國立臺灣大學漁業科學研究所

碩士論文

Institute of Fisheries Science
College of Life Science
National Taiwan University

Master Thesis

呂宋鰻在台灣及菲律賓的生物地理分佈

Biogeographic distributions of *Anguilla luzonensis* in Taiwan and Philippines

羅敏睿

Min-Jui Lo

指導教授：韓玉山 博士

Advisor: Yu-San Han, Ph.D.

中華民國 102 年 7 月

致謝

在這段碩士的學程當中，讓我學到了非常的多，由原本進實驗室像個愛玩的小孩不知道長大，到後來拼命的量魚做實驗，轉變非常的大，從小就好動的我，家人一直擔心我是否無法去面對實驗室的生活，我自己也非常的存疑，這之中的轉變最大的感謝真的是韓老師，在月報的幾句簡單的訓誡，能讓一向坐立難安好玩的我，真正的有去思考未來，進一步的去拚實驗，而接下來的每個月報，也都是皮繃得緊緊的希望能得到認可，老師的幾句稱讚，能讓簡單的我高興半個月，從懵懂的我，變成跟朋友聊天話題卻時常離不開鰻魚的我，真的是個很大的轉變，學生敏睿是真心的謝謝這位專精與廣泛能二者唯一的恩師。

當然，在實驗室之中每一位都是不可或缺的，從剛進實驗室的時候，敬華學姊一步一腳印的教我量魚、鑑定、歸檔，這些都歷歷在目，是非常盡責且不吝指導的學姊，而婉之、何敏更是有任何的好東西，都與我們這些學弟妹分享，讓我們能在實驗室有像在家一樣的感覺，明惠及小陶更是實驗室說說笑笑的好夥伴，就連我碩士論文不懂的地方在網路上詢問小陶，他也是耐心的一一解答，有這群這樣好的學姐們，也讓我們碩士過得更加的精彩。而現在碩一可愛的學弟妹們，真的個個都是寶，做實驗跟雅棻聊的天花亂墜，昱如學妹每次的聽我發牢騷，翔奕不但是個好球友，更是個好學弟，下午茶後門的行動，他總是不會缺席，最有緣的是寧比學妹了，我們不但同樣是苗栗人，連國中高中也都是讀同樣的學校，真的有一見如故稱兄道弟的感覺，讓人心曠神怡。新進實驗室的一群，雖然相處時間不久，但我相信你們一定能非常順利的!而同屆的阿旗、搞搞、阿梅、邦又、鳳娸、觀元，我們一起走過一起分享一起緊張一起開心，這些不需要言語並且永遠忘不了的!最後我要謝謝一路陪著我不管是高興生氣吵架的咪咪醬以及我可愛的奇丁跟桔子還有老媽，不用多語，沒有你們，我走不到今天，也非常謝謝口委們熱誠熱心的幫助我們完成論文，順利畢業!

中文摘要

鰻魚為東亞國家重要的養殖魚種之一，但近年鰻魚的資源量都在急速的下降，不論是鰻苗或是成鰻，數量都比以往來的少，而鰻魚養殖所需的鰻苗，完全都是從漁民捕撈而來，在資源量減少的情況下，物種復育、新養殖鰻種的興起甚至取代，都成為研究的目標之一，而呂宋鰻（*A. luzonensis*，又名黃氏鰻 *A. huangi*）於 2009 年被公布為第 19 種鰻魚，然而此新鰻種雖然已被公布，但對於它的生物地理分布、資源量、生物特徵等相關的研究資料是少之又少，因此本篇想針對呂宋鰻的生物地理分布與生物形態特徵，建立較完整的資料庫。

本篇的樣本來源分成兩個部分，以呂宋鰻被發現的時間（2008）做為分界點，第一部分是 2008 年至 2011 年，採自菲律賓、台灣採集之鰻苗，第二部分是 1984 年至 2008 年採自台灣之鰻苗。利用型態與 DNA 鑑定方法，鑑定出各採樣點之鰻苗組成，由此建立出呂宋鰻的生物特徵及生物地理分布。結果指出，呂宋鰻的高捕獲量主要出現在菲律賓呂宋島的 7、8、9 三個月，而在這三個月中，其數量甚至超過鱸鰻，而在台灣方面，呂宋鰻非常稀少，捕捉量 8、9、10 月較其他月份為高。生物形態特徵方面，鱸鰻及呂宋鰻的鰭差比分布圖之比較，可看出有少部分重疊的情況發生，而導致有可能誤判。呂宋鰻色素與體長的相關變化，從 VA 時期至 VIA_3 時期呈現緩慢下降或是停滯，趨勢與日本鰻類似；月份與體長之比較，趨勢並不明顯且無規律。呂宋鰻的生物地理分布於本篇中確

立出以菲律賓為主。

關鍵字：呂宋鰻、生物地理分布、生物型態、鰭差比

Abstract

Eels have been an important category in aquaculture in East Asian countries. But in recent years the stock size of eels has been in rapid decline. No matter it is eel fry or adult eels, their quantities are much less than the past. All the eel fry needed in eel farming come totally from the catches by fishermen. Under the circumstance of reduced stock size, things like species rehabilitation, emergence of newly farmed eel species or replacement by new species have become one of the targets of the researchers. *A. luzonensis,* also known as *A. huangi*, has been announced as the 19th species in 2009. Although this new species has been announced, the relevant research information on its biogeographic distributions, stock size, and biological features is quite meager. As such, this paper aims at establishing a more comprehensive database of the biogeographic distributions and early life history of *A. luzonensis*.

Samples used in this paper are from two parts. The first part is collections of *A. luzonensis* from 2008 to 2011. The second part is collections of *A. marmorata* from 1984 to 2008. The demarcation is the time (2008) when *A. luzonensis* was discovered. Collections made after 2008 are mostly in places in the Philippines and Taiwan. Prior to 2008, they were mainly collected in Taiwan. After that, pattern identification is employed to establish the biological features and biogeographic distributions of *A. luzonensis.* The results indicate that high catches of *A. luzonensis* are mainly in the three months of July, August, and September in the Philippines. In the three months the amount of *A. luzonensis* caught is even more than that of *A. marmorata*. In Taiwan, *A. luzonensis* is extremely rare and from September to January more catches are seen. In terms of biological patterns and features, we can see that there is an overlap that leads to possible misidentifications from the comparison of fin difference ratio in *A. marmorata* and *A. luzonensis*. The associated change of pigmentation of *A. luzonensis* and its total length shows resemblance in the run chart compared to that of *A. japonica* in that there is a slow downward slope or even a standstill from VA to VIA_3 stages. The comparisons of months with total lengths showed no obvious upward or downward trend.

In this paper we have established the biogeographic distributions of *A. luzonensis* in the Philippines primarily.

Keywords: *Anguilla luzonensis*, biogeographic distribution, biological pattern, fin difference ratio

Contents

Figure legend

Table contents

Appendix table

Introduction

Eel resource decline

The eels (*genus Anguilla*) are important economic species in Southeast and Northeast Asia. Due to artificial reproduction techniques of eel fry (glass eel) haven't been found yet, all of the eel fry needed for aquaculture depends on the wild catch.

In recent years the Japanese eel *Anguilla japonica*, European eel *A. Anguilla* and American eel *A. rostrata* stock sizes have been significantly declined owing to overfishing, habitat destruction, global climate change, dams, and other unknown factors (Tatsukawa 2003; Hitt 2012). How to restore the abundance of these eels or discover a new aquaculture species has become an important task.

There are 19 species of anguillids distributed around the world in the coastal areas of North Atlantic Ocean, Indian Ocean and West Pacific Ocean (Aoyama 2009). All anguillids spawn in the tropical or subtropical oceans. After eggs are hatched, leaf-like bodies called leptocephali are transported by ocean currents from their spawning area to continental habitats then metamorphose into glass eels (Miller 2009; Aoyama 2009). After metamorphosis, glass eels enter estuaries, rivers or lakes. They grow for years and then become silver eels to return to their birthplace to spawn and then die (Tsukamoto 2006).

Biogeographic distributions

In Taiwan, there are four species of freshwater eels: *A. japonica*, *A. marmorata*, *A. bicolor pacifica* (Tzeng 1983) and *A. luzonensis* have been reported (Han 2010). The biogeographic distributions of tropical eel, *Anguilla mamorata*, larvae are transported by the North Equatorial Current (NEC), then into either the northward flow of Kuroshio or the southward flow of Mindanao Current and recruit to Taiwan, Southern Japan, the Philippines and northern Indonesia. In Taiwan, *A. marmorata is* found throughout the year and dominant between March and July. In the Philippines, *A. marmorata* is dominant between February and June (Han 2013) . *Anguilla japonica* is transported by the NEC and then enters the northward flow of Kuroshio towards East Asia. In Taiwan, *A. japonica* glass eels mainly occur in winter in Yilan River, Danshui and Tungkang River. Aoyama (2009) suggested that the spawning areas of *A. bicolor pacifica* and *A. luzonensis* were also located in the NEC region. The mid- and large-size leptocephali of *A. bicolor pacifica* are collected in the east of New Guinea, Indonesia and west of the Mariana Islands. This finding indicates that *A. bicolor pacifica* may spawn in the western North Pacific (WNP) region and might have multiple spawning areas in the western Pacific (Kuroki *et al.* 2006) .

The new species, *A. luzonensis,* also known as *A. huangi*, was found in Cagayann River, on the northern Luzon Island of the Philippines (Watanabe *et al.* 2009; Teng *et* *al.* 2009) . The leptocephali of *A. luzonensis* were found in the west of the Mariana Islands, which supported that *A. luzonensis* also spawned in the NEC (Aoyama 2009; Watanabe *et al.* 2009; Kuroki *et al.* 2012). Although *A. luzonensis* has spawning sites in the NEC, the precise spawning area, migratory routes, biogeographic distributions and early life history of *A. luzonensis* are still poorly understood. The experiment of this study is to understand the biogeographic distributions of *A. luzonensis* in Taiwan and the Philippines.

Pigmentation stage

The progress of glass eel growth and development can be measured by pigmentation stages, which commence as water temperature rises and physiological changes take place (Strubberg 1913). Previous studies revealed that the changes in glass eel size and pigmentation proceed in parallel (Tesch 2003a). Water temperature determines the pigmentation and correspondingly TL decrease and later growth as well (Strubberg 1913). As the glass eel continues to grow, external pigments develop from sub-epidermal layers. Glass eel is defined in this paper as all developmental stages from the completion of the leptocephalus metamorphosis to full pigmentation. The process includes stages VA to VIB. Aside from progressive pigmentation, the total length changes simultaneously to some extent. Knowing the stage of development of recently arrived recruits is also important since there is considerable variation in the stages of glass eels as they arrive in coastal areas after their long migration in the ocean (Sugeha 2001; Aoyama 2009). Their stages and body condition when they arrive are important information for evaluating seasonal and interannual recruitment variations especially for anguillid species that have shown recruitment declines in recent decades. An alternative but much more laborious way to evaluate the development stage is to estimate the age using analysis of their otolith rings. However, for young eels this method may not always be reliable because the deposition of otolith material can stop at water temperatures below 10ºC, which is a temperature that anguillid glass eels recruiting to the higher latitude regions would encounter during the winter months (Fukuda 2009). The experiment of this study is to understand the TL variation of all the pigmentation stages in detail for *A. luzonensis*.

Therefore the main purpose of this paper is to conduct a further research on the biogeographic distributions of *A. luzonensis* in Taiwan, Luzon Island, and Mindanao. Their monthly catches would be used to do pattern identification and survey of pigmentation stages. Then clearer temporal and spatial distributions of *A. luzonensis* could be established. Furthermore a comparison is made between the relative abundance of *A. marmorata* and *A. luzonensis* to understand the differences between the two species in the peak season as well as the distinction between the places they are caught. Lastly a trend comparison of pigmentation stages and total lengths is made in the hope of completely establishing the biogeographic distributions and biological features of *A. luzonensis*in Taiwan, the North Luzon Island and Mindanao.

Materials and methods

Sample collection

Glass eels in the northern Luzon Island were collected monthly from May 2008 to September 2009 of the Philippines of Cagayan River, Wangag River, and Abulug River (Fig.1), all in the province of Cagayan in northern Luzon. Samples were collected with the use of a fyke net (Fig. 2b) dipped to the bottom of the river. With glass eels from Taiwan (Fig.1), we can divide them into 2 parts. Part1: were caught using fyke nets (Fig. 2a, c) at night between February 2009 and December 2011 between 1 and 3 times monthly from the estuaries of Danshui River (Northwestern Taiwan), Donggang River (Southwestern Taiwan), Yilan River (Northeastern Taiwan), and Siouguluan River (Eastern Taiwan). All glass eels were purchased from local fishermen. Part 2: were caught using fyke nets between 1984 and 2008 from estuaries in Taiwan. Glass eels in areas around the Celebes Sea were collected using hand nets from January 2009 to April 2010 from Buayan River, General Santos City in southern Mindanao.

Species identification based on morphological measurement

Eel species were identified using morphological methods. The total length, pre-dorsal length, and pre-anal length (to the nearest 0.1 mm) of each glass eel were measured under a stereomicroscope. The following equation was used to calculate the fin difference as an index to classify whether an eel was long finned or short finned: Fin difference = AD/TL × 100%, where AD is the vertical distance from the origin of the dorsal fin to the anus and TL is the total length. Morphological measurement were also checked by the presence or absence of caudal cutaneous pigmentation (Strubberg 1913) (Fig. 3). *A. japonica* and *A. bicolor pacifica* were easily identified by using morphological methods. Samples with fin difference <13% from Taiwan and Luzon Island were identified by molecular methods. All samples from Mindanao were identified by molecular methods.

Species identification based on molecular methods

The fin difference value of *A. marmorata* and *A. luzonensis* of 12-13% was usually ambiguous (Fig. 4), so molecular identification was used. A small piece of muscle was purified and extracted by a commercial DNA kit (Bioman Scientific Ltd.). The sample tissue was grounded and homogenized by using a micropestle and 200μl GT buffer. After reaching homogenity, 20μl Proteinase K was added and the sample was incubated at 60ºC for 30 min to lyse the sample. After that, 200μl GB buffer was added and the sample was incubated at 70ºC for 20 min until the sample lysate was clear. Then 200μl ethanol was added to the sample lysate and vortex immediately to mix the sample. The mixture was then transferred to GD column and centrifuged at 13,000 rpm for 5 min. After centrifuge, the flow-through was discarded and 400μl W1 buffer was added for centrifugation at 13,000 rpm for 1 min. After 600μl wash buffer washing at 13,000 rpm for 1 min and again for additional 3 min to dry the column, the GD column was transferred into a new microcentrifuge tube and 100μl preheated Elution buffer was added. Wait 3-5 min for the elution buffer to be absorbed by the matrix, then the GD column was centrifuged at 13,000 rpm for 1 min to elute purified DNA. The extracted DNA was stored at -20ºC before polymerase chain reaction (PCR) occurred. PCR was performed in 25μl reaction volume consisted of 11μl of double distilled water (ddH2O), 12.5μl 2X Taq DNA Polymerase Mastermix-Red (Bioman Scientific Ltd.), 0.5μl forward primer, 0.5μl reverse primer, and 0.5μl of total genomic DNA for a total of 25μl solution. Amplification parameters were as follows: initial denaturation at 94ºC for 3 min, followed by 30 cycles of denaturation at 94ºC for 30s, annealing at 57ºC for 30s and extension at 68ºC for 30s, followed by final extension at 68ºC for 10min and 4ºC for 10min. The PCR products were electrophoresed and sent for sequencing for direct species identification. Universal Anguilla primer set for cytochrome b (forward:5′-GATGCCCTAGTGGATCTACC-

3′; reverse: 5′TATGGGTGTTCTACTGGTAT-3′) was used for PCR.

Results

Fin difference distribution of A. luzonensis and A. marmorata

A distribution chart is made against the fin differences between *A. luzonensis* and *A. marmorata* in the three major rivers of Northern Luzon, namely Cagayann River, Wangag River, and Abulug River. Comparing the two species and the result after analysis shows there are 829 eels of *A. luzonensis* with fin differences in the range of 11.5~12.5, and 82 eels have a fin difference above 12.5 (Fig. 5). As to *A. marmorata*, there are 38 eels with a fin difference below 12.5. There are another 494 eels in the sample with fin differences in the range 12.5~13.5. It clearly shows an existence of overlapped region in the distribution chart against the fin differences between *A. luzonensis* and *A. marmorata*. For this, molecular identification has become the most accurate way of differentiating between *A. luzonensis* and *A. marmorata* for now (Fig. 5).

Monthly glass eel composition by location

4 *Anguilla* species were identified in Taiwan and Luzon Island: *A. japonica*, *A. marmorata*, *A. bicolor pa*cifica, and *A. luzonensis*. In Taiwan, glass eels of *A.*

marmorata are found all the year, and are much more dominant than *A. luzonensis*. In the Philippines, *A. marmorata* is dominant between February and June in Luzon Island, *A. luzonensis* is dominant half of the year, mainly from July to September, in northern Luzon Island (Table 1, Fig 6a,6b,6c). In Taiwan, *A. marmorata* is dominant between March and July and *A. luzonensis* are rare all year round (Table 2). In contrast to the wild distributions of *A. marmorata* in Southeast Asia, *A. luzonensis* seems to be mainly distributed in Luzon Island but is rare in Taiwan and Mindanao (Table 3).

Correction of Past Misidentifications

Pattern identification of eels before 1984 and up to 2008 also determined 6 and 5 *A. luzonensis* separately in 2001 and 2000. It is quite obvious that even *A. luzonensis* was discovered in 2009, Taiwan had been one of its habitats prior to that. One thing worth mentioning is that *A. celebesensis* was also discovered.

Pigmentation stages of A. luzonensis and its changes with TL

After measurement of its total length and distinction of pigmentation stages, the result is a relationship chart of total lengths vs. pigments for *A. luzonensis* in the three rivers in Luzon Island (Table4). In these three rivers, we could find that the VA and VB stages of *A. luzonensis* showed a significant difference in total lengths in

Cagayan river and Wangag river ($P<0.05$) (Table5). *A. luzonensis* in two of the rivers show a slow decrease of total lengths. The two rivers that are more significant are Cagayan River and Wangag River (Fig. 7a,7b). The analysis chart of Wangag River shows a slightly significant slow downward slope and then a gradual upward slope (Fig. 7c). As to Cagayan River and Abulug River, there is no clear indication of changes with relation to pigmentation stages and total lengths after VIA_3 as there is no sample available.

The pigmentation analysis chart from Taiwan indicates a slow gradual decrease or gradual increase after the VA stage. Right after the VIA_4 stage, it shows a more rapid growth of total lengths. However, the quantity of *A. luzonensis* is quite scattered in Taiwan (Fig. 7d).Yet if the analysis is conducted using a larger scale (Abulug River+Cagayan River +Wangag River) , the analyzed result would show that total lengths are actually shorter in stage VA than in stage VB and it would exhibit significant differences as well. However when viewed from VIA_2 to VIA_4, there is no significant change in total lengths as they either are at a standstill or decrease slightly. (Fig. 7e)

When we use the VA stages of *A. luzonensis* to do analysis of the variation in total lengths between the months of May 2008 and August 2009, we can see that there's no obvious pattern in the total length between each month (Fig. 8a,b). As to

Wangag river, there is no way to see if there is an upward or downward trend since the quantities collected in many of the months were too low or even none (Fig. 8c). From the aggregated data of the three rivers, the condition is also level with no obvious upward or downward trend. (Fig. 8d). As to analysis for VB, it does not show any obvious pattern or change either (Fig. 9a, b, c). Generally speaking, there is no significant change in total lengths of *A. luzonensis* between each of the months.

Comparison of the biological patterns and features of A. luzonensis in Taiwan, Luzon Island and Mindanao

From Table 6 we can see that *A. luzonensis* in the three major Philippine rivers do not show significant differences in pre-dorsal fin length, pre-anal fin length and fin difference ratio and so forth and if we do not take pigmentation stages into consideration. The same is true in Taiwan with differences shown only in pre-dorsal fin length and fin difference ratio. As to Mindanao, the sample was only 30 and they showed significant differences with those of Luzon Island in total length, pre-anal length, and pre-dorsal length. However as the sample was extremely small in size, more samples are needed to verify the accuracy of the analysis.

Discussion

Misidentification of A. celebesensis

The new anguillid species *A. luzonensis* was identified in the Cagayan River System (Watanabe *et al.* 2009). Meanwhile, Teng *et al*. (2009) also found this new species, named *A. huangi*. To identify this species, identification based on morphology such as variegated markings, maxillary bands, numbers of vertebrae, and the location of the origin of the dorsal fin were not reliable enough due to its similar and overlapping morphometric characteristics with those of *A. celebesensis* and *A. marmorata*. Molecular-based technique was a better method for species identification than morphometric characteristics but this method needs more time to be spent than identification on morphology and is expensive (Aoyama 2009; Teng *et al*. 2009; Watanabe *et al*. 2009). In the previous paper, *A. luzonensis* is misidentified as *A. celebesensis* in 1976 and 1982, because they have similar fin difference ratio between 10%-12.5% and indistinguishable morphological characteristics, such as marbled dark brown on the back of adults and caudal cutaneous pigmentation for glass eels (Tabeta *et al*. 1976; Tzeng 1982 ; Teng *et al*. 2009; Watanabe *et al*. 2009) Glass eels of *A. celebesensis* could have come from a spawning area near the mouth of the Celebes Sea, recent data on the distribution of leptocephali of this species indicate that spawning occurs in both the Celebes Sea and in Tomini Bay (Aoyama *et al*. 2003). However, the northern Philippines is further north than the known distribution range of *A. celebesensis* (Ege 1939), so it now appears fairly certain that the glass eels originally identified as *A. celebesensis* from this area were actually *A. luzonensis*, since no *A. celebesensis* has been identified using DNA analysis from the northern Philippines, whereas *A. luzonensis* glass eels and yellow eels are clearly present there (Teng *et al*. 2009, Watanabe *et al*. 2009). Regardless of how often *A. luzonensis* leptocephali or glass eels may have been historically misidentified, there appear to be very few reports of "*Anguilla celebesensis*" collected in the regions north of the Philippines (Ege 1939, Arai *et al*.2003, Teng *et al*. 2009, Watanabe *et al*. 2004).

One report from Taiwan (Tzeng 1982) and some previously reports of *A. marmorata* leptocephali that were not genetically identified, could have actually been those of *A. luzonensis* (Jespersen 1942, Miller *et al*. 2002, Miller and Tsukamoto 2004). Nevertheless in this paper, we could see that the total lengths, pre-anal lengths and pigments of the two types of glass eels are very similar from pattern identification of *A. marmorata* and *A. luzonensis* in the Philippine region and the distribution chart of their fin difference ratio. Furthermore the fin difference ratio also shows there is an overlap between the two species. Therefore we could deduce that some *A. marmorata* identified in the past through pattern identification but not yet through molecular identification could have been *A. luzonensis* as well.

Spawning area and Species composition of Anguilla glass eels

In previous studies, the leptocephali of *A. luzonensis* were collected offshore or at the edge of the subtropical gyre of the western North Pacific. The offshore presence of these leptocephali supports the hypothesis that this species may have a spawning area similar to the three other anguilliform eels in the region (*A. japonica, A. marmorata* and *C. myriaster*) (Kuroki 2012). From the analysis of this paper, the biogeographic distributions of *A. luzonensis* also coincide with previous studies by other scholars. The *A. luzonensis* collected in this study are from regions of Taiwan, Luzon Island, and Mindanao. Among them, most of the *A. luzonensis* are from Luzon Island and fewer are from Taiwan and Mindanao. This matches the paper of Han *et al*.(2013) in that their hatching grounds correspond to those of *A. marmorata* and *A. japonica* and that *A. luzonensis* are drifted with the NEC. When they reach the southern part of Luzon Island, they are split into two branches that are the Mindanao current (minor) and the Kuroshio (major) separately. Therefore the major distributions are found in Luzon Island. As to the rare sample in Mindanao, they are transported by the Mindanao current (minor). On the other hand, *A. luzonensis* occurred dominantly during July, August and September but the total collection was rare in Taiwan and Mindanao. As to why most of the distributions of *A. luzonensis* are centered around Luzon Island and not like *A. marmorata* to be plenty in amount in Taiwan as well, the conjecture is that it could be related to the biological features of the leptocephalus stage of *A. luzonensis.* A likely guess is that the larva duration of *A. luzonensis* is not as long as that of *A. marmorata*. Therefore during the drifting process, they could not be transported to areas like Taiwan in large amounts as *A. marmorata* due to its ability to move, leading to its distributions to be limited to northern Luzon Island only and not to be drifted further to Taiwan. This is also why there are only small amounts of *A. luzonensis* in Taiwan and Mindanao.

Pigmentation v.s Total-length

In a previous study the body measurements of *A. japonica* indicate that the TL and BW decreases or stays about the same until VIA_3 and VIA_4 and then starts to increase rapidly, which is similar to observations of *A. anguilla* from Tunis Lake, Tunisia, whose TL and BW decreased until VIA_3 before increasing rapidly during the VIA_4 stage (Tesch 2003). *A. japonica*, the TL and BW slightly decreased until VIA_2 and then increased. The TL–BW relationships of each pigmentation stage showed developmental changes in their body proportions. The body proportion changed gradually to the slender body form from VA to VIA_3 and VIA_4, and became the thicker body form at VII. This was because the eels with larger TL had a thicker body form during VIB, suggesting that VIB was the transition time of the change from the slender body (VIA_3 and VIA_4) to the thick body (VII). (Fukuda *et al*. 2013).

The relationship of pigmentation stages and total lengths in this study shows a similar pattern to studies previously made. *A. luzonensis* in VA and VB stages did not show a significant increase in total lengths. Among them, total lengths in Wangag River and Cagayan River show a decrease from the VA to VB stages. In VIA_2 to VIA_3 stages, it is a situation of ups and downs with no significant increase. But as the lab does not have pigmentation stage *A. luzonensis* samples after VIA_4, we could not make sure if there is a relevance to previous studies made by others in stages after VIA_4 to VII. The analysis of the total length and pigmentation of *A. luzonensis* in this study shows that even the total lengths from stages VA to VIA_3 show a condition of slow decrease or even standstill, it is obvious the variation is not so much as those of *A. japonica*. Among them, the total length in stage VB for those from Abulug River is even longer than that in stage VA. The explanation concerning this could be that as *A. japonica* are temperate eels and *A. luzonensis* are tropical eels, there is difference in the habitats they choose. Furthermore we usually see more production and resources in tropical regions than in temperate regions. Therefore the conjecture that variation in the total lengths of *A. luzonensis* is not so noticeable is because they have more food choices and lead to little impact on their growth. The explanation for the total lengths of eels and change in the pigmentation stage is that the transition between body forms and growth may correspond to their ecological change from using passive transport in glass eels with tidal flow into rivers, to their settlement and further movements of elvers upstream (Fukuda *et al.* 2013). But it is still little known whether these changes in pigmentation stages and body growth are due to the effect of salinity change, a time-delay effect of recruitment, ecological change or all of them (Leander *et al.*2012). As to the fact that there is no significant change in total lengths of *A. luzonensis* between each of the months, the conjecture in this study is that as Luzon Island is situated in the temperate zone with a mean annual temperature of 27 degrees C. In tropical regions the latitudinal diversity gradient is higher than that of temperate regions. Furthermore as the climate in Luzon Island is not as changeable as in Taiwan, but rather is one with a constant high temperature. Therefore there is no significant change in total lengths of *A. luzonensis* as they keep a constant length when they live in an environment with abundant food and a higher growth rate.

Conclusion

Through analysis we could find that the Philippines is the major distribution region for *A. luzonensis.* The three months of July, August, and September are the time with a higher trend. As to Taiwan and Mindanao, there is only a small amount of *A. luzonensis*. In the identification between *A. marmorata* and *A. luzonensis* afterwards, we need to be more cautious as there is an overlapped morphometric index between them. Molecular identification is still the top choice nowadays. Even it is higher in cost, it has the best accuracy. As to pigmentation stages and changes in total lengths of *A. luzonensis,* we can see there is no significant different from VIA_2 to VIA_4 and stage VA to VB show a decrease that is similar to that of *A. japonica*.

References

Aoyama J (2009) Life history and evolution of migration in catadromous eels (genus *Anguilla*). Aqua-BioScience Monographs 2:1-42.

Arai T, Miller MJ, Tsukamoto K (2003) Larval duration of the tropical eel *Anguilla celebesensis* from Indonesian and Philippine coasts. Marine Ecology Progress Series 251:255-261.

Fukuda N, Kuroki M, Shinoda A, Yamada Y, Okamura A,Aoyama J, Tsukamoto K (2009) Influence of water temperatureand feeding regime on otolith growth in *Anguilla japonica* glasseels and elvers: does otolith growth cease at low temperatures? Journal of Fish Biology 74:1915–1933.

Fukuda N, Miller MJ, Aoyama J, Shinoda A, Tsukamoto K(2013) Evaluation of the pigmentation stages and body proportions from the glass eel to yellow eel in *Anguilla japonica*.Fisheries Science 79:425-438

Han YS (2010) Study of production of potential aquaculture species – *Anguilla marmorata*. Project report of Council of Agriculture, Executive Yuan.

Haro AJ, Krueger WH (1988) Pigmentation, size, and migration of elvers (*Anguilla rostrata* (Lesueur)) in a coastal Rhode-Islandstream. Canadian Journal of Zoology 66:2528–2533.

Kuroki M, Aoyama J, Miller MJ, Wouthuyzen S, Arai T, Tsukamoto K (2006) Contrasting patterns of growth and migration of tropical anguillid leptocephali in the western Pacific and Indonesian Seas. Marine Ecology Progress Series 309:233-246

Kuroki M, Miller MJ, Aoyama J, Watanabe S, Yoshinaga, Tsukamoto K (2012) Evidence of offshore spawning for the newly discovered anguillid species

Anguilla luzonensis (Teleostei: Anguillidae) in the western North Pacifc. (early view) Pacific Science 66:4

Kuroki M, Aoyama J, Miller MJ, Yoshinaga T, Shinoda A, Hagihara S, Tsukamoto K (2009) Sympatric spawning of *Anguilla marmorata* and *Anguilla japonica* in the western North Pacific Ocean. Journal of Fish Biology74:1853-1865

Leander NJ, Shen KN, Chen RT and Tzeng WN (2012) Species Composition and Seasonal Occurrence of Recruiting Glass Eels (*Anguilla* spp.) in the Hsiukuluan River, Eastern Taiwan. Zoological Studies, 51: 59-71.

Luers DF, Love JW, Bath-Martin G (2011) Settlement and pigmentation

of glass eels (*Anguilla rostrata* Lesueur) in a coastallagoon. Environmental Biology of Fishes 90:19–27

Strubberg A (1913) The metamorphosis of elvers as influenced by outward conditions. MeddrKommn Havunders. Serie Fiskeri 4: 1-11.

Sugeha H, Arai T, Miller MJ, Limbong DKT (2001) Inshoremigration of the tropical eels *Anguilla* spp. recruiting to thePoigar River estuary on north Sulawesi Island. Marine Ecology Progress Series 221:233–243

Tatsukawa K (2003) Eel resources in East Asia.In : Aida K, Tsukamoto K, Yamauchi K (eds.) Eel biology, Springer, Tokyo. 293-298

Teng HY, Lin YS, Tzeng CS (2009) A new *Anguilla* species and a reanalysis of the phylogeny of freshwater eel. Zoological Studies 48:808-822

Tesch FW (2003a)The eel. Oxford, UK: Blackwell Science 130-131.

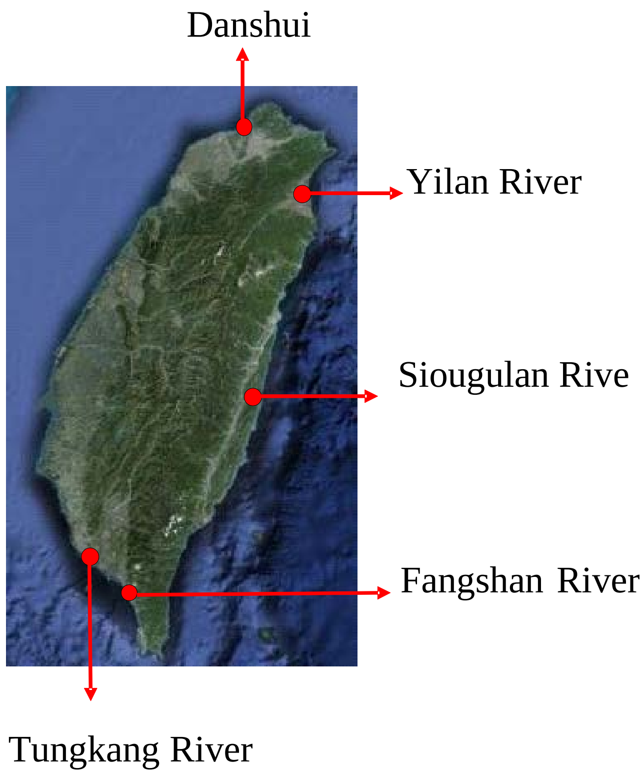
Tsukamoto K (2006) Spawning of eels near a seamount. Nature 439:929

Tzeng WN (1983) Species identifiecation and commercial catch of the anguillid elvers from Taiwan. China Fish Month. 366:16-23 (in Chinese)

Watanabe S, Aoyama J, Tsukamoto K (2009) A new species of freshwater eel *Anguilla* *luzonensis* (Teleostei: Anguillidae) from Luzon Island of the Philippines. Fish Science 75:387-392

Fig. 1 Glass eel samples collected from the rivers. (a) Map of the collection sites in Taiwan; (b) Map of the collection sites in northern Luzon Island

(a)



(b)

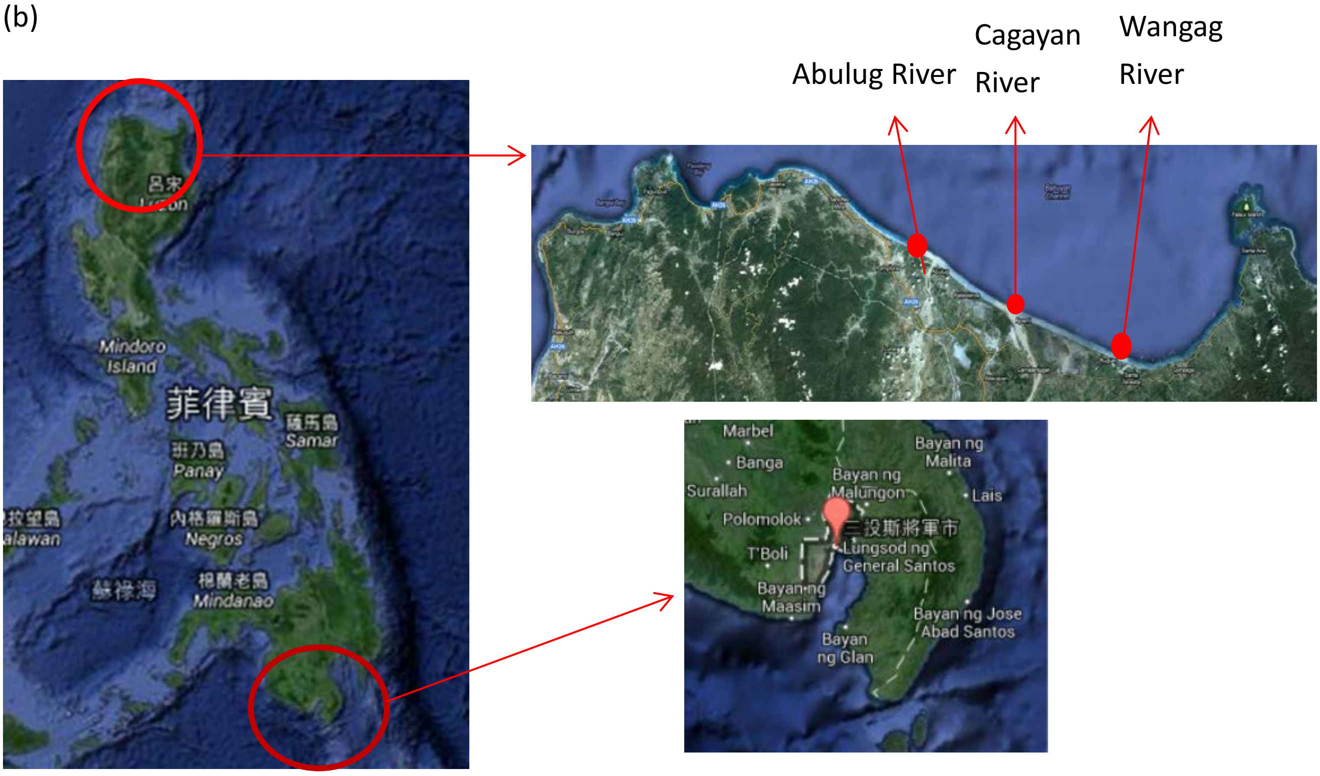
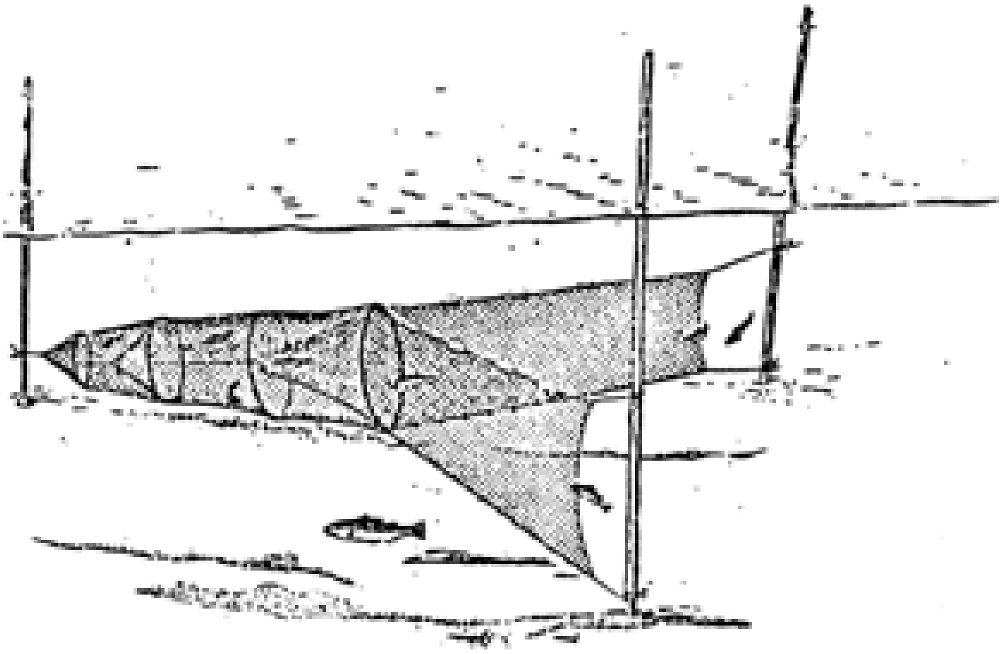


Fig. 2 The fishing tackle for capturing glass eel in the estuaries. (a) The traditional triangle nets were used in Siouguluan and Fengshan River; (b) the fixed fyke nets were used in Company Tian creek of Danshui, Cagayan, Wangag and Abulug River; (c) the mobile fyke nets were used in Yilan and Tungkang River

(a)

(b)



(c)

Fig. 3 Identification of different development stages of pigmentation on the *Anguilla luzonensis* glass eels. VA, VB, VIA1, VIA2, VIA3 and VIA4 refer to Strubberg (1913) and Fukuda *et al.* (2013)

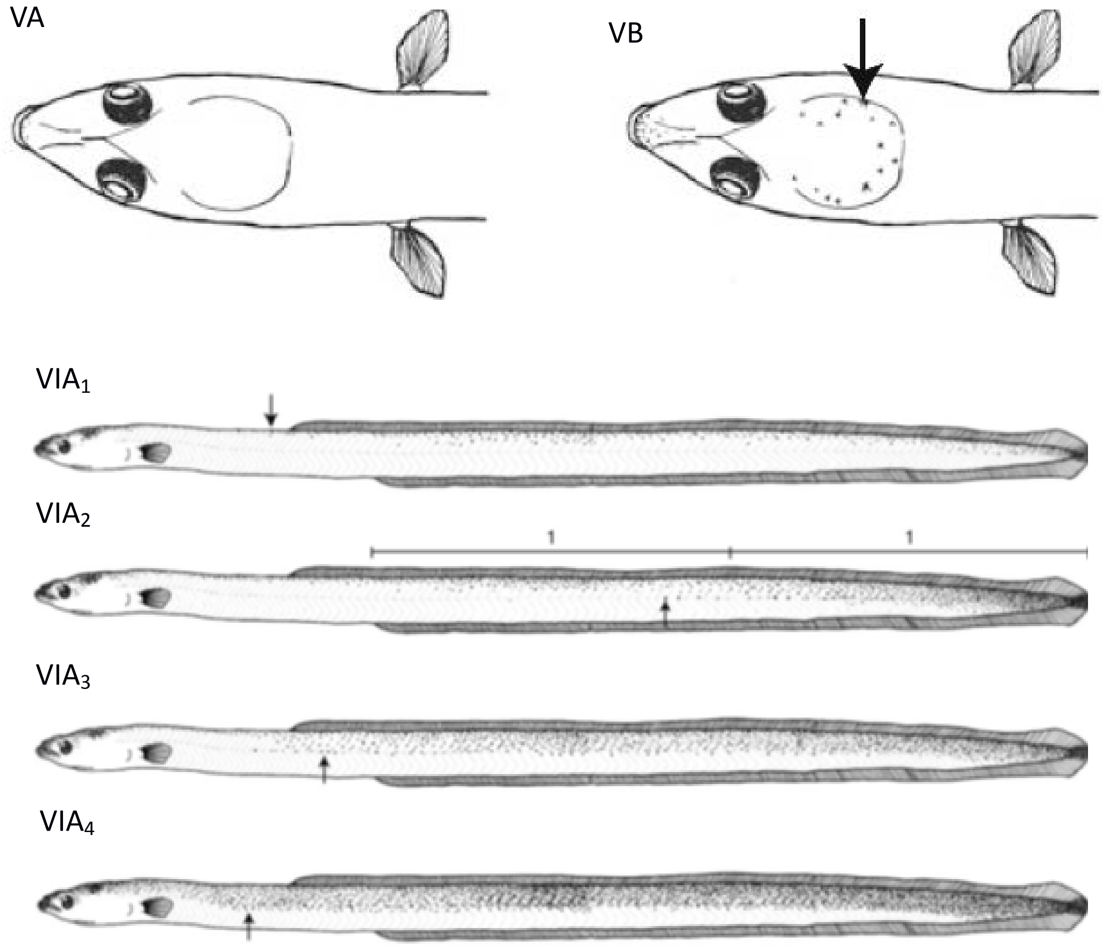


Fig. 4 Identification of *Anguilla* glass eels based on morphology

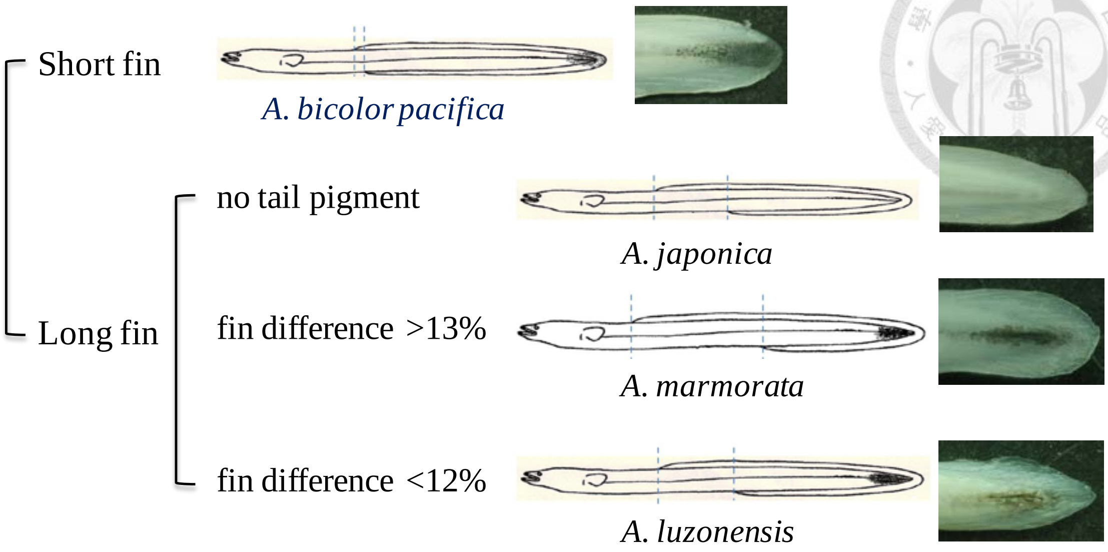
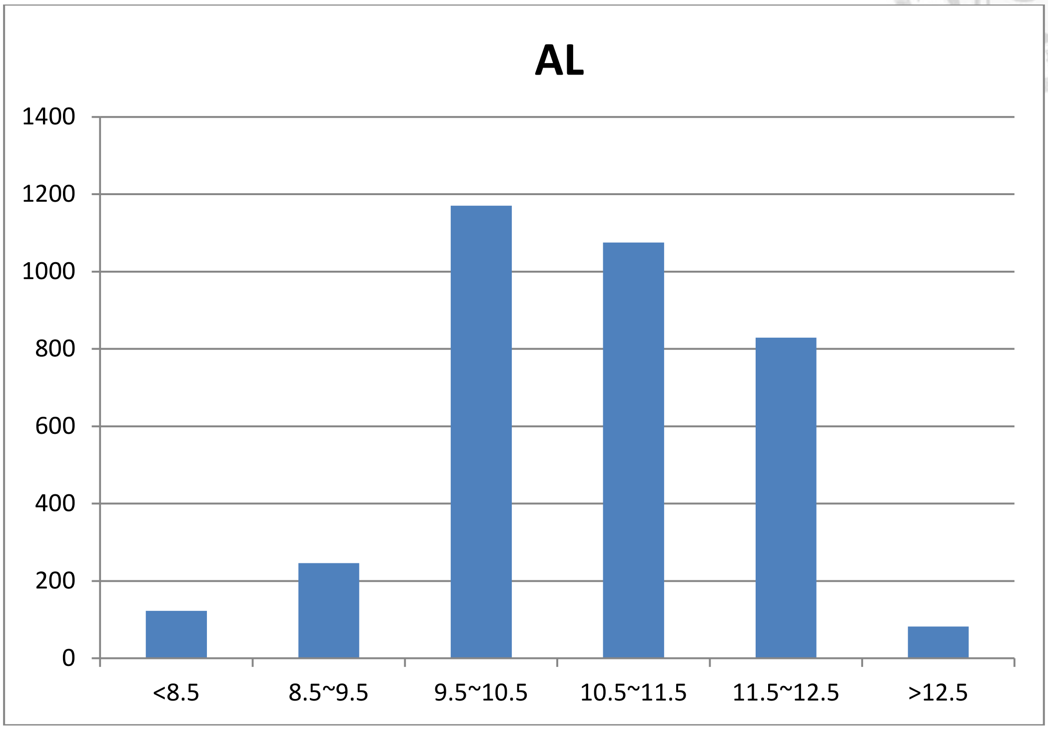


Fig. 5 Fin difference distribution with AM (*Anguilla marmorata*) and AL (*Anguilla luzonensis*). (X=Fin difference , Y=Catch)



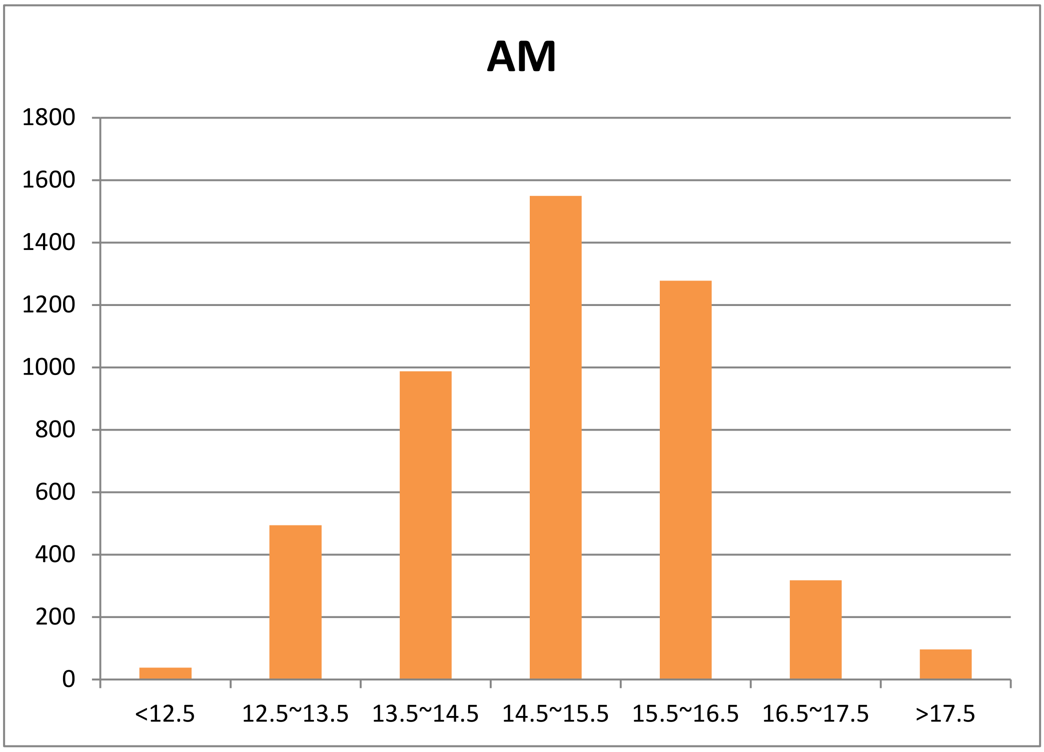
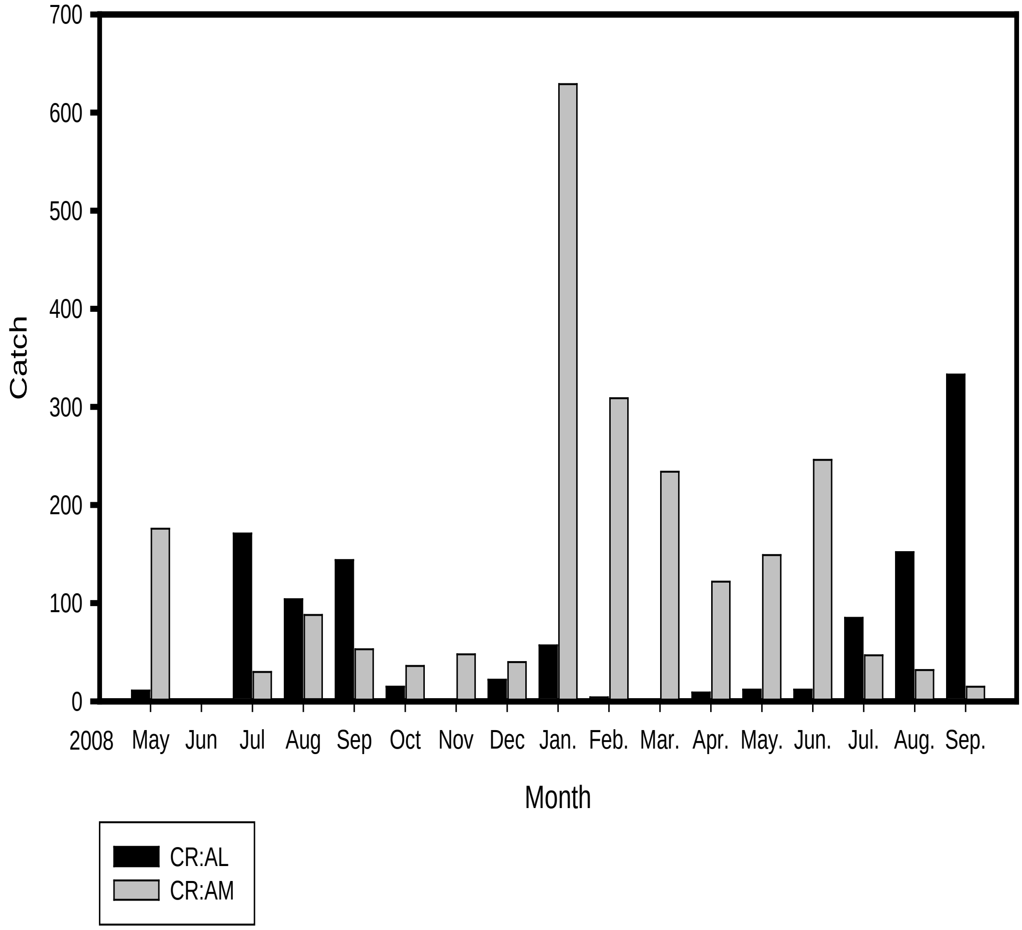
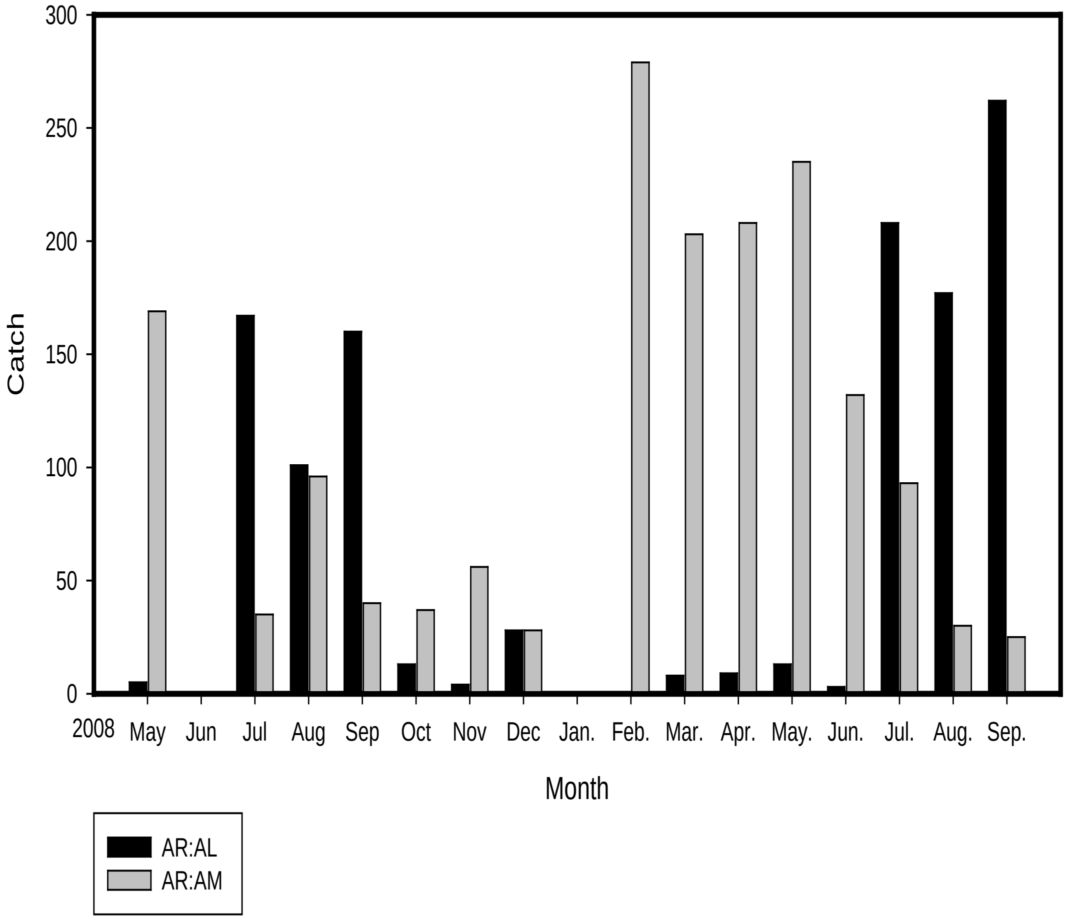


Fig. 6 Sample collection from north Luzon Island from May.2008 to Sep. 2009 in (a) Cagayan river (CR) (b) Abulug river (AR) (c) Wangag river (WR)

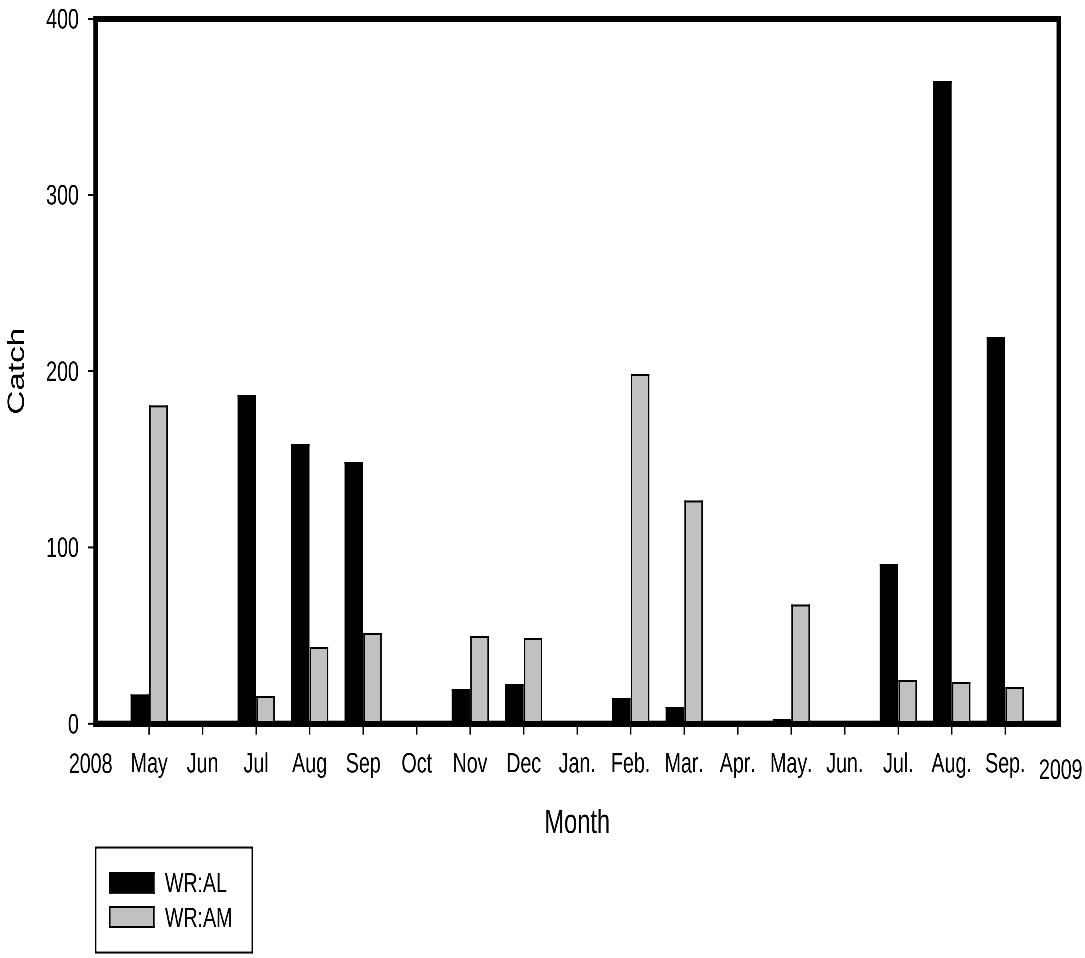
(a)



(b)


300
250
200
150
100
50
0
Catch
2008
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan.
Feb.
Mar.
Apr.
May.
Jun.
Jul.
Aug.
Sep.
Month
AR:AL
AR:AM

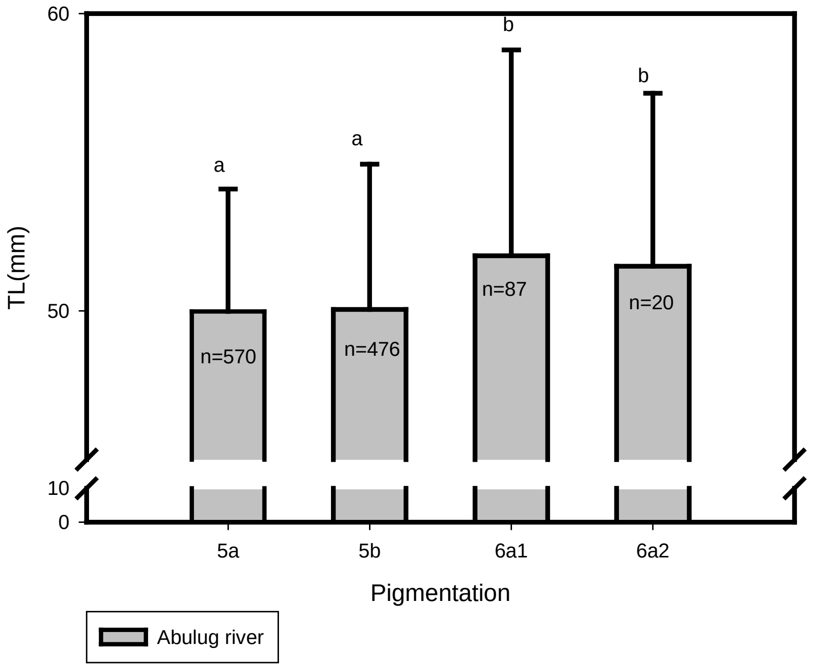
(c)



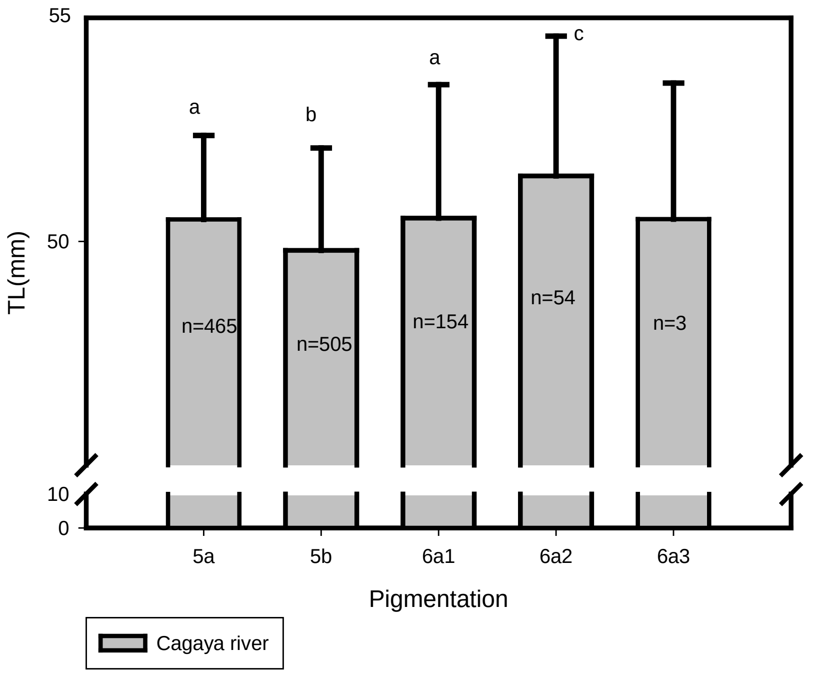
400
300
200
100
0
Catch
2008
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan.
Feb.
Mar.
Apr.
May.
Jun.
Jul.
Aug.
Sep.
2009
Month
WR:AL
WR:AM

Fig. 7 Pigmentation stage v.s Total length (mm) of *Anguilla luzonensis* (a) Abulug river (b) Cagayan river (c) Wangag river (d) Taiwan (e) Philippines (3 rivers)

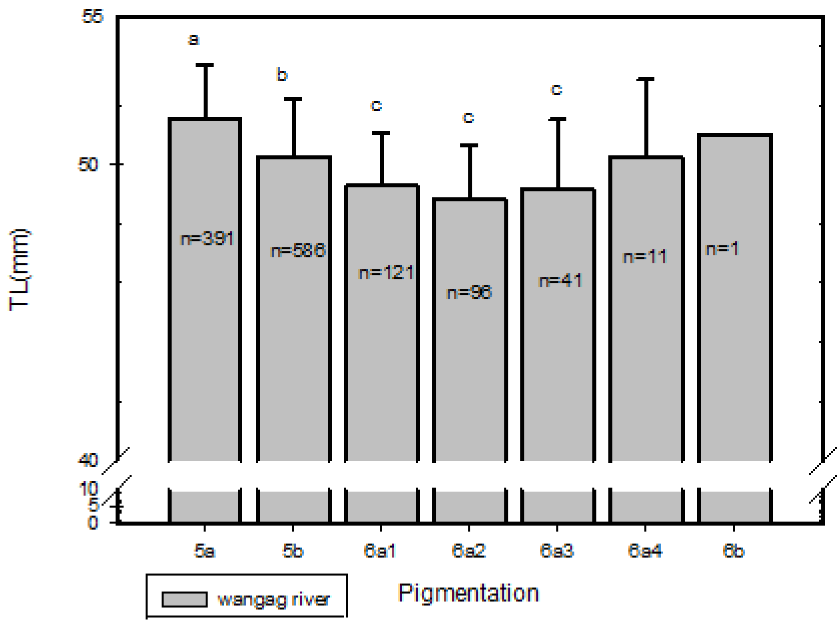
(a)



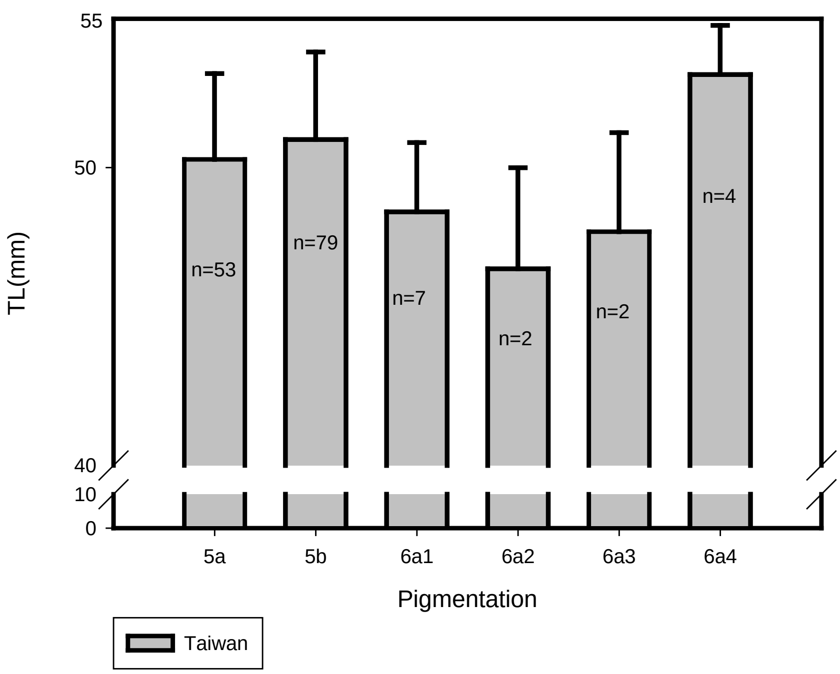
(b)



(c)


55
50
40
10
5
0
TL(mm)
a
b
c
c
c
n=391
n=586
n=121
n=96
n=41
n=11
n=1
5a
5b
6a1
6a2
6a3
6a4
6b
wangag river
Pigmentation

(d)


55
50
40
10
0
TL(mm)
n=53
n=79
n=7
n=2
n=2
n=4
5a
5b
6a1
6a2
6a3
6a4
Pigmentation
Taiwan

(e)

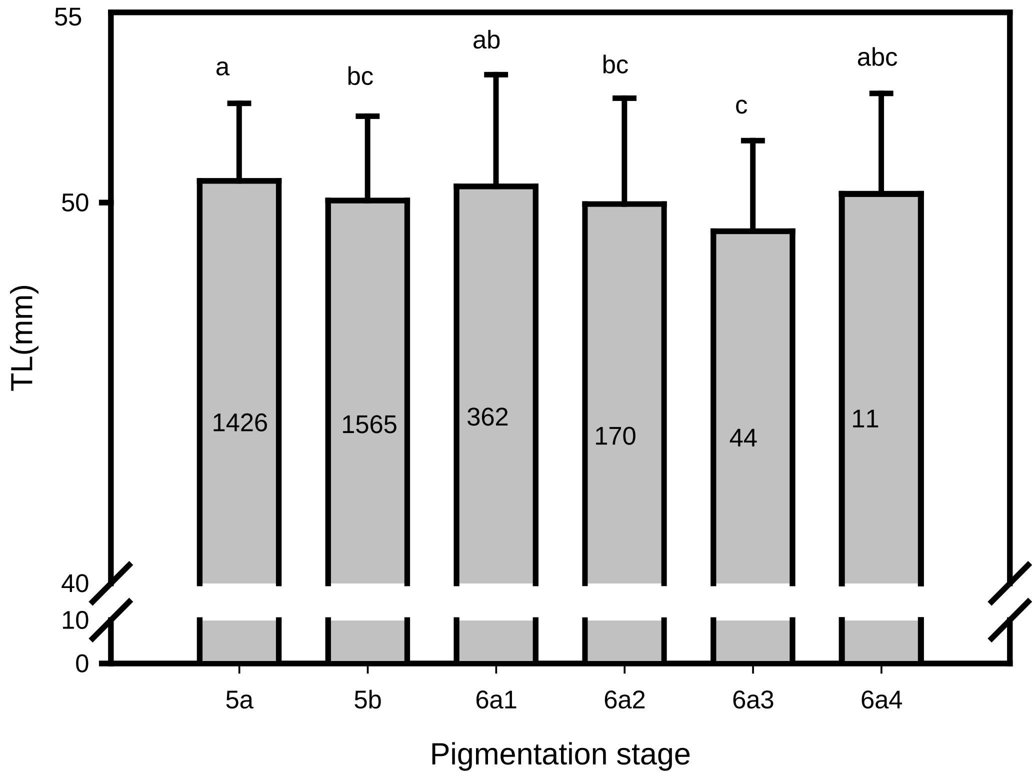
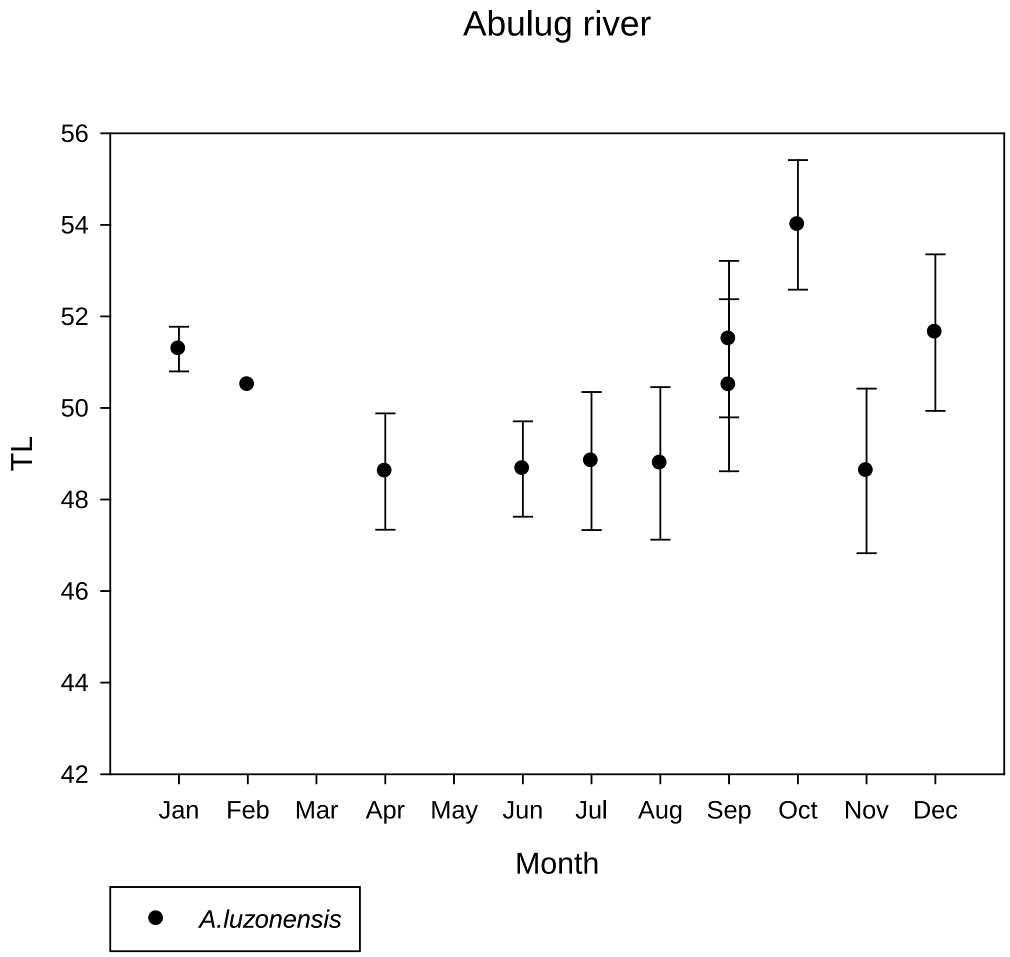
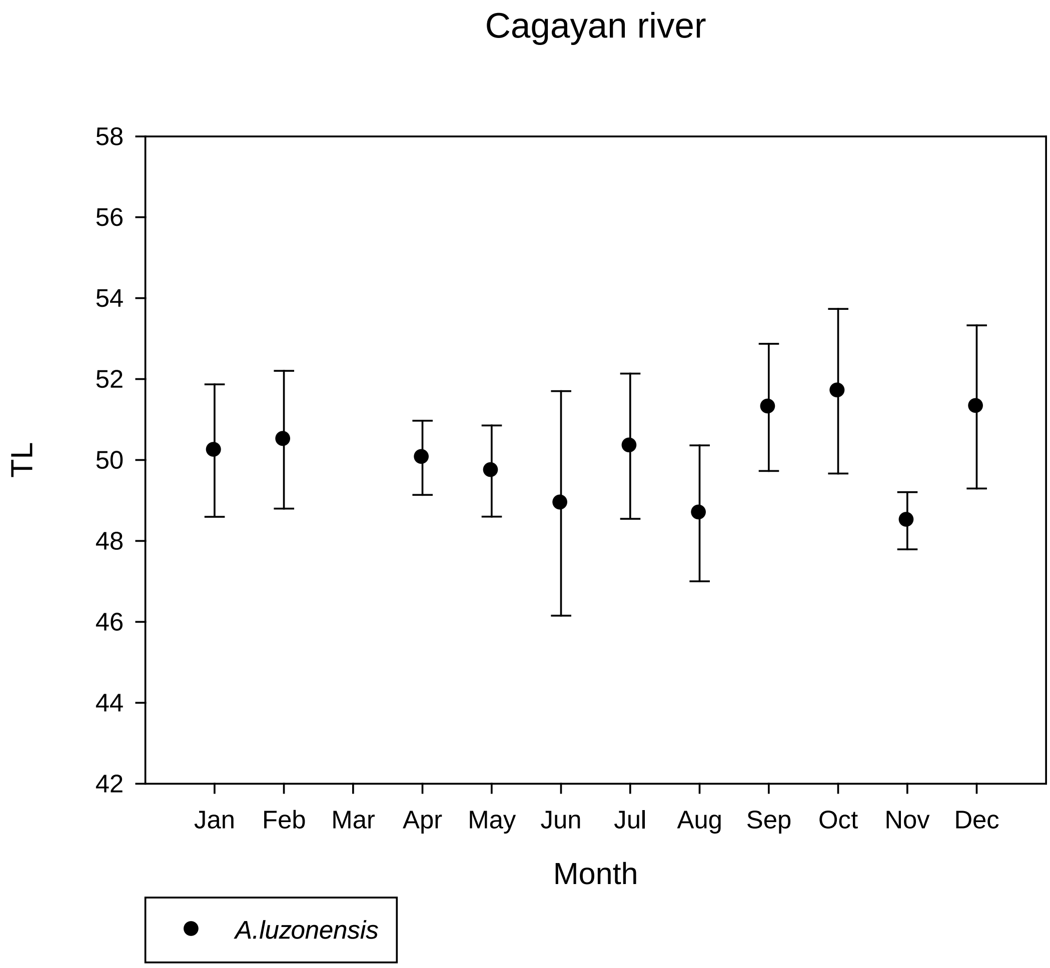

55
50
40
10
0
TL(mm)
a
bc
ab
bc
c
abc
1426
1565
362
170
44
11
5a
5b
6a1
6a2
6a3
6a4
Pigmentation stage

Fig. 8 Means Total length (mm) of *A.luzonensis* (VA) from May.2008 to Sep.2009 (a) Abulug river (b) Cagayan river (c) Wangag river (d) Abulug river+Cagayan river + Wangag river (VA+VB)

(a)

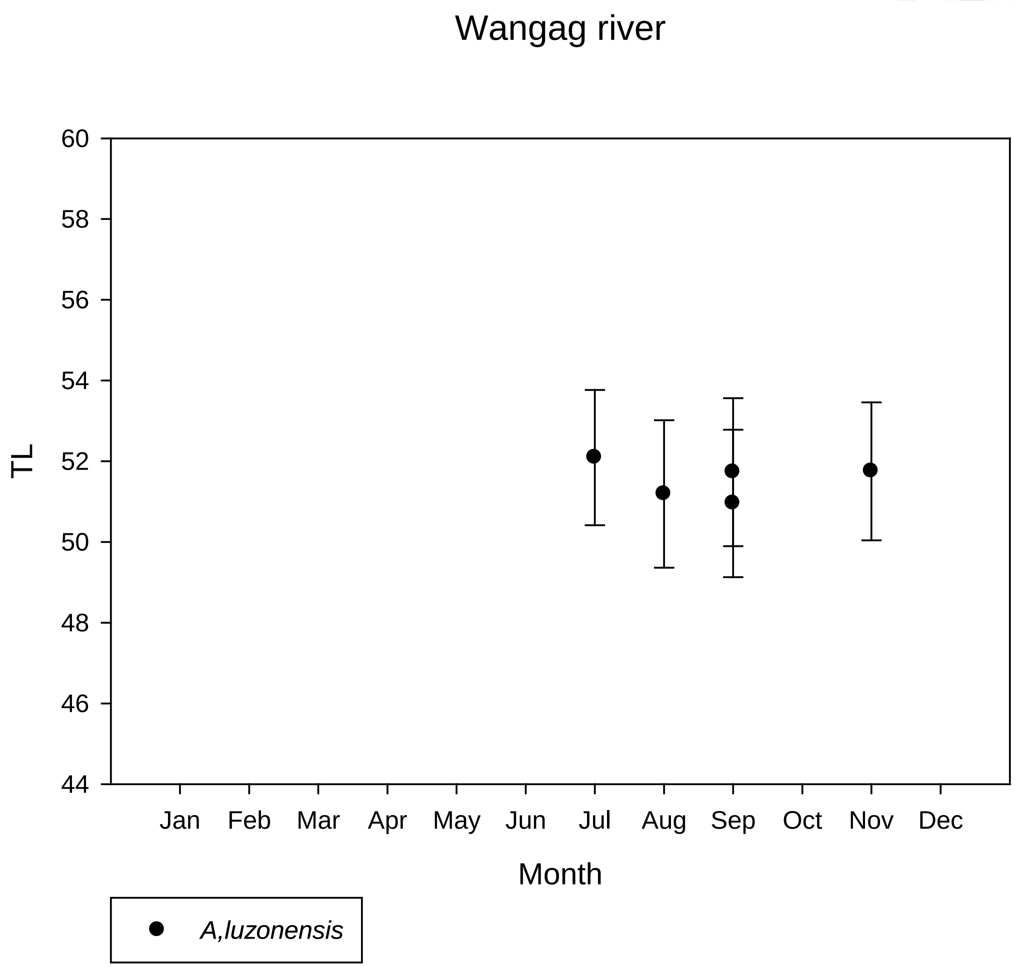


(b)



Cagayan river
58
56
54
52
50
48
46
44
42
TL
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month
A.luzonensis

(c)


Wangag river
60
58
56
54
52
50
48
46
44
TL
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month
A,luzonensis

(d)

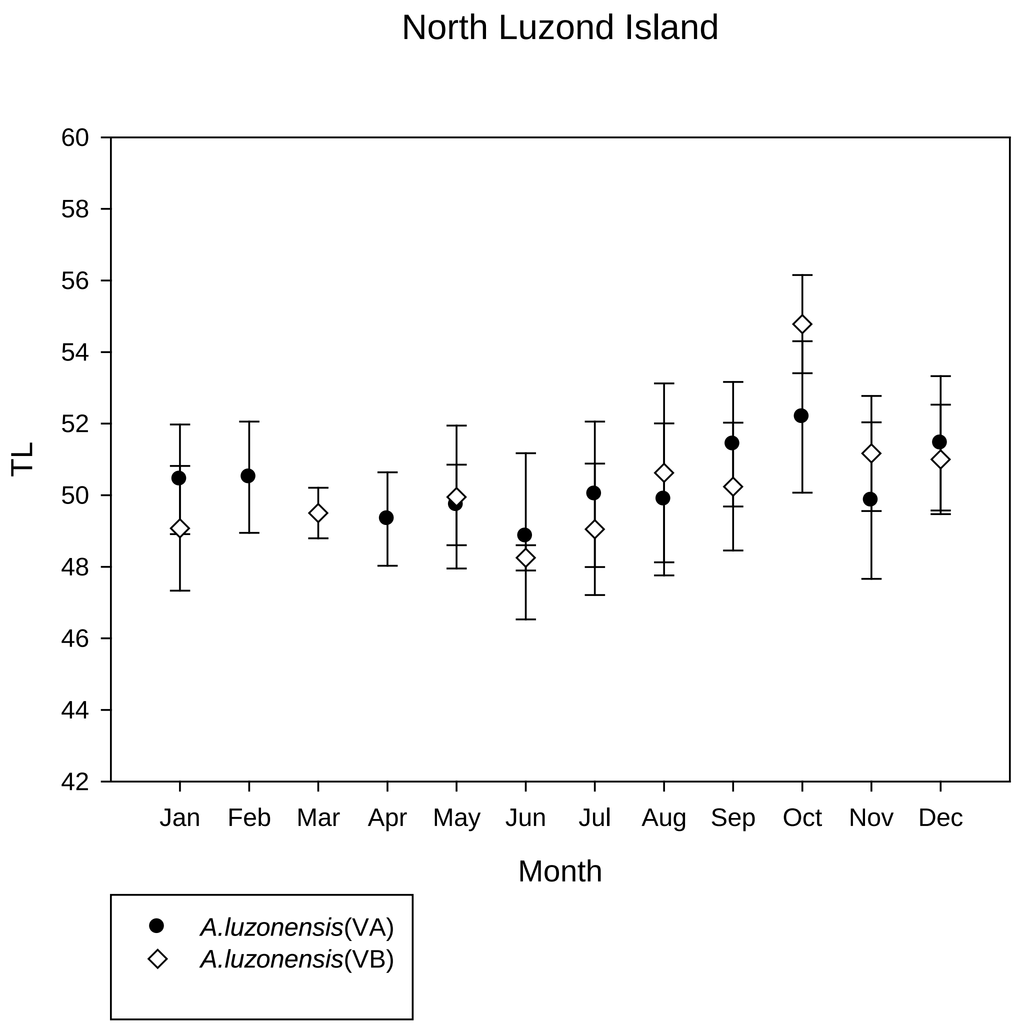
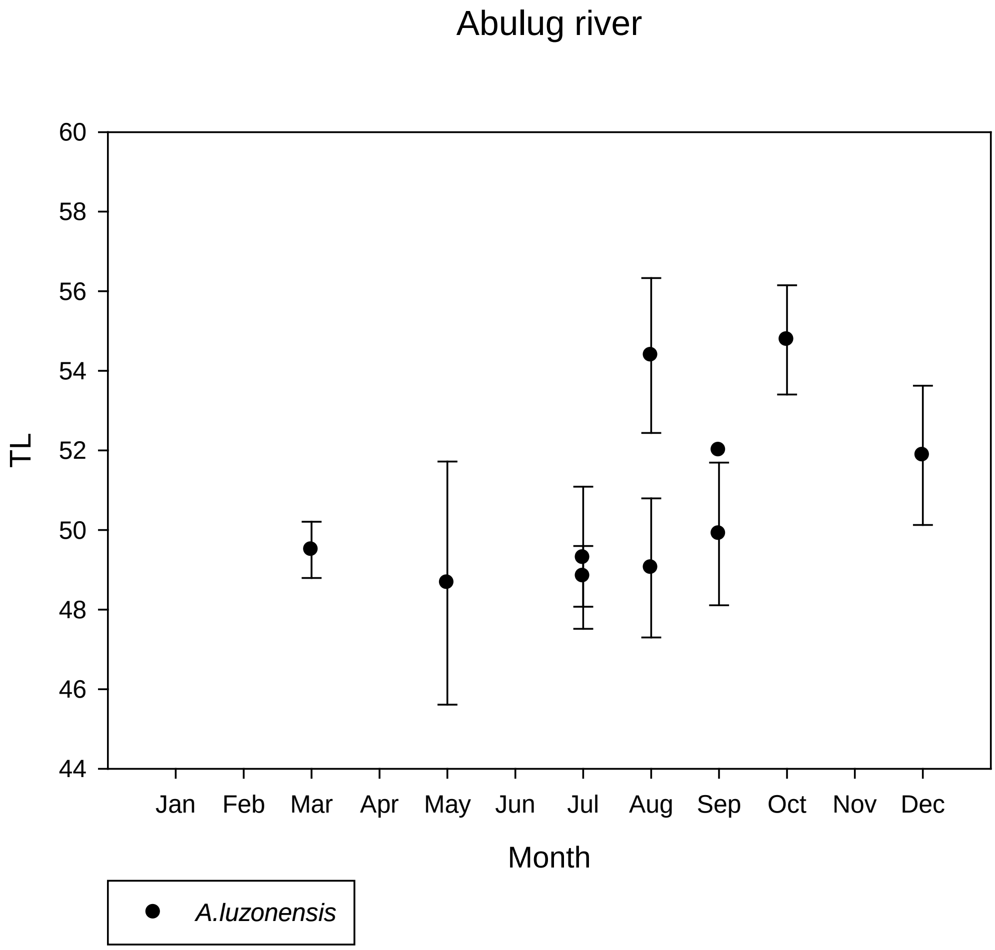
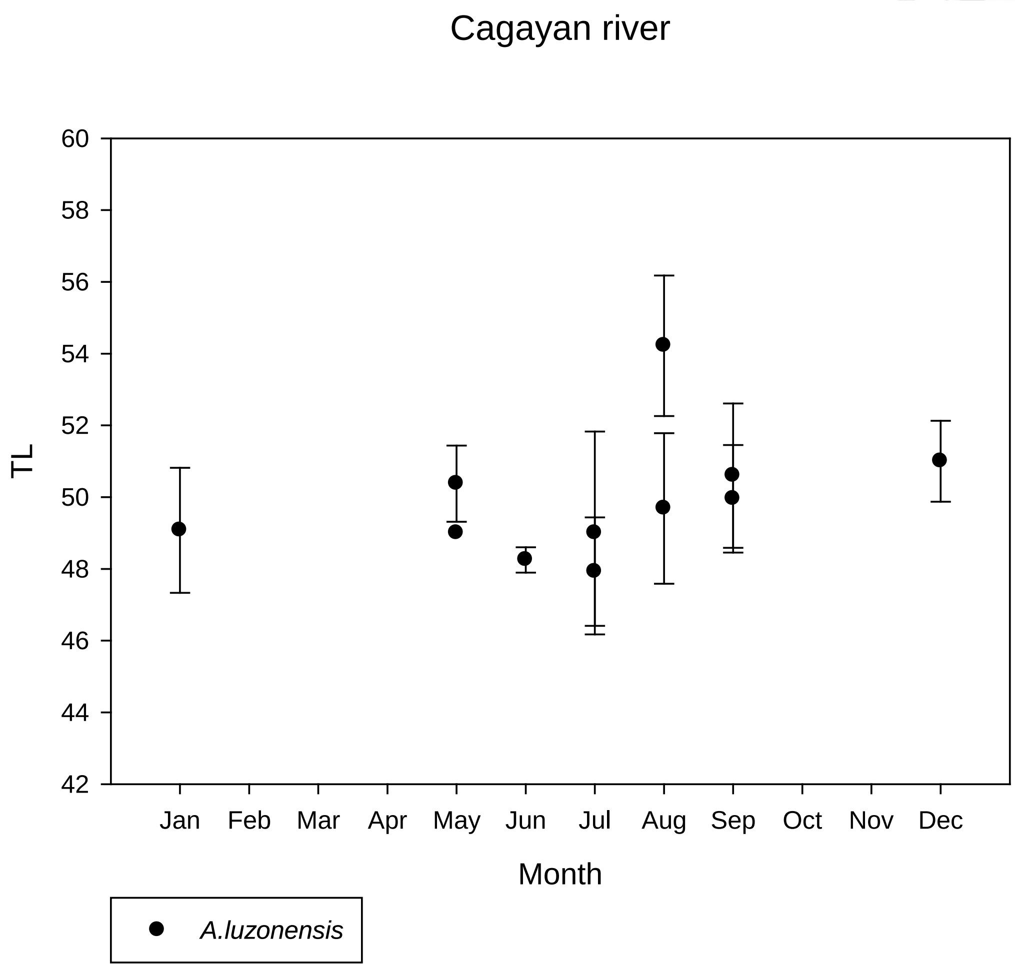

North Luzond Island
60
58
56
54
52
50
48
46
44
42
TL
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month
A.luzonensis(VA)
A.luzonensis(VB)

Fig. 9 Means Total length (mm) of *A.luzonensis* (VB) from May.2008 to Sep. 2009
(a) Abulug river (b) Cagayan river (c) Wangag river

(a)



(b)


Cagayan river
60
58
56
54
52
50
48
46
44
42
TL
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month
A.luzonensis

(c)

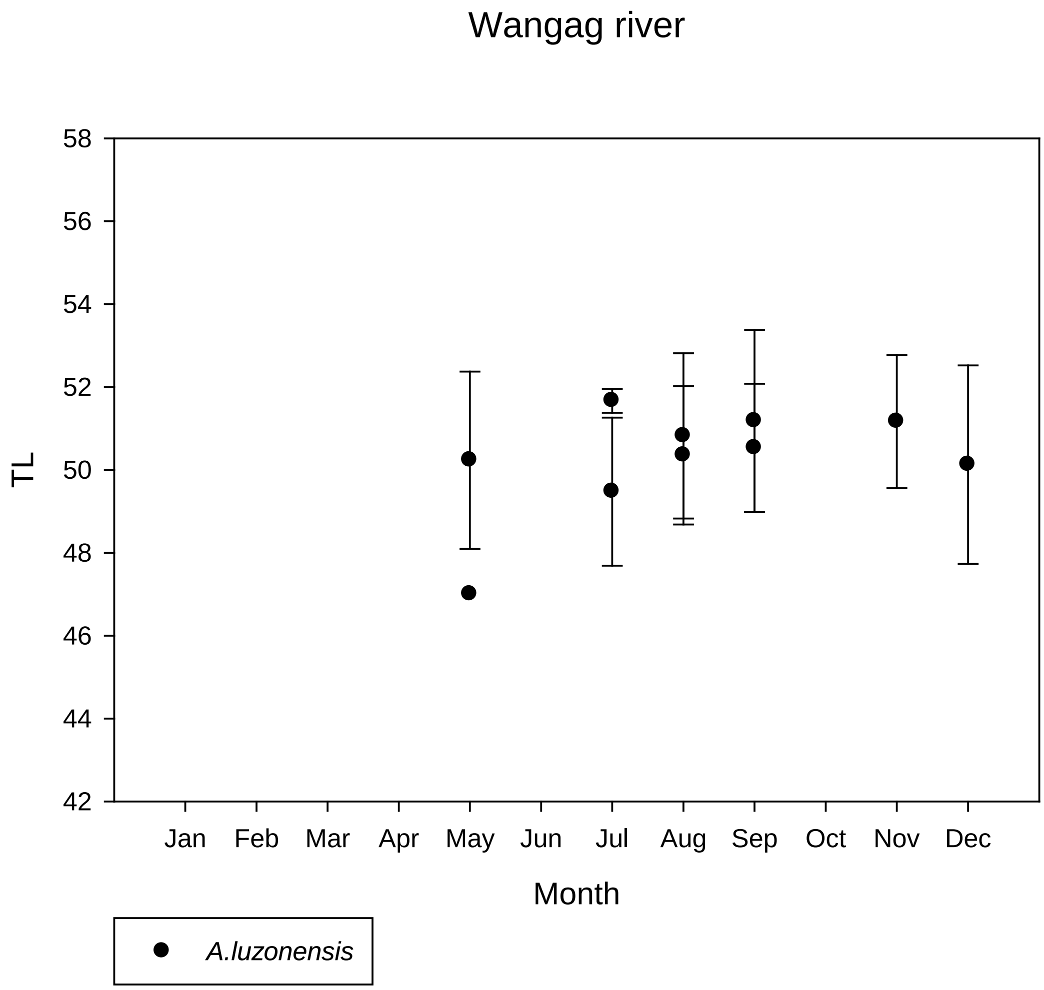

Wangag river
58
56
54
52
50
48
46
44
42
TL
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month
A.luzonensis

Table 1 Sample collection from north Luzon island from May. 2008 and Sep. 2009

	Cagayan river (AL) : (AM)		Abulug river (AL) : (AM)		Wangag river (AL) : (AM)	
2008.MAY	11	176	5	169	16	180
2008.JUL	171	30	167	35	186	15
2008.AUG	104	88	101	96	158	43
2008.SEP	144	53	160	40	148	51
2008. OCT	12	36	13	36	0	0
2008. NOV	3	48	4	55	19	49
2008. DEC	40	40	28	27	22	48
2009. JAN	57	629	0	0	0	0
2009. FEB	4	309	1	279	14	198
2009. MAR	2	234	2	203	9	126
2009. APR	9	122	9	208	0	0
2009. MAY	12	149	13	235	2	67
2009. JUN	12	246	3	132	0	0
2009. JUL	85	47	208	93	90	24
2009. AUG	152	32	177	30	364	23
2009. SEP	333	15	262	25	219	20

Table 2 Monthly composition of glass eel samples of *A. marmorata* (AM) and *A. luzonensis* (AL) collected from Taiwan

Month	AL	AM
JAN	14	436
FEB	16	1247
MAR	3	1901
APR	1	1251
MAY	1	661
JUN	0	991
JUL	14	1439
AUG	11	872
SEP	19	1036
OCT	46	1177
NOV	20	1101
DEC	2	587

Table 3 Sample collection from Mindanao from Jan. 2009 and Apr. 2010

Month	AL	AM
JAN	0	195
FEB	-	-
MAR	-	-
APR	0	97
MAY	-	-
JUN	-	-
JUL	-	-
AUG	-	-
SEP	27	106
OCT	2	163
NOV	1	102
DEC	0	94

Table 4 Pigmentation stage of *A.luzonensis* from Abulug River, Cagayan River, Wangag River and Taiwan.

	Abulug river	Cagayan river	Wangag river	Taiwan
Pigmentation				
5A	570	465	391	53
5B	476	505	586	79
6A1	87	154	121	7
6A2	20	54	96	2
6A3	0	3	41	2
6A4	0	0	11	4
6B	0	0	1	0
Total	1153	1181	1247	147

Table 5 Pigmentation and TL±SD of *A.luzonensis* from Abulug River,Cagayan River, Wangag River and Taiwan.

	Abulug river	Cagayan river	Wangag river	Taiwan
Pigmentation	TL ± SD	TL ± SD	TL ± SD	TL ± SD
5A	49.97±2.0[a]	50.49±1.8[a]	51.52±1.8[a]	50.27±2.8
5B	50.04±2.4[a]	49.79±2.2[b]	50.27±1.9[b]	50.94±2.9
6A1	51.8±3.4[b]	50.5±2.9[a]	49.27±1.7[c]	48.51±2.3
6A2	51.5±2.9[b]	51.46±3.1[c]	48.79±1.8[c]	46.6±3.3
6A3	-	50.5±3.0	49.15±2.3[c]	47.85±3.3
6A4	-	-	50.22±2.6	53.12±1.6
6B	-	-	51	-

Table 6 Morphology of *Anguilla luzonensis* from Luzon Island,Taiwan and Mindanao

Morphology	Abulug river	Cagayan river	Wangag river	Taiwan	Mindanao
TL(mm)	50.17±2.4^{a}	50.24±2.3^{a}	50.40±2.0^{a}	50.38±3.5^{a}	48.83±2.0^{b}
Fin difference	10.77±1.0^{a}	10.70±1.1^{a}	10.61±1.1^{a}	11.12±1.4^{b}	10.36±2.1^{a}
Total samples	1153	1181	1247	147	30

Appendix table 1. Data of *Anguilla luzonensis* eels used for analysis in this thesis.

Year	Country	Sampling sites	Month	n	Total length	Pigmentatoin					
					Mean ± SD	V_A	V_B	VI_{A1}	VI_{A2}	VI_{A3}	VI_{A4}
2008	Philippne	Abulug river	May	5	48.4 ±2.3	0	3	1	1	0	0
2008		Abulug river	Jul	167	49.2 ±1.7	0	165	1	1	0	0
2008		Abulug river	Sep	160	51.5 ±1.7	159	1	0	0	0	0
2008		Abulug river	Oct	13	54.5 ±1.3	4	9	0	0	0	0
2008		Abulug river	Nov	4	48.6 ±1.7	4	0	0	0	0	0
2008		Abulug river	Dec	28	51.6 ±1.6	24	4	0	0	0	0
2009		Abulug river	Feb	1	50.5	1	0	0	0	0	0
2009		Abulug river	Mar	2	49.5 ±0.7	0	2	0	0	0	0
2009		Abulug river	Apr	9	48.6 ±1.2	9	0	0	0	0	0
2009		Abulug river	May	13	49.4 ±0.6	13	0	0	0	0	0
2009		Abulug river	Jun	3	48.6 ±1.0	3	0	0	0	0	0
2009		Abulug river	Jul	208	48.8 ±1.4	205	3	0	0	0	0
2009		Abulug river	Aug	177	48.9 ±1.7	67	109	1	0	0	0
2009		Abulug river	Sep	262	49.9 ±1.9	81	131	40	10	0	0
2008		Cagayan river	May	11	50.7±3.0	0	8	0	0	0	0
2008		Cagayan river	Jul	171	48.0±1.6	0	111	52	8	0	0
2008		Cagayan river	Aug	104	54.0±1.8	0	34	44	25	1	0
2008		Cagayan river	Sep	144	51.2±1.6	133	10	1	0	0	0
2008		Cagayan river	Oct	15	51.7±2.0	15	0	0	0	0	0
2008		Cagayan river	Nov	2	48.5±0.7	2	0	0	0	0	0

Appendix table 1. Data of *Anguilla luzonensis* glass eels used for analysis in this thesis.

Year	Country	Sampling sites	Month	n	Total length	Pigmentatoin					
					Mean ± SD	V_A	V_B	VI_{A1}	VI_{A2}	VI_{A3}	VI_{A4}
2008	Philippne	Cagayan river	Dec	22	51.1±1.4	8	14	0	0	0	0
2009		Cagayan river	Jan	57	49.6±1.7	28	27	0	0	0	0
2009		Cagayan river	Feb	6	50.5±1.7	6	0	0	0	0	0
2009		Cagayan river	Apr	9	50.0±0.9	9	0	0	0	0	0
2009		Cagayan river	May	12	49.6±1.0	11	1	0	0	0	0
2009		Cagayan river	Jun	9	48.7±2.4	7	2	0	0	0	0
2009		Cagayan river	Jul	199	50.3±1.8	197	2	0	0	0	0
2009		Cagayan river	Aug	153	49.4±2.0	39	114	0	0	0	0
2009		Cagayan river	Sep	228	49.8±1.5	0	155	52	20	1	0
2009		Cagayan river	Dec	39	51.2±1.8	9	28	0	0	0	0
2008		Wangag River	May	16	50.1±2.0	0	15	1	0	0	0
2008		Wangag River	Jul	186	49.4±1.7	0	177	7	2	0	0
2008		Wangag River	Aug	158	50.3±1.6	0	153	4	1	0	0
2008		Wangag River	Sep	148	50.7±1.7	78	70	0	0	0	0
2008		Wangag River	Nov	19	51±1.4	4	3	2	3	4	3
2008		Wangag River	Dec	22	49.6±1.3	0	4	15	2	1	0
2009		Wangag River	Feb	14	50.5±2.7	0	0	0	4	6	3
2009		Wangag River	Mar	9	49.5±3.5	0	0	0	1	3	5
2009		Wangag River	May	2	45.5±2.1	0	1	0	0	1	0

Appendix table 1. Data of *Anguilla luzonensis* glass eels used for analysis in this thesis.

Year	Country	Sampling sites	Month	n	Total length	Pigmentatoin					
					Mean ± SD	V_A	V_B	VI_{A1}	VI_{A2}	VI_{A3}	VI_{A4}
2009	Philipine	Wangag River	Jul	90	52.0±1.6	87	3	0	0	0	0
2009		Wangag River	Aug	364	49.8±2.1	92	92	84	77	19	0
2009		Wangag River	Sep	219	51.4±1.9	130	68	8	6	7	0
2009	Taiwan	Danshui River	Feb	4	46.1±1.6	3	1	0	0	0	0
2009		Danshui River	Apr	1	51.5	1	0	0	0	0	0
2009		Danshui River	Jul	1	48	0	1	0	0	0	0
2009		Danshui River	Sep	3	45.5±2.7	3	0	0	0	0	0
2009		Donggang River	Jul	5	47.5±1.4	5	0	0	0	0	0
2010		Donggang River	Mar	1	53	1	0	0	0	0	0
2010		Fengshan River	Oct	1	45	0	1	0	0	0	0
2011		Fengshan River	Jul	2	52.5±0.7	0	0	0	0	0	2
2011		Fengshan River	Sep	2	52.2±4.5	0	0	0	1	0	1
2011		Fengshan River	Oct	1	52	0	0	0	0	0	1
2011		Fengshan River	Nov	1	45.5	0	0	0	0	1	0
2010		Yilan River	Jul	4	46.9±2.8	1	1	0	1	1	0
2010		Yilan River	Aug	3	53.0±3.0	0	3	0	0	0	0
2010		Yilan River	Nov	6	49.3±2.1	0	5	1	0	0	0
2011		Yilan River	Jan	5	49.1±1.9	2	3	0	0	0	0
2011		Yilan River	Feb	10	52.0±2.0	1	8	1	0	0	0

Appendix table 1. Data of *Anguilla luzonensis* glass eels used for analysis in this thesis.

Year	Country	Sampling sites	Month	n	Total length	Pigmentatoin					
					Mean ± SD	V_A	V_B	VI_{A1}	VI_{A2}	VI_{A3}	VI_{A4}
2010	Taiwan	Yilan River	Sep	1	48.8	1	0	0	0	0	0
2011		Yilan River	Aug	1	47	0	1	0	0	0	0
2011		Yilan River	Nov	1	49	0	1	0	0	0	0
2009		Siouguluan River	Feb	1	49.5	0	1	0	0	0	0
2009		Siouguluan River	Mar	2	55.5±0.7	0	2	0	0	0	0
2009		Siouguluan River	May	1	47.9	0	1	0	0	0	0
2009		Siouguluan River	Jul	1	53	1	0	0	0	0	0
2009		Siouguluan River	Aug	2	49.5±0.7	2	0	0	0	0	0
2009		Siouguluan River	Sep	1	46	1	0	0	0	0	0
2009		Siouguluan River	Oct	2	46.2±0.3	2	0	0	0	0	0
2009		Siouguluan River	Dec	2	47.2±1.7	1	1	0	0	0	0
2010		Siouguluan River	Jan	1	47.5	0	0	1	0	0	0
2010		Siouguluan River	Jul	1	45.5	0	1	0	0	0	0
2010		Siouguluan River	Aug	5	50.7±3.1	2	3	0	0	0	0
2010		Siouguluan River	Sep	11	50.1±3.0	7	2	2	0	0	0
2010		Siouguluan River	Oct	42	51.8±2.3	5	35	2	0	0	0
2010		Siouguluan River	Nov	12	51.4±2.8	7	5	0	0	0	0
2011		Siouguluan River	Jan	8	50.6±3.0	1	7	0	0	0	0
2011		Siouguluan River	Feb	1	55	1	0	0	0	0	0