*, morphology, stomach content analysis, prey items, %IRI

ABSTRAK

Sebuah kajian morfologi dan analisis kandungan perut telah dijalankan terhadap ikan buntal Tetraodontidae, Lagocephalus lunaris. Sebanyak 101 individu L. lunaris telah digunakan bagi mengidentifikasi morfologi dan analisis kandungan perut. Sampel-sampel ini telah ditangkap pada bulan Ogos dan Oktober, 2010 di Muara Tebas, Kuching, Sarawak. Sampel-sampel ini telah disuntik sebanyak 1 mL menggunakan 10% formalin pekat pada dubur bagi mengelakkan isi perut samnpel-sampel daripada hancur dan rosak. Ciri-ciri fizikal sampel dikenalpasti dan berat badan, jumlah panjang dan panjang piawai sampel juga telah diukur. Hasilnya, semua sampel memiliki rata-rata jumlah panjang sebanyak 8.7 ± 0.2 cm, panjang piawai 6.93 cm. Nilai -nilai ini tidak menunjukkan sebarang perbezaan yang ketara (p > 0.05) antara sampel yang diambil daripada Stesen 1 Manakala, nilai bagi sample yang diambil daripada Stesen 2 pada bulan Ogos dan Oktober menunjukkan pebezaan yang ketara di mana sampel dari bulan Ogos mempunyai saiz yang lebih besar. Julat bagi sirip ekor, dubur, dada dan punggung adalah 7-17, 9-12, 7-11 dan 5-12. L. lunaris juga memiliki duri yang kecil dan pendek pada bahagian atas tubuhnya. Nilai Peratus Kepentingan Relatif (%IRI) telah dijalankan terhadap isi perut bagi mengenalpasti item mangsa yang terpenting dimakan oleh L. lunaris. Ketam ialah item terpenting telah dimakan oleh L. lunaris dan menunujukkan nilai %IRI paling tinggi iaitu 51.51% (S1) dan 53.13% (S2). Ini diikuti oleh udang dengan nilai %IRI ialah 34.57% (S1) dan 37.39% (S2) dan ikan mempunyai nilai %IRI sebanyak 12.70% (S1) dan 6.66% (S2). Item-item yang kurang dimakan oleh sampel ialah sotong (%IRI = 0.19% (S1), 1.17% (S2)) dan bivalvia mempunyai nilai %IRI yang rendah iaitu 0.94% (S1) dan 0.64% (S2). Kedudukan item-item mengikut kadar kepentingan dimakan oleh L. lunaris adalah ketam, udang, ikan dan kedudukan terendah adalah sotong dan bivalvia.

Kata kunci: Lagocephalus lunaris, morfologi, analisis kandungan perut, item mangsa, %IRI

1.0 INTRODUCTION

Lagocephalus lunaris is a puffer fish that belonging to Tetraodontidae family and genus of *Lagocephalus*. It can be found in marine habitat and resides at tropical and subtropical water regions. Morphologically, it has known with having four strong teeth and has distribution of small spines in its dorsal body, also has a box-like of body shape. Its body is unhydrodynamic makes it swims slowly but capable of delicate maneuvering (Berra, 2001). It also named "puffer" because of its habit of inflating itself with water or air when threatened, creating a unique defensive mechanisms to prevent the predator.

There are many marine puffer fish that considered containing high concentration of toxicity such as *Takifugu niphobles, T. flavidus* and *L. inermis* and (Noguchi and Arakawa, 2008). These are known as puffer toxins because they are having extremely poisonous neurotoxin which is known as tetrodotoxin (TTX) in their body. This non-protein organic compound (aminoperhydroquinazoline) is the most violent toxins in puffer fish and can block voltage-dependent sodium channels (Sabrah *et al.*, 2006). Symptoms of TTX are salivation, weakness, diarrheal and abdominal pain, nausea and vomiting, respiratory paralysis and can cause human death.

TTX in puffer fish is influenced by two main sources which the first is produced by puffer fish itself (endogenous), mostly from its organs such as liver, skin, intestines and gonads. Second, TTX-source is taken from outside and accumulated in fish body (exogenous), usually by its feeding habits. It has TTX toxins in its body when it feeds on a variety of stationary or slow-moving invertebrates (Miyazawa and Noguchi, 2001) and marine bacteria (Noguchi *et al.*, 1986; Hashimoto *et al.*, 1990).

Then, TTX are believed derived from food chain and not endogenously produced (Noguchi *et al.*, 2006). Due to this situation, *L. lunaris* possibly accumulate TTX in its body by feeding TTX-organisms. There are many cases of human poisoning due to consume of puffer toxin are being reported in Taiwan (Hwang *et al.*, 1992), China (Yu & Yu, 1997), Japan (Kodama *et al.*, 1984) and Bangladesh (ICDDR, 2008). Therefore, the consumption of this species has been being prohibited for a certain period in order to reduce the amount of intoxication cases among people.

Lagocephalus species are widely being marketed and consumption of *L. lunaris* can lead to food poisoning cases for human. There are three of *Lagocephalus* species which are *L. wheeleri, L. spadiceus and L. lunaris* that considered as having similar morphological characteristics. However, *L. lunaris* is the only *Lagocephalus* species known as toxic while the others two species are non-toxic puffer fish (Ngy *et al.*, 2008). Morphological differences based on distribution patterns of small spines in their dorsal body can be used to differentiate among these three species of *Lagocephalus* (Ngy *et al.*, 2008).

This study has been carried out in Muara Tebas Sea in Kuching because fishing activities mostly done and the yield being marketed commercially in Kuching area. Therefore, status of *L. lunaris* or locally known as "Buntal" can be documented and it is important to identify the occurrence of this species in Kuching water thus give information on the risky of this species especially to local people. This species can be eaten safely when cooked by experienced people. However, it is harmful to inexperienced people who consume *L. lunaris* because intoxication can be occurred.

According to Bachok *et al.* (2004), stomach content analysis usually is being conducted in order to determine dietary compositions and food habits of fish species. The Index of Relative Importance can be used to verify of food items in the stomachs. Thus, the composition in of food that contained in stomach of *L. lunaris* can be analyzed through this study and the contribution of prey items fed by *L. lunaris* to TTX accumulation in its body can be estimated.

In addition, stomach content analysis also has been used by Bascinar and Saglam (2009) to identify the effect of sex, seasons and also size characteristics to the diet of fish. The types of prey items that commonly found in stomach of fish are plants parts, insects, crustaceans, rotifers, worms, red mullet, mud shrimp, sea horse, harbor crab, copepods bivalve and green algae (Bachok et al., 2004; Bascinar and Saglam, 2009; Ikpi and Okey, 2010; Krumme et al., 2007).

The objectives of this study are; (1) to describe morphological structure of *Lagocephalus lunaris* that have been collected from Kuching, Sarawak and (2) to determine the type of its diet composition through stomach content analysis. Therefore, based on the objectives of this study, methods used are morphometrical identification and fish stomach contents analysis.

2.0 LITERATURE REVIEW

2.1 Morphology and distribution

Lagocephalus lunaris was having elongated body with a broad head and slightly box-shaped (Attack, 2006). Its eyes were large and rounded with free orbital margin on lower side with the nasal organ in front of eyes consist of a short papilla with two large openings. Upper side of lateral line was conspicuous, extending from the top of head and curves down to flank where it reaches the caudal peduncle.

In addition, dorsal fins and anal fins were short and set far back on the body also the caudal peduncle compressed and fins slightly forked. It had attractive skin with metallic gold colour, darker on the back and sides to an almost silvery gold colour underneath. The dorsal fins have 11 to 13 soft rays and anal fins have 10 to 12 soft rays. Typical scales were absent, but many spinules often present on back and/or belly, sometimes on sides.

Distribution patterns of small spines in its dorsal body had been differentiated between three *Lagocephalus* species that considered in having similar morphological characteristics (Ngy *et al.*, 2008). For *L. wheeleri*, the distribution of small spines was apart from the base of dorsal fin ray with elliptical shape. While the small spines distributed in tadpole-like shape and the tail reached to the base of dorsal fin ray for *L. spadiceus*. Lastly, there was elliptical shape of small spines distributed on *L. lunaris* which the small spines were extended to the base of dorsal fin ray.

Commonly, most species mottled and variegated on back and side, sometimes with spots of various sizes and colours, whereas others uniformly coloured. It is marine organisms and can be found throughout the Indo-Pacific Ocean and common around Peninsular Malaysia and Sarawak.

2.2 Feeding habits study

Previously, there were two types of analysis had been carried out in order to determine the feeding habit of fishes which were stomach content analysis and stable isotope analysis.

2.2.1 Stomach content analysis

According to Fanelli and Cartes (2010), prey items of samples were identified to the lowest possible taxonomic level. Then, they were counted under binocular microscope and then weighed with the precision of 0.0001g, after removed the excess moisture by blotting with tissue paper. The percentage frequency of occurrence (%F), numerical abundance (%N), and gravimetric composition (%W) were used to estimate the contribution of each prey items in the fishes' diet. The Index of Relative Importance (IRI) also was used, where IRI = (%N + %W) x %F and expressed as percent IRI, where (%IRI) = [(IRI/∑IRI) x 100].

Then, prey items sorted according to their %IRI in decreasing order and cumulative %IRI was calculated based on Hyslop (1980). The stomach fullness (stomach weight/fish weight x 100) and trophic diversity indices which was Shannon-Wiener diversity index *H'* were calculated. Torres-Rojas *et al.* (2010) stated that Levin standardized index "Bi" was used to evaluate the trophic-width niche of fish and data were pooled, and then separated by gender. Index values range from 0 to 1, with high values (>0.6) indicating generalist diets and lower values (<0.6) indicating diet dominated by few prey items (Labropoulou and Eleftheriou, 1997).

2.2.2 Stable isotope analysis

According to Renones *et al.* (2002), this study shows reflected of the isotopic values of food consumed after fractionation through feeding, food processing and excretion process to the carbon and nitrogen isotope ratios of an organisms. ^{15}N used as indicator of trophic level of an organism through its life cycle and ^{13}C provided information in materials at the base of food web.

This study also used to determine individual diet specialization, within- and among-population variation in trophic regimes. Based on Fanelli and Cartes (2010), analysis of stable isotope composition; nitrogen (N) and carbon (C) served an indication of the origin and transformation of organic matter. Stable N-isotope data had been used to estimate the trophic levels of organisms while ^{13}C might act as indicator for primary organic carbon sources in organisms' diet.

Stable isotope also had been practiced to detect seasonal changes in fish diet and had been applied in deep-sea ecosystem to explain temporal changes in trophic levels were limited. Then, isotope composition gave the average indication of trophic level of food that had been assimilated into tissue.

2.2.3 Study cases on stomach content analysis

A study of dietary composition and feeding ecology on banded puffer fish, *Colomesus psittacus* had been done by Krumme *et al.* (2007). The samples were collected from intertidal mangrove area during diurnal neap tides. After that, stomach content analysis was formed to the samples where the contents of stomach were observed and identified to the lowest classes, and then the numbers of prey were calculated. Most of the prey items identified were small in size which believed were being broken as they reached the intestine. The patterns in diet composition and feeding strategy of this puffer fish also had been examined using non-parametric multidimensional scaling (MDS). The result showed that Brachyuran crab and Cirripedia were recorded the most number of diet composition. The pattern in feeding, energy flow, interaction between plant and animal and the behavior of puffer fish during feeding season were identified in this study.

Besides that, a study on stomach content analysis had been conducted related to marine fishes along east coast Malaysia and the South China Sea (Bachok *et al.*, 2004). This study was focused on both commercial demersal and pelagic fishes from Terengganu waters. The purposes of this study were to verify fishes' dietary compositions and food habits. It also aimed to discover better understanding on the biology of predator and prey species and for stock- and ecosystem-level analyses. Dominant prey items that had been identified fed by fishes in Terengganu waters were cephalopods, crustaceans, mollucs and enchinoderms.

Percentage of IRI also had been identified to define the feeding ecology of scalloped hammerhead shark, *Sphyrna lewini* (Torres-Rojas and Hernandez-Herrera, 2009). In stomach content analysis done in this study, gender factor had been considered to differentiate the composition of diet in this fish. The results showed that 85% of stomachs were contained with food and the rest were empty. There were three main species composed on the diet which were cephalopods, crustaceans and other marine fishes. Value of %IRI recorded by cephalopods, Carangidae fish and Synondotidae fish were 18%, 25 and 19%.

2.3 Tetrodotoxin (TTX) in aquatic organisms

2.3.1 Properties of TTX

Arakawa *et al.* (2010) stated that tetrodotoxin (TTX) was a potent neurotoxin of low molecular weight, named after puffer fish order which was Tetraodontiformes. TTX was insoluble in organic solvents and water, but became soluble in water when acidic was added. It also stable toxin in neutral to weakly acidic and did not decomposed by cooking. TTX inhibited action potential by plugging the sodium channels on nerve or muscle at extremely low concentrations.

As shown in Figure 1, the positive charged of nitrogen (N^+) ion acted as sodium ion (Na^+) which was allowing TTX to dive into the channel and bound well to certain peptides. The main symptoms of human intoxication were numbness of lips, tongue and limbs, respiratory distress and TTX might cause death due to respiratory failure in most critical cases.

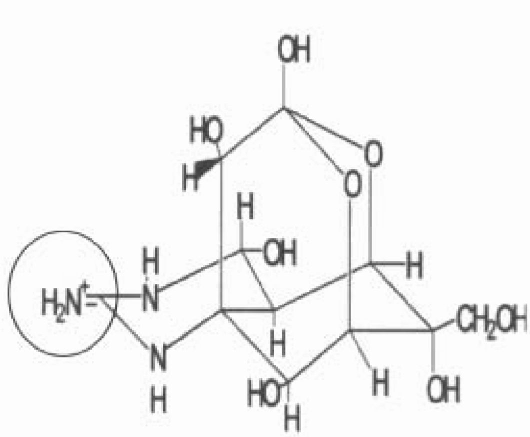

Figure 1. Chemical structure of TTX (Noguchi *et al.*, 2006).

2.3.2 Distribution of TTX

Based on Noguchi and Arakawa (2008), there were 22 species of Tetraodontidae family listed as toxic puffer fish in Japan including *Takifugu niphobles*, *Tetraodon alboreticulatus*, *Chelonodon patoca*, *Canthigaster rivulata*, *Lagocephalus inermis* and also *L. lunaris*. Commonly, the distribution of TTX toxin in body parts of puffer fishes were in their skin and liver. However, Noguchi and Arakawa (2008) had been reported that *L. lunaris* was composed high amount of TTX toxin in muscle with more than 1000 MU/g tissue which considered as strongly acid.

Japanese Ministry of Health, Labour and Welfare had prohibited those organs from being used for food. In Taiwan, there were 23 species of marine puffer fish were listed as toxic including *Takifugu oblongus* and *T. niphobles* and also *L. lunaris*. Then, *L. lunaris* from Thailand and Cambodia was considered as potential causative species of puffer fish poisoning (Ngy *et al.*, 2008).

In addition, TTX had been detected in other marine animals containing TTX in their body. For examples, goby *Yongeichthys criniger*, blue-ringed octopus *Hapalochlaena maculosa*, carnivorous gastropods *Charonia sauliae*, starfish of genus *Astropecten*, xanthid crabs, horshoe crab *Carcinoscorpius rotundicauda*, flatworms and ribbonworms (Noguchi and Arakawa, 2008). TTX productivity also had been found in certain marine bacteria which were *Vibrio alginolyticus*, *Shewanella alga* and *Alteromonas tetraodonis*. They were isolated from TTX-bearing organisms such as pufferfish, toxic starfish and the xanthid crab.

2.3.3 Accumulation of TTX in puffer fish

Previous studies had been proved accumulation ways of TTX in puffer fish. TTX was distributed over various organisms including food animals of puffer fish, the trumpet shell accumulated TTX by ingesting toxic starfish, marine bacteria primarily produced TTX, puffer fish became non-toxic when they were fed on the TTX-free diets and such non-toxic puffer fish efficiently accumulated TTX when exposed to TTX diets.

These suggested that puffer fish did not synthesize TTX by itself but accumulated through food chain and mostly started from marine bacteria. Moreover, Noguchi *et al*. (1981, 1982) stated that in some TTX-bearing gastropods, the food was determined to be the source of the TTX. While intestinal bacteria of TTX-bearing organisms and other marine bacteria are being found to be producers of TTX (Hashimoto *et al*., 1990), although some were found to produce TTX. Marine bacteria as the primary TTX producer are the initiator of TTX accumulation in puffer is through food web.

2.3.4 TTX intoxication

There were two types of TTX intoxication which were due to puffer fish and due to marine animals other than puffer fish. Examples of puffer fish that could be given intoxication effect was *Takifugu poecilonotus* as a case had been reported and recorded in 2008 by Japanese Ministry of Health, Labour and Welfare (Arakawa *et al*., 2010).

It was necessary to identify the causative species as well as investigation of leftover fish toxicity. Species identification mostly done based on morphological characteristics such as pattern of the skin, shape of fins, and distribution of small spines. In addition, there was a case of TTX intoxication caused by scavenging gastropod *Nassarius glans* in July, 2007. It composed of 4.290 MU/g of TTX in cooked muscles and digestive glands (Taniyama, 2009). These both of puffer fish and gastropods were considered to obtain TTX toxin exogenously, from their toxic prey organisms then accumulated the toxin in specific organs.

2.3.5 Seasonal variations

A study about seasonal variations effected to the toxic ratio and toxicity of seven species of puffer fish was done by Hwang *et al.*, (1992). The species were *Lagocephalus gloveri, L. wheleri, L. inermis, Fugu oblongos, F. xanthopterus* and *Amblyrhynchotes hypselogenion* and also *L. lunaris.* These experiments were taken during spring (March-May), summer (June-August), autumn (September-November) and winter (December-February) season.

Most species were having higher percentage of toxic ratio in autumn and winter season. Toxic ratio for *L. lunaris* was having 83.3% of toxic ratio in autumn and 85.7% in winter. The toxicity of livers and ovaries also had higher value in autumn and winter. Moreover, *L. lunaris* was having higher amount of toxic in ovary compared to liver over the year.

3.0 MATERIALS AND METHODS

3.1 Sampling area and Field collection

Sampling areas were located in Muara Tebas, Kuching where the Global Positioning System (GPS) (model 60 CSX GARMIN) reading between 01^0 38' N, 110^0 31' N and 01^0 39' N, 110^0 33' E. This area also near to Bako National Park, Kuching and mostly resided by fishery communities.

Depth range of the water was approximately 3.8 to 4.3 m while salinity was 15 psu. Water temperature of the sampling area was between 30.6 to 32.5°C and pH recorded was 7.86 to 8.02. Then, value of turbidity and dissolved oxygen were 15.0 to 22.9 NTU and 5.03 to 6.32 mg/L. Instruments that had been used to measure those parameter were EUTECH multiparameter, turbidity meter, Secchi disk, hand refractometer and depth finder.

In addition, the sampling collections were done by using trawling boat and size of mesh was 1 inch. There were also two stations (Station 1 and Station 2) had been established during sampling activities on August and October, 2010.

Then, 101 samples of puffer fish caught were injected using 1 ml of 10% buffered formalin on their anus. After that, the specimens had been kept in cooler box with ice and brought back for laboratory analysis. They also had been kept in -20^0C freezer until used.

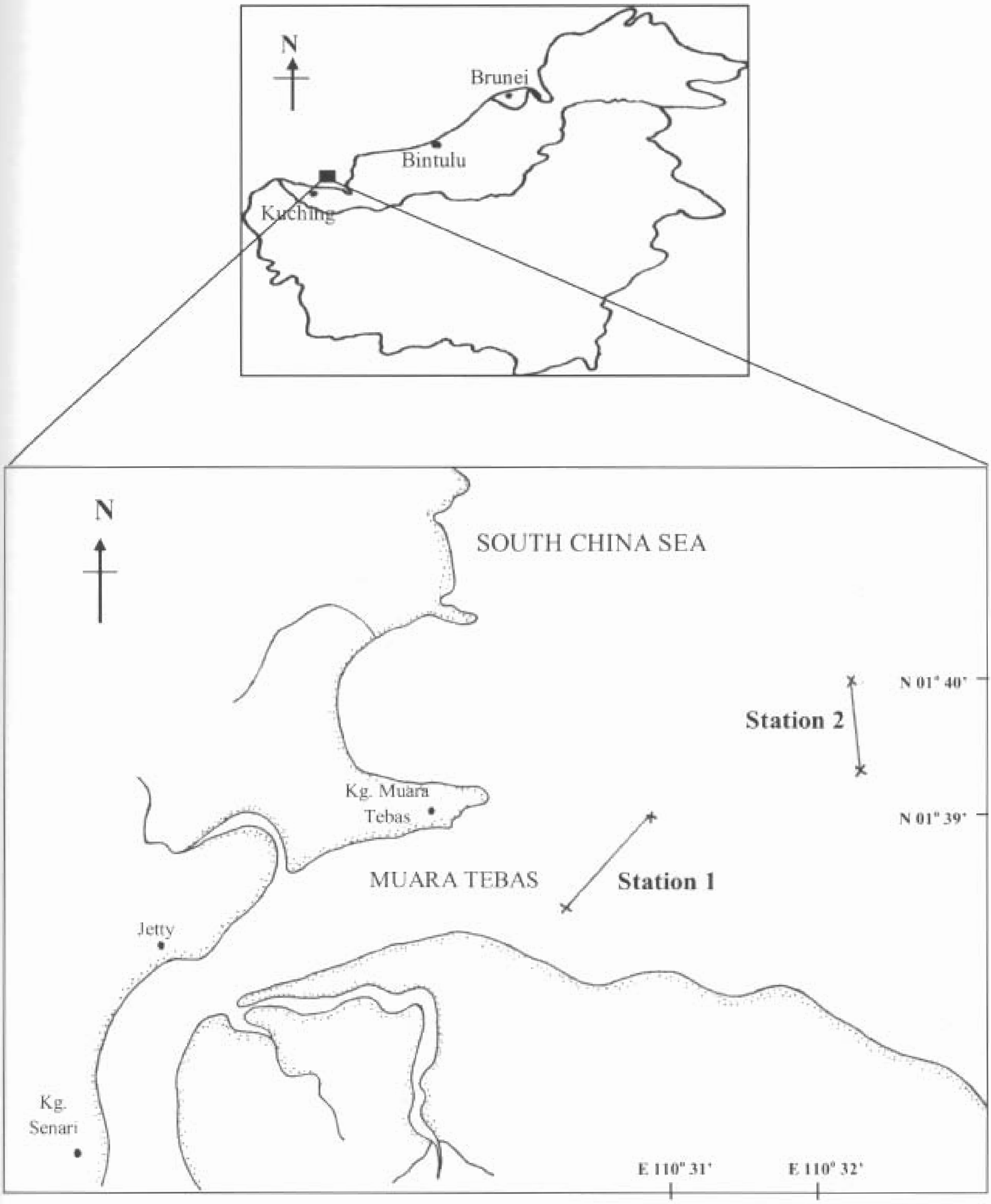

Figure 2. Map of Sarawak and location of sampling sites where samples of *Lagocephalus lunaris* were collected.