12. Analyses of Marine Bioresources for Development of Potential Local Resources in Bicol Region, Philippines

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1. Introduction

The Philippines, being an archipelago, was dubbed as the center of the center of marine biodiversity[1]. The Philippines has ranked 9th among the top producing countries constituting 2.01% of total world production of 205.6 million metric tons (MT). On the other hand, it ranked 11th in the world aquaculture production of fish, crustaceans and mollusks with a 1.03% share to the total global aquaculture production of 80.01 million MT. The Philippine's volume of fisheries production has reached 4.30 million MT in 2018 from the sector of aquaculture (52.9%), municipal (25.0%) and commercial (21.7%) fisheries[2].

The Bicol region has a total share of 5.9% (256,589.96 MT) in the total Philippine fisheries production in 2018[2]. The Bicol region's municipal fisheries contributed the highest percentage of 49.4%[2]. Researches focusing on stock assessments, reproductive biology and morphological studies of fish species like siganids, crab (mud crab and blue crab), and mollusks had been carried out[3-5]. Various finfishes, elasmobranchs and invertebrates are reported to be caught[6,7] which are sold fresh or in dried form[8]. The region is also one of the sources of tuna and tuna-like species which are processed in Mindanao[9]. In addition, not only for fresh fish caught, the Bicol region one of the important sources of dried fish in the Philippines[8]. Two of the most important fishing grounds in the Bicol region, San Miguel Bay (SMB) and Lagonoy Gulf (LG) (Figure 1), have been source of various aquatic resources[6,7]. SMB is characterized as a large, shallow estuarine water body with muddy and sandy substrate[10] have been known for its catch on tigertooth croaker (*Otolithes ruber*) fish which are being processed in the region. Moreover, commercially important finfishes are caught in LG[7]. LG's geographical location near the spawning ground of Anguillid eels is advantageous for its recruitment however very limited information on its fisheries were available.

Tigertooth croaker (*O. ruber*), locally known as *abo* (Figure 2A), is one of the major species in SMB[11]. Other croaker species with similar morphological characteristics often caused confusion, therefore, species identification needs to be confirmed via molecular approaches. Tigertooth croaker is commercially important

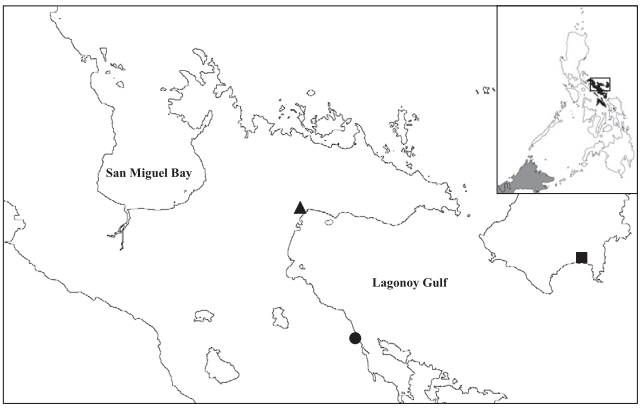

Figure 1 Map of Bicol Region showing the two fishing sites, San Miguel Bay and Lagonoy Gulf.
●: Comun river, Albay; ▲: Lagonoy river, Camarines Sur;
■: Bato river, Catanduanes.

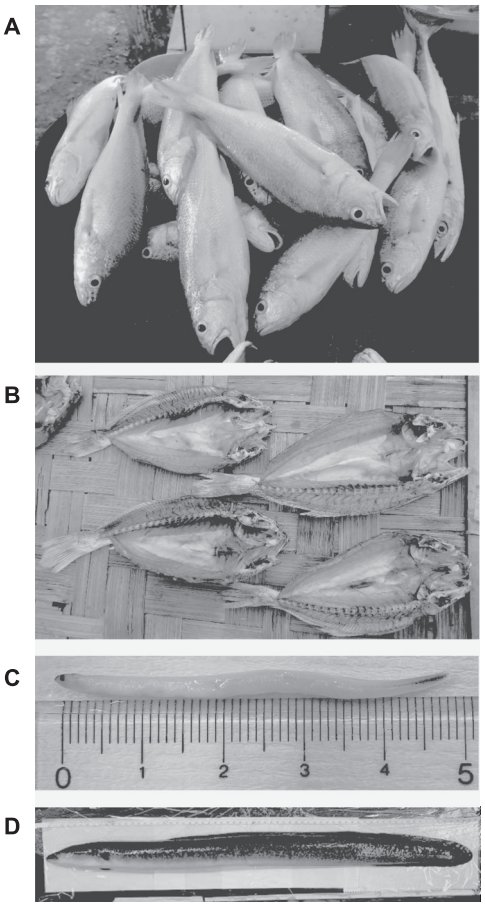

Figure 2 Target commodities: (A) fresh *abo*; (B) dried abo; (C) glass eel; (D) adult freshwater eel (*A. marmorata*)

and processed as a popular dried fish or *"badi"* (Figure 2B). A major landing site of *abo* is located at Calabanga, Camarines Sur where at least 100 dried fish processors are found in the area. Yater et al.[12] reported that dried fish processing in SMB involved soaking in brine for 12 h and sun drying for 4 -5 h, hence, dried fish had high salt content and short drying time. At present, commercial dried tigertooth croaker are produced with long drying time and brining at high concentration of salt, as highly dried and salty *abo* products are more popular to the market. Nowadays, consumers also demand low salt dried products due to health concerns. Thus, there is an opportunity to develop a dried fish product that might improve the quality, taste and consumer acceptability. Information on the biochemical and sensory quality of the dried tigertooth croaker is of interest to retailers and consumers, however, limited data is available.

Current available information on glass eels (Figure 2C) were on its trade by the fishers of LG to other parts of Luzon and in Mindanao. Glass eels commonly caught at the river mouth undergo several steps prior to packaging and transport such as sorting, rapid salinity shocking, methylene blue treatment and groupings as to species[13]. On the other hand, adults collected at the upstream of the rivers are commonly sold directly to consumers within the region[14]. Issues on glass eel discards (non-marketable weak individuals) and the low number of adult freshwater eels caught by traditional fishing practices needed consideration for effective management of available resources.

Anguilla marmorata was reported to have comparable nutritional value and superior preference test in terms of texture[15,16], although market value is lower than that of *A. bicolor pacifica*[17]. Since these species is of similar genus with *A. japonica* which was reported to have high collagen content compared to other fishes belonging to 24 species[18], *A. marmorata* and *A. bicolor pacifica* may also have high collagen. Collagen, which

is a dominant protein in fish muscle, is denatured and solubilized as gelatin, unlike other proteins which precipitates upon cooking[19]. Gelatin is reported as a taste enhancer[20]. *Anguilla marmorata* and *A. bicolor pacifica* cooked as *paksiw*, which is a famous cuisine in the Philippines, may have gelatin in the soup that can enhance its taste, therefore investigated.

Dried salted *abo* in SMB and Anguillids in LG have high potential for product development. For effective utilization and management of available marine bioresources of the region, science-based information is important. To assess the current status of tigertooth croaker and Anguillid fisheries in SMB and LG, an interview was conducted given limited information on the fishing practices (gears, collection, fish production); confirm identification of tigertooth croaker at the species level; clarified the species composition of Anguillids recruited and its composition; improve the processing methods for dried salted tigertooth croaker production; investigate the changes occurred during *abo* processing and the potential utilization of *A. marmorata* (Figure 2D) through the presence of gelatin.

2. Methods

2.1 Interview

A preliminary survey was conducted to assess the dried tigertooth croaker processing situation in SMB and optimize the processing conditions to be employed in the laboratory preparation of dried salted *abo*. Face-to-face interviews were carried out in three batches in Calabanga, Camarines Sur, Philippines. Nine dried fish processors selected by purposive sampling were the respondents for the first batch of interview to document their existing dried tigertooth croaker process flows. To supplement and confirm the information gathered, key informant interviews were also conducted to dried fish processors, small-scale fisherman and officer from the local government unit of Calabanga, Camarines Sur. Secondary data from the National Stock Assessment Program of the Bureau of Fisheries and Aquatic Resources (NSAP-BFAR) Region 5 was obtained through email communication.

An interview was also conducted to elicit information about fishing practices limited to gears and their uses from[37] glass eels and adult eels fishers and collectors of Balza, Malinao Albay, Lagonoy Camarines Sur, and Bato, Catanduanes. In addition, consumption and cooking ways of adult freshwater eels were also documented.

2.2 Sample Collection

Fresh *abo* were purchased in the local market of Calabanga, Camarines Sur. The pectoral fin was excised and preserved in 95% ethanol for molecular analysis.

For the Anguillids, glass eels were collected using a modified fyke net installed near or at the river mouth of Comun river and Lagonoy river, while a push net was used in the Bato river. The collection was only conducted for a day at 18:00 h for 2-4 h during the new moon phase between July 2018 and June 2019. The glass eels collected (n = 4,801) were preserved in 95 % ethanol for morphological identification. Approximately 10 % (n = 554) of the morphologically identified specimens were randomly selected for molecular analysis.

Adult *A. marmorata* and *A. bicolor pacifica* were also collected at Teima River, Nago City, Japan, and procured at Bric's ECO JAPAN, Hyogo, Japan, respectively, for *paksiw* preparation to investigate gelatin content.

2.3 Morphological Identification of Anguillids

The glass eels tail part was observed under a stereomicroscope (2 x magnification) with an external light source and photographed. The pigmentation patterns on the tail and the caudal fin was used for morphological identification[21].

2.4 Molecular Identification of *Abo and Anguillids*

2.4.1 DNA Extraction and Polymerase Chain Reaction (PCR)

Fish tissue or fin were cut into small pieces and lysed overnight using conventional commercially available kits [DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) or FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp., MI, USA)]. The target fragment genes such as 16S rRNA and cytochrome c oxidase I (COI) were amplified using the adapted and designed primers so with the thermal cycler profile used[22,23]. Successful amplicons were visualized on agarose gel electrophoresis stained with GelRed Nucleic Acid Gel Stain (Biotium, CA, USA).

2.4.2 Restriction Fragment Length Polymorphism Analysis for Anguillids

Simulated RFLP patterns for six target Anguillid species *A. marmorata, A. bicolor pacifica, A. luzonensis, A. japonica, A. bicolor bicolor*, and *A. celebesensis* found in the Philippines were used as reference of species identification. The complete mitochondrial genome of these Anguillid downloaded from the National Center for Biotechnology Information (NCBI) aligned by MEGA 7 or X software[24,25] was used for virtual digestion of 32 restriction enzymes. Based on its ability to cut DNA into fragments and the patterns produced, *Msp* I was used for species identification. *Dde* I was used to further distinguish *A. bicolor pacifica* from *A. bicolor bicolor*. The RFLP patterns were visualized by gel electrophoresis were then compared with the simulated RFLP patterns for species identification.

2.4.3 DNA Sequencing

The designed and adapted oligonucleotide primers[26-29] (see Table 1), were used to sequence the 16S rRNA and/or COI target fragment genes. The PCR product purified by Agencourt AMPure XP (Beckman Coulter, CA, USA) was used for direct cycle sequencing with the ABI BigDye v.3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA). Sequences generated from the 3130 Genetic Analyzer (Applied Biosystems, CA, USA) were edited and manually aligned using Chromas version 2. 6. 6 and the MEGA 7 or X software[24, 25]. Phylogenetic tree were constructed using MEGA 7 or X[24, 25] with 1,000 bootstrap probabilities for species identification of *abo* and the glass eels with unknown RFLP patterns and confirmation of representative specimens with expected ones.

2.4.4 Comparison of the Morphological and Molecular Identification of Anguillids

The data on the correct and misidentified species by morphology as confirmed by molecular analysis were presented in percentage. In addition, re-observation of the pigmentation patterns of 140 individuals identified by molecular analysis and groupings based on the pigmentation patterns was also expressed in percentage.

Table 1 PCR primers used for DNA sequencing and PCR-RFLP analysis

Specimen	Sequence (5'-3')	Oligo name	Target Fragment Gene	Step/Analysis Used	Reference	Amplification profile
Abo	*CGCCTGTTTATCAAAAACAT*	16SAR	16S rRNA	PCR amplification; Sequencing	Palumbi et al.[24]	Lakra et al.[26]
	CCGGTCTGAACTCAGATCACGT	16SBR		PCR amplification; Sequencing		
	TCGACTAATCATAAAGATATCGGCAC	CoxI Fish F2	COI	PCR amplification; Sequencing	Ward et al.[25]	Lo et al.[27]
	ACTTCAGGGTGACCGAAGAATCAGAA	CoxI Fish R2		PCR amplification; Sequencing		
	CTTAGTTAACAGCTAARCGC	5346R		PCR amplification; Sequencing	Designed primers	initial denaturation 94 °C, 5 min; 35 cycles: denaturation 94 °C, 30 s; annealing 50 °C, 30 s, elongation 72 °C, 60 s; final extension 72 °C for 10 min
	GTRTCTACGTCTATTCCGAC	6446R		PCR amplification; Sequencing		
Glass eel	*CCGCTTAAACATTCAGCC*	5503F1	COI	PCR amplification, RFLP		
	GGGGGTTCAATTCCTTCC	7138R1		PCR amplification, RFLP		
	ACATTCAGCCATCTTACC	5511F2		Sequencing		
	CAATTCCTTCCTTTCTTG	7131R2		Sequencing		
	VCCAGTCCTAGCTGCAGG	6126F3		Sequencing		
	TGCRATGATTATTGTGGC	6468R3		Sequencing		

2.5 Chemical Analyses

2.5.1 Materials

Fresh tigertooth croaker were also purchased from the local fish market in Calabanga, Camarines Sur, Philippines. Chemicals (analytical grade) used in all experiments were obtained from Nacalai Tesque, Inc. (Japan) or Thermo Fisher Scientific (USA).

2.5.2 Overview of Dried Fish Processing Methods

For the laboratory-preparation of dried tigertooth croaker, approximately seven 7kg of tigertooth croaker was transported in ice to the Fish Processing Laboratory of Partido State University, Sagñay, Camarines Sur, Philippines. The average weight and length of tigertooth croaker were 70.0 ± 11.1 g and 20.0 ± 1.06 cm, respectively. Collected tigertooth croaker were divided into six groups, with combination of three salt concentrations (4%, 8% and 12% w/w) and two drying time conditions (6 h and 12 h). Fish were washed with tap water, degutted, cut into butterfly fillet and soaked in prepared brine solutions (for 30 min). Fish were then rinsed, drained, placed in racks for sun-drying at 31-34 ˚C, and vacuum packed. (Figure 3).

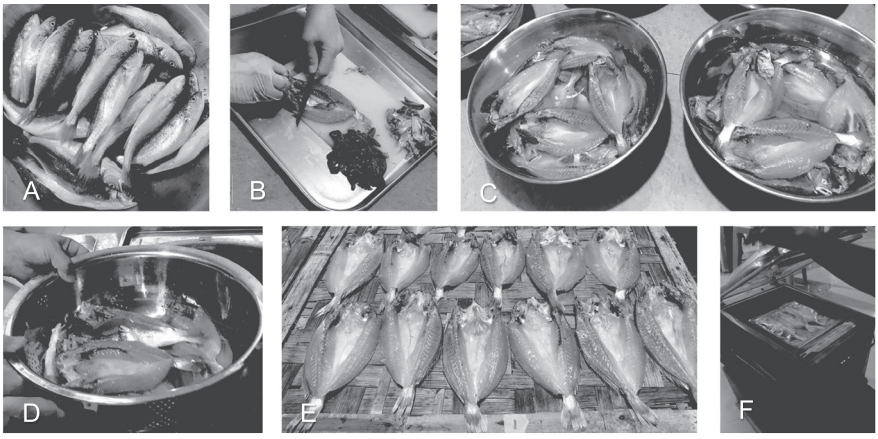

Figure 3 Process flow of laboratory processed *abo* (A) fresh *abo*; (B) degutting; (C) brining; (D) rinsing and draining; (E) sun drying; (F) packaging.

2.5.3 Proximate Analysis and Salt Content Analysis

The laboratory-produced dried tigertooth croaker were subjected to proximate analysis for moisture, ash, crude protein, and crude lipid following the official methods described by the Association of Official Analytical Collaboration (AOAC) International[30]. The salt content was determined by the using a PAL-SALT Probe digital salt meter (ATAGO CO. LTD., Japan).

2.5.4 *Paksiw* Preparation and Gelatin Content Determination

Paksiw for Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was prepared by downscaling the eel samples and water, based on Tiangson-Bayaga and Deveza[31]. The skin, muscles, and bones were separated from a slice of the middle and tail regions of *A. marmorata* and *A. bicolor pacifica* and then weighed. Weight if each eel part (92.2-154.5 mg) for *paksiw* preparation was based on its percentage composition (9.1-76.3%) of the sliced regions. The eel parts were cooked individually and centrifuged to separate the supernatant liquid. A 100-mL supernatant of each of eel parts were mixed to represent the *paksiw* soup. The gelatin in the paksiw soup was estimated through Coomassie Protein Assay with gelatin as the standard.

2.5.5 SDS-PAGE Analysis

Tricine SDS-PAGE was carried out according to the method of Schägger and von Jagow[32] with some modifications. Five ug of protein was mixed with sample buffer solution with or without 2 x 2-Mercaptoethanol (2-ME; Nacalai Tesque, Inc., Japan) and electrophoresed in 10% polyacrylamide gel. Laemmli method[33] was used to visualize gelatinous proteins in the *paksiw* soup of *A. marmorata* and *A. bicolor pacifica* wherein 1 uL of each eel parts and 3 uL of the mixed sample with equal volume of sample buffer solution was loaded in 7% polyacrylamide gel. Gels were stained with 0.04% Coomassie Brilliant Blue R-250 and destained with 10% acetic acid.

2.5.6 LC-MS/MS

Gel pieces were excised, digested in trypsin and analyzed using a Finnigan LTQ XL mass spectrometer (Thermo Fisher Scientific, Inc.), coupled with liquid chromatography (Michrom BioResources, Inc. AUBURN CA, USA), equipped with a nanoelectrospray ion source (Thermo Fisher Scientific, Inc.). The MS/MS data were analyzed with an in-house database by Mascot server, using Proteome Discoverer software version 1.2 (Thermo Fisher Scientific, Inc.).

3. Results

3.1 Interview

3.1.1 Status of Tigertooth Croaker Fishing and Dried *Abo* Processing in SMB

The highest landed catch of tigertooth croaker (40.56%) was recorded in the landing center at Sabang, Calabanga, Camarines Sur. From 2016 - 2018, landed catch was observed to have increased with 104.78 MT of overall catch in SMB in 2018. Varying peak and lean months of landed catch of tigertooth croaker were recorded during the four-year period (2015 - 2018) (NSAP-BFAR data, unpublished). On the other hand, as reported by respondents, the peak and lean seasons could be observed between August and September and

between January to March, respectively.

3.1.2 Current Status of Freshwater Eel Fishery

Gears used for collection of freshwater eels depends on the life stages. Glass eels are commonly caught by the modified fyke net (MFN) (Figure 4A) assisteduy scoop net (Figure 4B) set at the river mouth which coincides with the new moon phase. Adult freshwater eels are mostly caught during rainy season at the river upstream. Among the gears used by the adult freshwater eel fishers, it was quite alarming to note that electrofishing gears (Figure 4C) were still used, considering that fishing using electricity in all water of the Philippines is illegal as stated in the Republic Act 10654 Section 92.

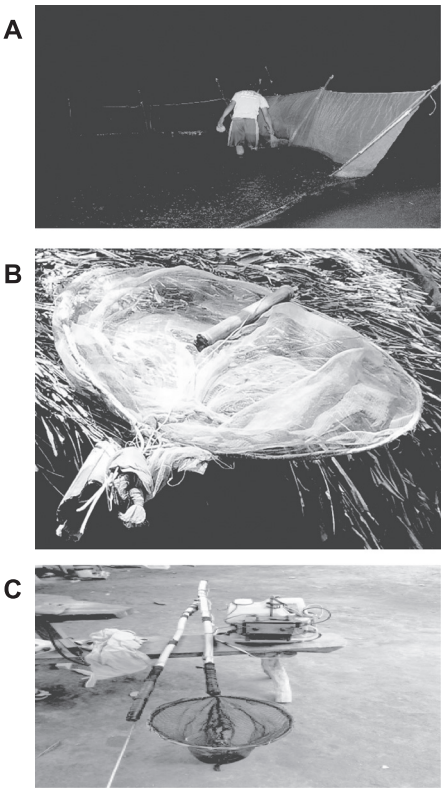

Figure 4 Gears used for eel fishing
(A) Modified fyke net; (B) scoop net; (C) electrofishing gear

3.2 Species Identification

3.2.1 Identification of *Abo* Species

Both the COI and 16S rRNA sequences of three *abo* specimens have 100% nucleotide identity. For COI, the nucleotide differences between *abo* and *O. ruber* sequences (EF534126) was estimated to be 13.1% (85/651). On the other hand, for 16S, only 0.9% nucleotide differences (5/571) were found among *abo* and *O. ruber* (EF528214) sequences. The NJ tree based on COI sequences revealed that *abo* formed a separate subclade with the clustered *O. ruber* and *Otolithes cuvieri* (98% bootstrap support), whereas the *Otolithoides* species formed a separate group. On the other hand, NJ tree of 16S sequences clearly showed that *abo* is clustered with O. *ruber* (100% bootstrap support).

3.2.2 Species Identification and Composition of Anguillids

Our recent study on species composition based on morphology limited to pigmentation pattern revealed that *A. japonica* can be easily identified given the absence of pigments which was found in negligible quantity (0.1%). Whereas, A. *bicolor pacifica* seems to be almost distinguished based on the dense pigment patches on the tail reaching until the caudal fin tip. On the other hand *A. marmorata* and *A. luzonensis* were difficult to identify; hence, individuals with pigmentation pattern on the tail not reaching the caudal fin was grouped as *A. marmorata*. Consequently, among the 4,000 pigmented glass eels, 89.8% were grouped as *A. marmorata* and 10.1% as *A. bicolor pacifica*. The existence of A. *luzonensis* in the group of *A. marmorata* reported was possible. Clarification by molecular analysis has revealed the occurrence of *A. luzonensis* being the 2nd most dominant species that occurred in Comun and Lagonoy rivers next to *A. marmorata*, which is the most abundant species in all the rivers (71.1-98.0%) (Figure 5). *Anguilla bicolor pacifica* was the third frequent species in the Comun (7.7%) and Lagonoy (6.5%) rivers (Figure 5). In the Bato river, *A. luzonensis* and *A. bicolor pacifica* (1.0%) were recruited at a considerably low percentage. In addition, *A. celebesensis* (0.9%) was rarely observed only in the Comun river (Figure 5).

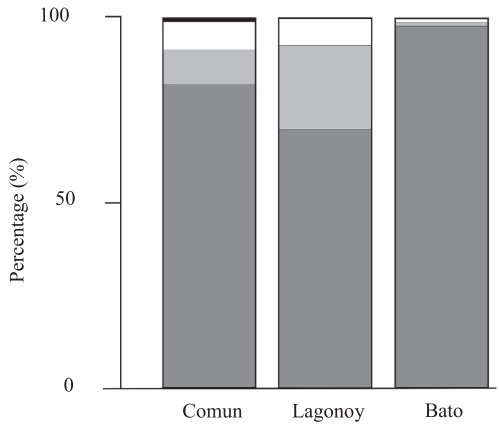

Figure 5 Percent (%) composition of freshwater eels recruiting in each of the provinces. ▩: *A. marmorata*; ▨: *A. luzonensis*; □: *A. bicolor pacifica*; ■: *A. celebesensis*.

3.2.3 Morphological and Molecular Comparisons

Molecular analysis confirmed that 81.7% of the morphologically identified *A. marmorata* and only 16.7% of *A. bicolor pacifica* were correct. However, the re-observation of the caudal pigmentation patterns revealed that *A. bicolor pacifica* and *A. celebesensis* exhibited pattern a (Figure 6a) with dense pigment patches on the tail reaching until the caudal fin tip. No individuals of *A. bicolor pacifica* and *A. celebesensis* exhibiting patterns b-g (Figure 6 b-g). Whereas *A. marmorata* and *A. luzonensis* individuals were found to exhibit patterns b-g (Figure 6 b-g).

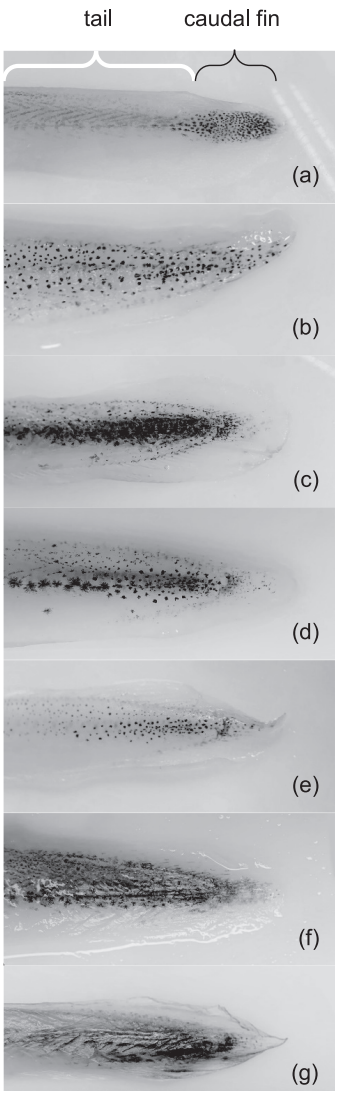

Figure 6 Photo of the caudal pigmentation patterns exhibited by the glass eel specimens. White bracket indicates tail and black bracket is for caudal fin. Scale bar size 5.0 mm.

3.3 Food Chemistry of Tigertooth Croaker

3.3.1 Salting and Drying Practices for Dried *Abo* in SMB

Landed catch of tigertooth croaker are processed as dried-salted product. Majority (88.9%) of the dried fish processors directly buy fresh tigertooth from the fish port by through "*bulungan*" or whisper bidding then transported in plastic tubs or *bañera* via tricycle. About 44.44% of the processors do not own a chilling equipment. Styrofoam fish boxes, often refilled with ice, are used as alternative cold storage. Upon arrival at the fish processing facility, *abo* are washed with tap water, degutted, then cut by butterfly splitting. Fish are submerged in prepared brine known as "*birok*". Salt concentration of the brine were found between 13.8- 50% (w/w). Including the fish weight, salt concentration was estimated to range from 7.1 to 12. 5% (w/w). After the brining period of 30 min, brined fish are rinsed in tap water twice, drained then subjected

to sun-drying for about 6 h to 12 h. Monitoring dryness is through observation and touching. Dried *abo* are packed in polyethylene plastics, vacuum packed or stored in boxes.

3.3.2 Proximate Composition and Salt Content

The processing conditions in the documented process flow in SMB was optimized for the laboratory-produced dried salted tigertooth croaker. The proximate composition of dried tigertooth croaker samples prepared with various salt concentrations (4%, 8% and 12%) and drying times (6 h and 12 h) was evaluated. Values of moisture, crude protein, ash, and crude lipid were found within the ranges of 38.34-66.28%, 26.4-47.9%, 3.94-12.79%, and 0.41-1.04%, respectively. Moisture content significantly decreased with increasing salt concentration in samples dried for 6 h. Highest reduction in moisture was observed at 12 h drying in 12% salt concentration. Ash content increased with increasing salt concentration in both 6 h and 12 h drying time with the highest ash content in samples dried for 12 h with 12% salt. No significant differences was found in the crude protein content except between 8% and 12% salt concentration samples at 6 h drying time. No significant differences was found in crude lipid in samples dried with different salt concentrations for both drying times. Salt contents of dried tigertooth croaker in 6 h and 12 h drying ranged from 4.20-7.87% and 3.31-12.5%, respectively.

3.3.3 Protein Profile

To examine the protein profile, SDS-PAGE was performed on proteins extracted from dried *abo* samples. Seventeen bands of different molecular weights (MW) were detected (Figure 7). In 12 h drying (lanes 5 -7), higher staining intensity was observed in band 1 while bands 2, 6, 8, 11,13 and 14 revealed lower intensities. However, at 6 h drying (lanes 2 - 4), staining intensity at band 1 and 2 were higher as compared to intensity of bands 4, 6, 8, 11, 12 and 14. Proteins identified by LC-MS/MS included the fragments of myofibrillar proteins (bands 1 - 6, 8 - 10, 12, 14) and sarcoplasmic proteins (bands 7, 11, 13, 15 - 17).

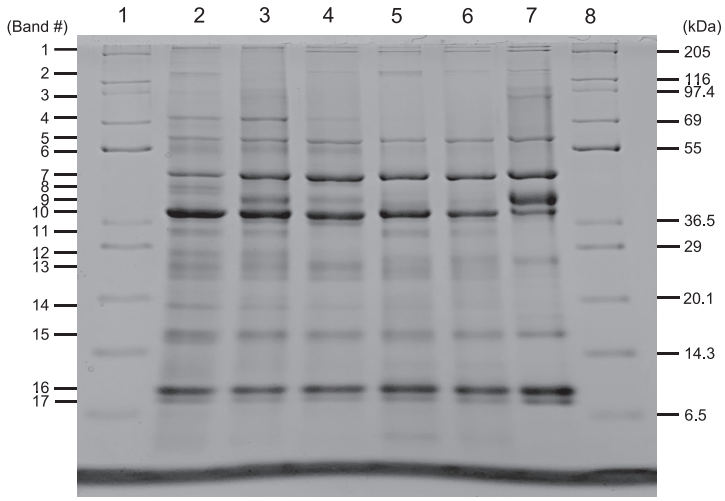

Figure 7 Protein band patterns in SDS-PAGE of *abo* with different salt concentration and drying time in 10% Tris-Tricine gel.
1,8 - protein marker; lane 2 - 4% salt concentration, 6 hours; lane 3 - 8% salt concentration, 6 hours; lane 4 - 12% salt concentration, 6 hours ; lane 5 - 4% salt concentration, 12 hours; lane 6 - 8% salt concentration, 12 hours; lane 7 - 12% salt concentration, 12 hours

3.4 Anguillid Eels as Food

3.4.1 Consumption of Freshwater Eels

In the Bicol Region, adult eels are commonly cooked and eaten as grilled, stewing with vinegar (*paksiw*), with coconut milk, or fried, which are served only during special occasions. In grilling, eels are marinated in vinegar and soy sauce with spices like garlic and pepper. *Paksiw* is prepared by boiling eel in water with vinegar added with ginger, garlic, onions, and pepper. It is also cooked with coconut milk, garlic, onion, ginger, and pepper. In addition, it is also served fried wherein eels are marinated in vinegar with salt before frying. These recipes are quite common not only for eels but also for fishes caught in the region.

3.4.2 Gelatin Presence and Content in the *Paksiw* Soup of *A. marmorata* and *A. bicolor pacifica*

Presence of gelatin in the was observed within the vicinities of 116 kDa and 205 kDa for *A. marmorata* and *A. bicolor pacifica paksiw* soup (Figure 8). Among the eel parts, the skin has the highest staining intensity of gelatinous bands. *Anguilla marmorat*a and *A. bicolor pacifica* cooked as *paksiw* was found to have slightly different gelatin content (27.92 ± 1.31 mg/ml and 30.41 ± 0.32 mg/ml respectively).

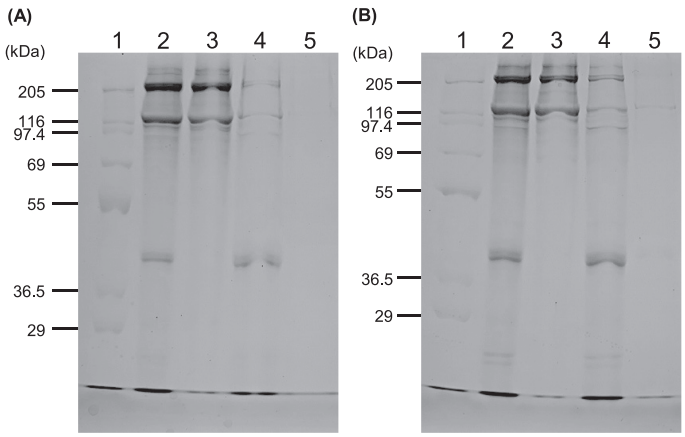

Figure 8 SDS-PAGE profile of cooked *paksiw* soup of (A) *A. marmorata* and (B) *A. bicolor pacifica* in 10% acrylamide gel.
L- ladder; lane: 1- mixed *paksiw* soup of skin, muscle and bones; 2- skin; 3- muscle; 4- bones; kDa- kilodalton

4. Discussion

4.1 Status of Tigertooth Croaker and Anguillid Eel Fishery

4.1.1 Status of *Abo* Fishing and Dried Tigertooth Croaker Processing in SMB

The increase in the landed catch of *abo* from 2016-2018 could indicate that the stock of tigertooth croaker in SMB is still in good condition. This supports the findings of the spawning potential ratio of tigertooth croaker that is still above the limit reference points, indicating that the stock of tigertooth croaker is still in good condition despite the fishing pressures experienced by this species[34]. The recorded peak and lean months of tigertooth croaker varied every year, hence, the varied responses of the respondents. Landed catch of tigertooth croaker are processed as dried salted fish year-round. Depending on the season and size of fish, price of dried fish products also varied.

4.1.2 Current Status of Freshwater Eel Fishery

Sustainability of fishery resources is an important aspect, hence, consideration on improving fishing practice for glass eel collection is needed to lessen discards. In addition, the alarming information on the electrofishing practice in the region suggested the need for stringent enforcement of laws related to fishing activities for sustainable management and utilization of freshwater eel resources in the region.

4.2 Identification of Fishery Resources

4.2.1 Species Identification of *Abo*

The nucleotide differences and the NJ tree inferred from COI sequences demonstrated that *abo* might belong to the *Otolithes* genus. On the other hand, 16S rRNA confirms that *abo* found in SMB might be *O. ruber*. Thus, to address the misidentification of *abo* from other croaker species, information campaign materials can serve as a guide for both sellers and consumers.

4.2.2 Species Identification and Composition of Anguillids

Morphological identification based on pigmentation patterns have been a common practice of fishers yet distinction between pigmented glass eels *A. marmorata* and *A. luzonensis* were difficult. The speculated existence of *A. luzonensis* was only confirmed by molecular analysis. *Anguilla luzonensis* was the second dominant species next to *A. marmorata. A. bicolor pacifica* was third frequent species recruited in LG. The rarity on *A. celebesensis* was also noted.

Inconsistencies on our initial assumption that *A. bicolor pacifica* could easily be distinguished was revealed on the high percentage of misidentification which may have been attributed to the pace of observation, large number of samples and the relative abundance of each of the species. However, re-observation of the caudal pigmentation revealed that distinction among Anguillids recruited in LG can only be between two groups- (a) group of *A. bicolor pacifica* and *A. celebesensis*; and (b) group of *A. marmorata* and *A. luzonensis*. Morphological identification limited to pigmentation pattern was not enough, and maybe combined with molecular analysis. Although, molecular analysis is better for use in precise distinction and estimate of composition of Anguillids and recruited in LG, fishers in the field will not be able to use it. The practical use of pigmentation pattern by fishers in the field setting could only be possible on separating *A. bicolor pacifica* from the group of *A. marmorata* and *A. luzonensis*.

4.3 Food Chemistry of Dried *Abo*

4.3.1 Salting and Drying Practices for Dried *Abo* in SMB

Generally, dried fish production in Calabanga, Camarines Sur are small-scale operations. Traditional sun-drying is still employed as no processing facility uses modernized equipment for drying. Highly salted dried tigertooth croaker are still highly produced despite increasing preference for low-salt dried fish products. The lack of cold storage and low income from processing are common problems faced by the processors. By improvement of the handling, processing and marketing techniques, high quality and high valued dried products can be produced. It is also interesting to note that establishment of association among dried fish processors is important to further develop the dried fish industry.

4.3.2 Proximate Composition and Salt Content

Proximate analysis provides information on the nutritional value of a particular organism used as a source of

food[35]. Moisture was the major constituent of the dried fish. In 6 h drying, moisture significantly reduced in 8% and 12% salt concentration samples. The lower moisture content in the 12% salt concentration samples can be attributed to the decrease in the water-holding capacity of proteins. The high values of ash of the dried tigertooth croaker samples correspond to the increasing salt concentration, suggesting that ash was derived from the added salt in the brine. This highlights the need for modification of salt content of the product. The 12% salt concentration samples at 6 h drying have significantly higher crude protein than 4% and 8% salt concentration samples which can be attributed to the aggregation of proteins. On the other hand, the low crude lipid values of dried tigertooth croaker could be due to characteristic of tropical fish species[36]. Overall, different salt concentration and drying time have significant role in the proximate composition. The study revealed that when processing dried salted tigertooth croaker, salt concentration can be controlled between 8 - 12% at 6 h drying to improve the texture of the product.

4.3.3 Protein Profile

Among the 17 bands detected in SDS-PAGE, nine proteins were identified. Among the identified proteins, tropomyosin, beta-enolase, L-lactate dehydrogenase, triosephosphate isomerase, nucleoside diphosphate kinase, and parvalbumin were found stable in their native MW. On the contrary, myosin heavy chain (MHC), actin, and keratin had dissociated into fractions. The original MW of the myosin heavy chain (200 kDa), actin (43 kDa), and keratin (62 kDa) were not observed in both reducing and non-reducing SDS-PAGE conditions (data not shown). This could be due to the loss of moisture imposed by soaking in the brine solution and exposure to sun-drying resulting in the denaturation of proteins. The action of endogenous enzymes during processing may have degraded the fish proteins[37]. In both 12 h and 6 h drying, between 8% and 12% salt concentration, fragmentation of MHC and polymerization of actin had occurred. Similar SDS-PAGE patterns of dried salted fish have shown degradation of myofibrillar proteins as influenced by different processing conditions[38,39]. Overall, this study demonstrated that myosin degradation and actin polymerization occurred in the tigertooth croaker muscle with the applied salting and drying time conditions. MHC and actin, the main myofibrillar proteins, could be useful indicators to monitor the changes in adjusting the salt concentration and textural properties of the product, hence can be further studied.

4.4 Anguillids as Food

4.4.1 Potential of *A. marmorata* for Food Development

The low-market value *A. marmorata* dominance not only in LG but also in the north and south part of the Philippines suggests that this has potential for food development. Although not famous, the interview showed that there is an existing way of consuming freshwater eels. With the comparable gelatin content of *A. marmorata* with *A. bicolor pacifica* and the reported comparable nutritional value and superior preference test[15] indicated the high potential of *A. marmorata* for food development, hence the need for further studies.

4.5 General Perspective

Abo is a commercially important fish species and popularly marketed as dried fish in the Bicol region. Our data suggest that management measures be directed towards the sustainability of the *Otolithes ruber* stock. Utilization of more efficient fishing gear could increase the catch rate and increase income of fishermen. However, the regulated use of fishing gears is important to prevent unsustainable fishing that could lead to depletion of fishery resources[7]. In addition, strong enforcement of fishery rules and regulations, specifically

on the closed season, is recommended. The possibility of misidentification of fish species could occur in the field and during marketing. Thus, raising awareness on the correct identification of fish species is also important to prevent the substitution of a less valuable species for a valuable fish species, which might result in an economic loss on the part of the fish trader or processor.

Moreover, improvement of the market value of the dried products by producing acceptable and highly graded dried *abo* may contribute to the increase of income of dried fish processors. By optimizing the processing conditions and studying the chemical composition, it was found that improvement on the salt concentration and texture of dried tigertooth croaker can be explored. Dried *abo* products with low salt and new texture may be introduced to the market. Observation of the drying facilities revealed that sanitation requirements and availability of cold storage needs to be complied to minimize microbiological contamination. Processors are also encouraged to take advantage of the opportunity to use vacuum packaging technology to maintain quality and extend shelf life of the dried fish. Product presentation will then be improved, thus attracting new market for this regional product.

With the Philippines being a source of freshwater eel, it is imperative for a stringent enforcement of laws related to fishing activities which are needed for sustainable management and utilization of freshwater eel resources in the region. *A. marmorata*, being the dominant catch not only in the Bicol region but also in the north and south part of the Philippines, focusing on its effective processing methods may entice aquaculture owners or establishment of an aquaculture facility in the region to grow glass eels until the adult stage that could be utilized to new products, and create new markets and avenues for the fishermen to improve their livelihood may be feasible. In general, resource management strategies in support to effective utilization, economic and market potentials, and sustainability of fishery resources in the Bicol region should be given ample attention and priority.

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