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Full Paper

SEXUAL DEVELOPMENT OF THE TROPICAL SHORT-FINNED EEL, *Anguilla bicolor bicolor* OF THE SEGARA ANAKAN WATERS, CENTRAL JAWA, INDONESIA

Hagi Yulia Sugeha[*)♣], Irwan Jatmiko[**)], Sahri Muhammad[***)]

Abstract

A total of 289 specimens of tropical short-finned eel, *Anguilla bicolor bicolor*, was collected from the Waters of Segara Anakan in May, September, October, and November 2004. Basic biological information including body weight (BW) and total length (TL), gonad morphology, gonad somatic index (GSI), and eye index (EI) among collecting specimen was examined in order to understand their sexual development. It was found that *A. b. bicolor* from Segara Anakan were greatly varied in body weight (10-900g) and total length (16-78cm), suggested their occured in different life stages (young eel and adult eel). Based on gonad morphology it was found that sexual development of the species could be separated into four groups, namely: female (17.6%), male (48.1%), intersex (32.2%), and undeveloped (2.1%). GSI were 0-3 in female, 1-9 in male, 0-4 in intersex, and 2-7 in undeveloped. EI were 3-10 in female, 1-7 in male, 2-8 in intersex, and 1-4 in undeveloped. Positive relationship was found between EI and body weight and length of the species in each sexual development, but no correlation between GSI and body weight and length, except for female. Different from temperate eels that bigger in size but smaller in GSI and EI, tropical eel *A. b. bicolor* was smaller in size but bigger in GSI and EI, suggested more earlier sexual maturation in the tropic than in the temperate.

Key words: *Anguilla bicolor bicolor*, sexual development, GSI, EI, Segara Anakan

Introduction

The freshwater eels of the genus *Anguilla* Schrank, 1798, are widely distributed in the world. From a physiological viewpoint the eel is a particularly popular for an experimental animal. This is due not only to its extremely marked resistance to many shorts of experimental conditions, but also to a large number of unusual characteristics which distinguish the eel from other species of fish. Included among these characteristics are its phases of differential activity and behavioral patterns, its multistage metamorphosis during ontogenic development; its great endurance and its ability to navigate during migration, and its unusual body shape. The freshwater eels have several groups of species that have very similar morphological features (Watanabe, 2001). However, in some species of temperate eels, the morphological characters were used as secondary characters on study the repoductive characters of the anguillid eels.

In most fishes the male and females are separate individuals, however, this tipical reproductive or life history strategy is open to many modification. Some species have internal fertilization but some species are hermaphrodite or change sex during their lifetime

[*)] Research Center for Oceanography - LIPI, Jakarta
[**)] Agency for Marine and Fisheries Research - DKP, Jakarta
[***)] Faculty of Fisheries and Marine Science - Brawijaya University, Malang
[♣] Penulis untuk korespondensi:email: hagi001@lipi.go.id

during their lifetime likes occur in the eel. The newly hacthed of young eel are a few millimeters long, usually in the form of larvae quite unlike the adult, with a yolk sac and relatively undeveloped body form. Once the yolk is resorbed, the larvae must find food on its own. After a period of growth the larvae metamorphose into juvenile, immmature adult, and mature form. The earliest stage it is possible to distinguish male from female gonads macroscopically when the fish reaches a length of about 20 cm (Walter, 1910 in Tesch, 1979) although according to some authors, definate identification cannot be made until a length of 30 cm has been reached (Satoh *et al.*, 1965; Sinha & Jones, 1966).

The gonad are mesodermal in origin and develop in close association with the nephric system. As teleost, the paired gonads of eel derived from a single region equivalent to the cortex. The location and determination of the gonads as male or female is often difficult in immature specimens, fatty tissue sometimes makes mis identified as gonad. The gonad are equal size, with the right extending further forward about 1cm in 30cm European eels and the left reaching further posteriorly (about 2 cm behind the cloaca in 30cm) also in European eels (Tesch, 1979). The left gonad is about 2-3% longer (Tesch, 1979), and is heavier and contains more egg than the right gonad (Matsui, 1952). Kochnenko (1959) in Tesch (1979) related head with to ovarian width; broad- headed specimens, had gonads about 5-6mm accross while eels with more pointed heads have ovaries about 9 mm wide (Tesch, 1979).

Those studies has been mainly conducted in the temperate eels, however, almost no study has been done in the tropical eels. Therefor, we propose to study on the sexual development of the short-finned eel, *A. b. bicolor*, as one of the representative tropical eels that inhabit in the Indonesian Waters. The objective of this study was to know the sexual development of *A. b. bicolor* from the Waters of Segara Anakan (Central Jawa, Indonesia) based on body measurements and gonad observation of that tropical short-finned eels.

Material and Method

Despite to its statue as National Conservation Area propagated by the Indonesian Government, the Waters of Segara Anakan were famous as favorable area for eel catch from long time ago. Fishing activities has been conducted by the local fisherman using various types of fishing gear including line and baits, traps, and fish nets. Eel catch were varied in life stage including glass eels, elvers, yellow eels, and silver eels for local consumption or even for both local and international trade. Based on its geographic position, the Waters of Segara Anakan was facing freshwater discharge from Cibaliung River as potential growth habitat for the tropical eels and the waters was also facing to the Indian Ocean as the potential spawning habitat of the catadromous eels (Fig. 01). This strategy environmental condition is favorable for eel living habitat.

Eel sample collection has been conducted in the Waters of Segara Anakan (Central Jawa, Indonesia) in May, September, October, and November 2004. In order to get representative sample, by conducting collaboration with local fisherman, the live specimen of yellow and silver eel in various size and number were collected using several fishing gears including line and bait, trap, and fish net that usually used on that area. The eels were kept alive after captured and transported to the laboratory for future examination.

Before morphological data collection of the body shapes, the specimens were overdosed with anesthesia. After weighing the body weight (g), the body length (cm) measurements were done including total length (TL), pre-dorsal length (PDL), pre-anal length (PAL), body depth (BD), head length (HL), head width (HW), head depth (HD), inter-orbital width (IOW), and pectoral fin length (Pf-L) to the nearest 1 mm. Other body part measurements such as horizontal and vertical eye diameter were measured to the nearest 0.1 mm. The morphological characters of ADL/%TL (Sugeha *et al*, 2001; Watanabe, 2001) and the dentition structure (Watanabe, 2001) were used as first confirmation for species identification.

Thereafter, specimens were dissected to pick up a piece of liver tissue for crossing check with genetic species identification using PCR-RFLP analyses (Sugeha *et al*, 2006; Sugeha *et al*, 2008). Gonad morphology and development were observed and described based on Satoh *et al.* (1962). After then, gonads were measured including gonad length (GL) and gonad width (GW) both of left and right side. After measurement, gonad were removed and weighed for adjustment the GSI of the specimen. The Gonad Somatic Index (GSI) was calculated as:

$$\text{Gonad Somatic Index} = (\text{gonad weight}/\text{body weight}) * 100$$

The eye index (EI) was also calculated as (Pankhurst, 1982a):

$$\text{Eye Index} = \{((A+B)/4)^2 \times \pi/L\} \times 100$$

Where A is the horizontal eye diameter, B is the vertical eye diameter and L is the total length.

Result

Specimen collection, species/sub species adjustment

A total of 289 specimens of tropical short-finned eels were collected from the Segara Anakan Waters and were used for this study. Based on morphology and genetic analyses,all the specimens were identified as *Anguilla bicolor bicolor*. Using external morphology character (ano-dorsal length as percent of total length), the range of ADL/%TL of the species was about -3 to 4 and using internal morphology character (number of ano-dorsal vertebrae) the range of ADV of the species was about -3 to 3. Using six restriction enzymes (*Alu* I, EcoT14 I, *Bbr*P I, *Hha* I, *Mva* I, and *Bsp*1286 I) as the specific enzymes to recognized the tropical eel species, the result performed 6 haplotypes of *A. b. bicolor* inhabit in the Indonesian waters (Sugeha *et al*, 2006; Sugeha *et al*, 2008). Additional morphology characters (broad maxillary band of the teeth structure and non-variegated marking of the skin type) also suggest that the specimens used in this study was *A. b. bicolor* only.

External morphology characters

Based on body skin coloration all specimens of *A. b. bicolor* collected from the Waters of Segara Anakan might belong to silver eel stage (25%) and yellow eel stage (75%). The silver eel was dark in dorsolateral portion, and the belly pectoral fin were dark black with a golden color at the base. The yellow eel was brown yellowish in dorsolateral portion, pectoral fin were yellow brownish with a white color at the base.

Biological information including body weight (BW) and body length (TL=total length; PDL=pre-dorsal length; PAL=pre-anal length; BD=body depth; HL=head length; HW=head width; HD=head depth; LG=length of gape; IOW=inter-orbital width; Pf-L=pectoral fin length) and gonad morphology (Gr-L=gonad right length; Gl-L=gonad left length) was examined and the results as could be seen in Table 1.

Anguilla b. bicolor from Segara Anakan were greatly varied in body weight (BW=10-900 g) were the female about 55-900 g, the male about 15-200 g, the intersex about 60-580 g, and the undeveloped about 10-70 g. The average of body weight was 310.76 g in female, 45.77 g in male, 219.95 g in intersex, and 30.3 g in undeveloped. Based on body weight measurement it was found that female and intersex *A. b. bicolor* was heavier than male, but male was heavier than undeveloped.

The total specimen of *A. bicolor bicolor* was also varied from 16.0-78 cm in TL. The range of TL was 33.5-77.8 cm, 24.5-54.5 cm, 32.5-71.5 cm, and 16.0-37.0 cm in female, male, intersex, and undeveloped, respectively. The average of TL was 55.23 cm, 31.70 cm, 50.97 cm, and 28.81 cm in female, male, intersex, and undeveloped, respectively. The results suggested that female and intersex was longest than male, but male was longest than undeveloped.

Inter orbital width (IOW) is usually greater in males than in females in the temperate eels, however, in the present study we found that IOW of female *A. b. bicolor* (16.97cm) was greater than male (9.08 cm). It is suggested that external morphological characters of IOW was different between species, so that the characters of IOW of temperate eels species could not be used as guidance for the same characters in tropical eel species.

Head length (HL) as the distance from the tip of the snout to the insertion of the pectoral fin was reported to be greater in female than in males, in temperate eels. In the present study we found that female *A. b. bicolor* was 44.5-105.2 cm HL, male was 20.7-62.2 cm, intersex was 43.8106.2 cm, and undeveloped was 29.0-51.7 cm with an average of 77.42 cm, 42.21 cm, 72.46 cm, and 38.70 cm in female, male, intersex, and undeveloped, respectively. It means, the similar with temperate eels, tropical eels of *A. b. bicolor* also greater than male in the character of head length. However, it was also found that intersex also greater than male in HL, but male was greater in HL than undeveloped.

The character of pre-dorsal length (PDL=the distance from the tip of the snout to the dorsal fin origin) was found to be greater in female than in male of the temperate eels. In the present study, it was also found that the PDL of *A. b. bicolor* was greater in female (23.00 cm) than male (12.85 cm), intersex (21.12 cm), and undeveloped (11.90 cm).

In general it could be seen in the Table 1 that all character of external morphology of *A. b. bicolor* was greater in female compared to male, intersex, and undeveloped eels. However, external morphological characters of intersex eels were greater than male eels, and external morphological characters of male eels were bigger than undeveloped eels.

Gonad morphology and sexual determination

Based on gonad morphological observation, it was observed that gonad of *A. b. bicolor* performed "like curtain shape" and that were recognized as the texture of female gonad while gonad performed "like split shape" were recognized as the texture of male gonad (Fig. 2). Both sexual development have milky color and softly texture of gonad tissue. Gonad intersex more transparent with jelly textured. In the present study, gonad intersex of *A. b. bicolor* was separated in three characters: C1 (gonad texture dominant male), C2 (female and male gonad fair), and C3 (gonad texture dominant female). Gonad undeveloped was combination between transparent and milky tissue with more jelly texture. The undeveloped gonad was separated in two characters: A1 (the appearance of two straight lines of gonad tissue), and A2 (the appearance of two straight lines of gonad tissue, but the other one with more advance development to perform "like split shape") that may have tendency to be male gonad texture.

Gonado Somatic Index and Eye Index

Gonad somatic index (GSI) of all collecting specimen was examined in order to understand their sexual development. Female silver eel *A. b. bicolor* that collected in the present study has relatively smaller ovaries compared to male silver eels. Gonado Somatic Index were 0-3 in female, 1-9 in male, 0-4 in intersex, and 2-7 in undeveloped. Further, male *A. b. bicolor* was more dominant and more mature than female of the species in the Waters of Segara Anakan. Intersex characters mostly observed in yellow eel stage with GSI 0-4 while undifferentiated mostly observed in silver eel stage with GSI (5-7) than in yellow eel stage (2-4) (Fig. 3).

All female *A. b. bicolor* in the present study was in yellow eel stage with GSI 0-3, suggesting collected specimens of female *A. b. bicolor* in the study mostly sexual immature stage and they still need time to develop well to be sexual mature adults before spawning in the ocean. Male *A. b. bicolor* consist of sexual mature adults (GSI=5-9) and sexual immature adults (GSI=0-4). Male in sexual mature stage were more abundant than male in sexual immature stage. Interestingly, GSI intersex eel (0-4) was almost similar with GSI female eel so they might be in sexual immature stage while GSI undeveloped eel (2-7) was almost similar with GSI male eel so they might be in both sexual immature and mature eel, like in male's case. Length of gonad left (Gl-L) proposed to be greater than length of gonad right (Gr-L), in the temperate eel. In the present study, it was found that Gl-L was greater than Gr-L in female, intersex, and undeveloped *A. b. bicolor*. However in male, Gl-L was similar with Gr-L.

Eye index (EI) of all collecting specimen was examined in order to understand their sexual development. Eye Index (EI) were 3-10 in female, 1-7 in male, 2-8 in intersex, and 1-4 in undeveloped. The result supporting the ideas above that female, male, and intersex in the present study was belong to sexual immature (EI $\leqslant$ 6.5) and sexual mature (EI $\geqslant$ 6.5), except for undeveloped eels that only belong to sexual immature stage with EI less than 6.5 (Fig. 3).

Positive relationship was found betwen GSI and EI of female *A. b. bicolor* (y=0.838x+4.4017), but negative correlation was found between GSI and EI of male (y=-0.4086x+5.6435), intersex (y=-0.4007x+5.8008), and undeveloped eels (y=-0.38089x+5.4785) (Fig. 4).

Positive relationship was found between body weight and EI of *A. b. bicolor* female (y=0.0094x+3.0008), male (y=0.0424x+1.5796), intersex (y=0.0041x+4.2428), and undeveloped (y=0.0275x+1.9337) (Fig. 5A). Positive correlation also found between body weight and GSI in female ($y=0.9788e^{-0.0014x}$), but negative correlation was found in male ($y=10.906e^{-0.0183x}$), intersex ($y=2.2558e^{-0.0022x}$), and undeveloped eels ($y=13.897e^{-0.0247x}$) (Fig. 5B).

Positive correlation was found between total length and EI of *A. b. bicolor* female (y=0.1573x-2.8925), male (y=0.2425-4.2436), intersex (y=0.0493x+2.6372), and undeveloped (y=0.1303x-1.1022) (Fig. 6A). Positive correlation was also found between total length and GSI of *A. b. bicolor* female ($Y= 0.4233e^{-0.023x}$), but negative correlation was found in male ($Y=183.43e^{-0.1158x}$), intersex ($Y=6.7004e^{-0.0307x}$), and undeveloped ($Y=164.87e^{-0.1113x}$) (Figure 06B).

Discussion

The sexuality of teleost fishes is extraordinary complex. Hermaphroditism is almost unknown in the freshwater group but it is common in marine fishes. In this case, it could be suggested that freshwater eel originally come from the ocean as marine organism and based on evolutionary scenario they become a unique marine creature as catadromous species of genus *Anguilla* (family Anguillidae, sub ordo Elophomorpha). Hermaphroditism may be successive where the individual starting as a male and becoming a female or synchronous where both parts of the ovitestis mature together (Bone *et al*., 1999). In the successive hermaphroditism, the males are

obviously smaller than the females (Krueger *et al*. 1997). Size is much more at premium for the females so that they can accomodate a large number of eggs. The male, having fertilized the eggs of its older and larger conspecifics, than can be saved to become a female itself. In anguillid eel, the hermaphrodite are protandrynous where the individuals change from males to females.
The tropical eel, *A. b. bicolor* and its closed sister *A. b. pacifica*, were belong to short-finned and non-variegated marking eels (plain eels), so that they were easy to external morphologically seperated from the other species of the genus with naked eye. However, the species was ecologically difficult to found in the upstream region or even in the river and lake. The species have tendency to living in the lower land or lower limit of freshwater area, especially in the brackishwater area, estuary, or mangrove area. However, other species of tropical eel (*A. marmorata*, *A. celebesensis*, *A. interioris*, and *A. borneensis*) has been reported to inhabit up to the river till the lake (Sugeha *et al*, 2001; Sugeha, 2003; Sugeha *et al*, 2006; Sugeha *et al*, 2008). There was no detail study has ever been done to clarified the phenomenon of species zonation in the tropical eel since the organism is quit difficult to collect in the field, but it could be done in temperate eels (Krueger and Oliveira, 1999). However, from this study could be clarified that the short-finned eel *A. b. bicolor* were inhabit in the lower area of freshwater ecosystem of the Segara Anakan Waters.

In the present study the species could be found in various life stages including glass eel, elver, yellow eel and silver eel, and suggested that they may living on that area and not migrate up to the river side until they are ready to back to the ocean for spawn. Such specific living bahaviour might be important adaption of the species to survive and to compete with the others species of tropical eels. The body size of *A. b. bicolor* is relatively smaller than the other species of tropical eel, so they may loose food and space when tried to migrate upstream as the others species of the genus. "Choice" for staying in the lower part of the river were supporting *A. b. bicolor* to life in savety and also allowing the species to keep energy before a long journey spawning migration from the lower inland water to the ocean and not from the lake to the ocean like the other species (Jessop, 1987; Oliveira *et al*., 2001; Sasai *et al*., 2001).

The detection of these secondary sexual characteristic is the result of statistical analysis of a great deal of data, and none can therefore be considered suitable as a ready means for distinguishing individual male and female on external features alone. There are nevertheless, experienced worked who, using these parameters, are almost always successful in separating males from females, even in the European eel (Pankhurst, 1982; Proman and Reynolds, 2000; Durif *et al*, 2006). Until the eels are at least 20 cm long, male are still largely undifferentiated externally (Tesch, 1979). But here in the present study, we found that undeveloped eel may reach until 37.0 cm long, while positive male may reach 54.5 cm long. Overlapping total length was occured from 24.5 cm to 37.0 cm between male and undeveloped, and could be suggested that most of the undeveloped eel would become male and not female. In contrast, total length of female and intersex was quiet overlapped (33.5 cm to 71.5 cm), so intersex eel have a tendency become females. If this is true than it could be said that related to body size, tropical eel in the same size with temparate eel male was smaller than females (Oliviera, 1998).

Different from temperate eels that bigger in size but smaller in GSI and EI, tropical eel *A. b. bicolor* was smaller in size but bigger in GSI and EI. It may be suggested earlier sexual maturation in the tropic than in the temperate. However, advance in sexual maturation could not be adjusted from external morphological observation alone. Histological study are required to confirm those ideas so that the maturation stage including gonad maturation stage as well as oogenesis and spermatogenesis stage of the species could be carried out (Aida *et al.*, 2003). Furthermore, study on the age of the species in each sexual development were important (Helfman *et al.*, 1987; Holmgren and Mosegaard, 1996; Svedang *et al.*, 1996; Homlgren *et al.*, 1997; Tzeng *et al.*, 2000; Jellyman, 2001) in order to adjust weather the differences of GSI and EI between temperate and tropical eels indicating that the tropical eel was the same age, younger, or older than temperate eels. If oldest than temperate eels, it means highest growth rate was occurred in the tropical eel, so maximum body size was not the important role to be mature in the tropical eel species. Different life history traits might be occurred between species (Oliviera and McCleave, 2000), but most seemingly that tropical eel have to perform highly growth rate compared to temperate eel (Sugeha, 2003). Fast growing phase were triggering fast sexual development in the tropical eel species and might be resulting on the multiple spawning ground and season through the year as proposed by previous authors that possible to occur in the tropical anguillid eels (Arai, 2000; Sugeha *et al.*, 2001; Sugeha, 2003; Aoyama *et al.*, 2003).

Most study on sexual development of marine fishes were based on histological analyses that required more technique, time, and budget. This study is the first study to examine sexual development of tropical eel based on combination between external morphology and gonad morphology analyses. The result shown important basic information on the sexual characters of tropical anguillid eel that may lead an advance study on the reproductive histological character of tropical eel including oogenesis and spermatogenesis studies of the species in the future.

Conclussion

The growth rate of the tropical eel was higher than temperate zone. Tropical eel was smaller in size but bigger in GSI and EI. They have got earlier sexual maturation in the tropic than in the temperate zone.

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Fig. 1. Map showing the sampling location of the *Anguilla bicolor bicolor* in the Waters of Segara Anakan (Central Jawa). Black circles represented two area of sampling activities (Muara Dua and Cangkring)

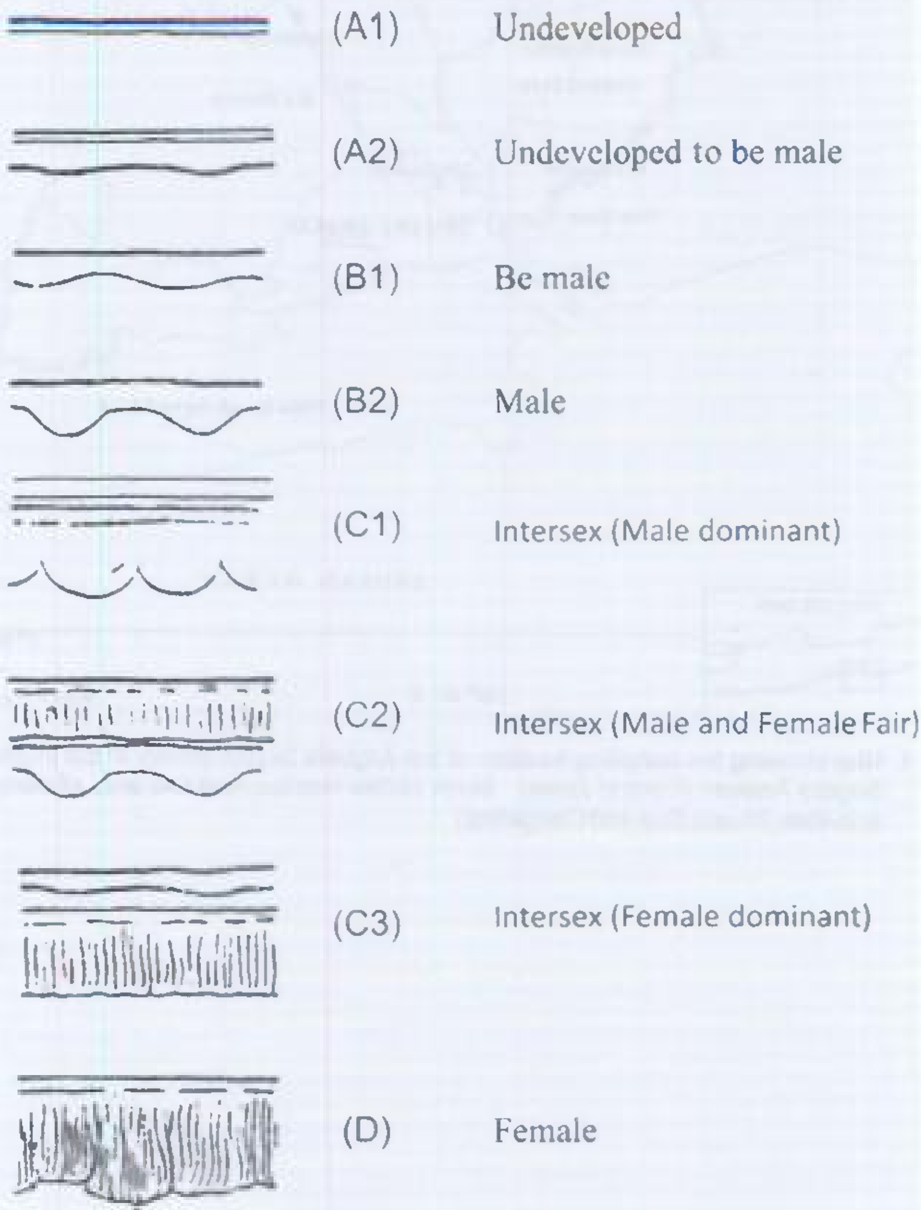


Fig. 2. Sexual development stages of the *Anguilla bicolor bicolor* from the Waters of Segara Anakan (West Jawa) based on gonad morphology. (A) Undeveloped; (B) Male; (C) Intersex; and (D) Female)

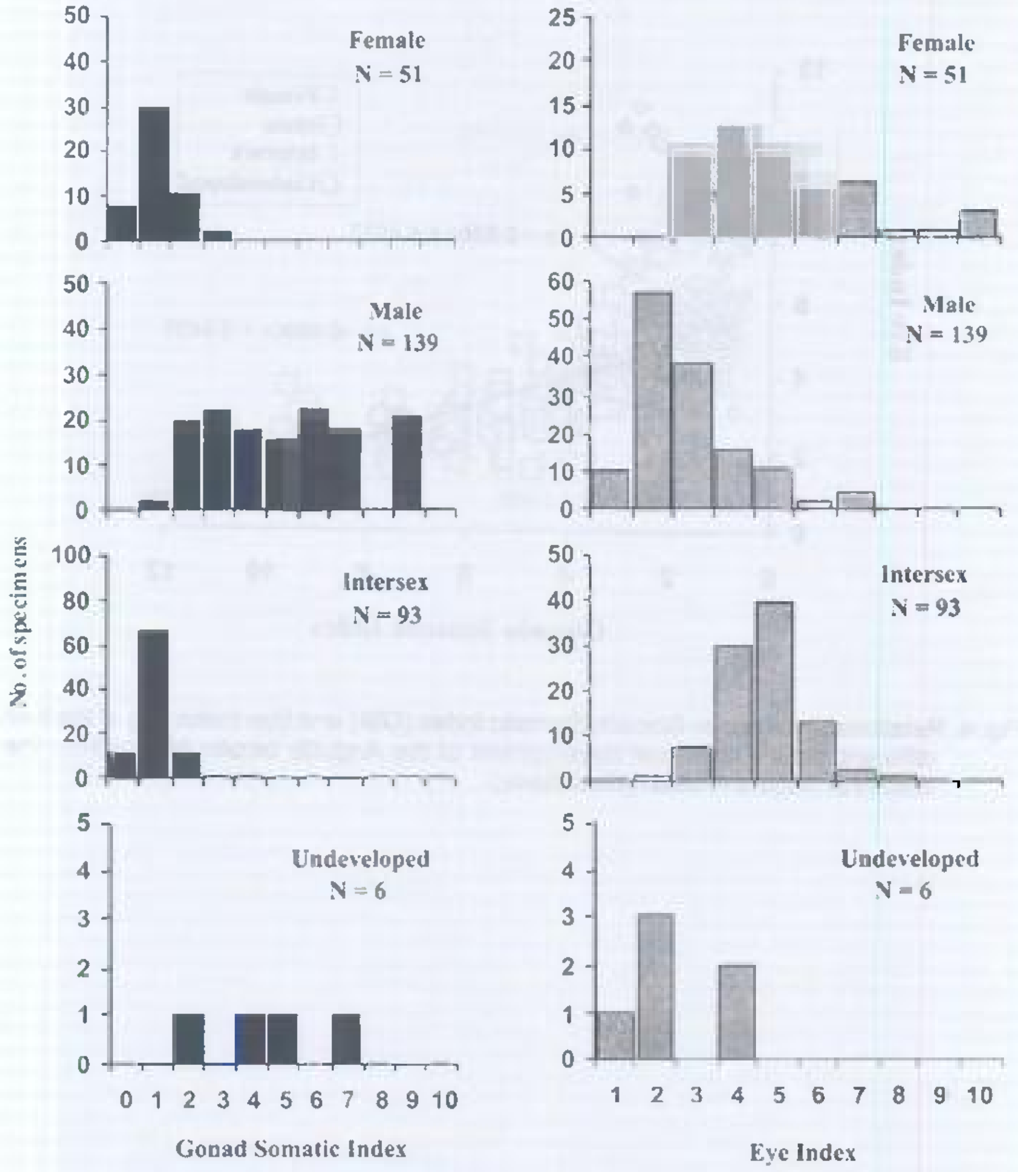


Fig. 3. Frequency distribution of the Gonad Somatic Index (GSI) and the Eye Index (EI) of four groups of sexual development of the *Anguilla bicolor bicolor* from the Waters of Segara Anakan (West Jawa)

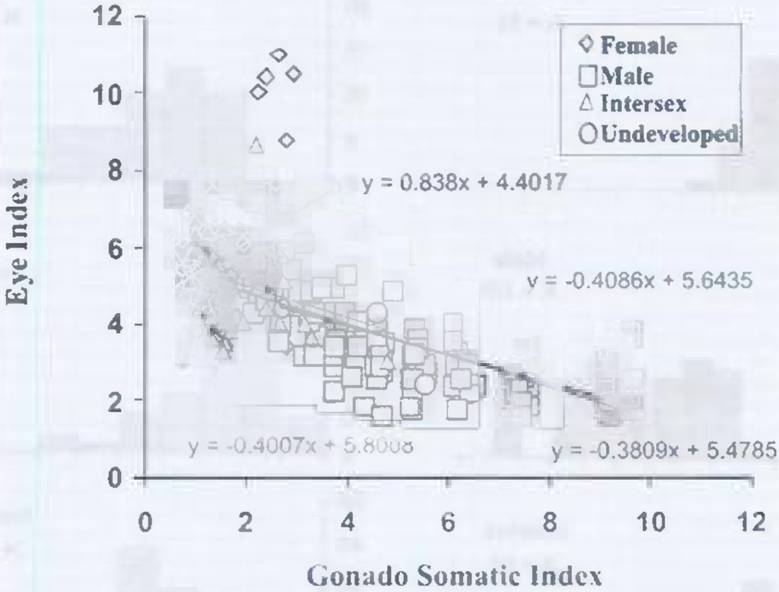
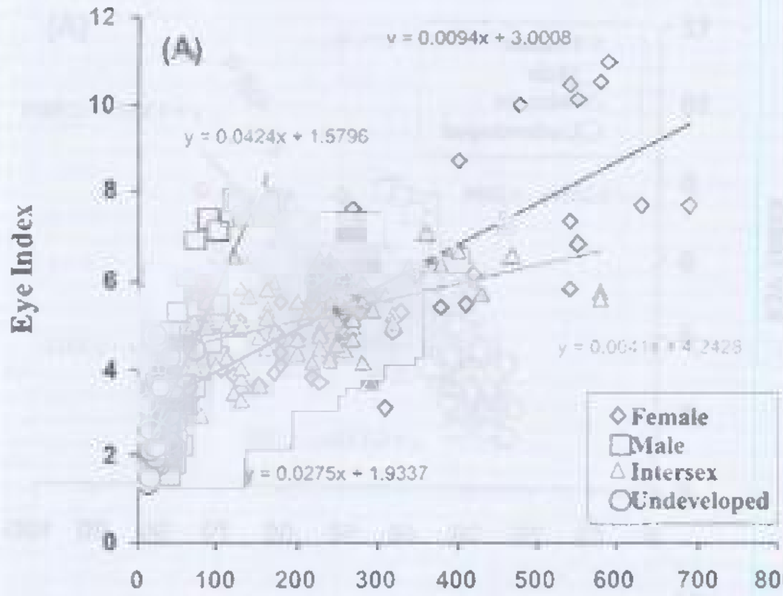


Fig. 4. Relationship between Gonado Somatic Index (GSI) and Eye Index (EI) of the four different groups of sexual development of the *Anguilla bicolor bicolor* from the Waters of Segara Anakan (West Jawa)



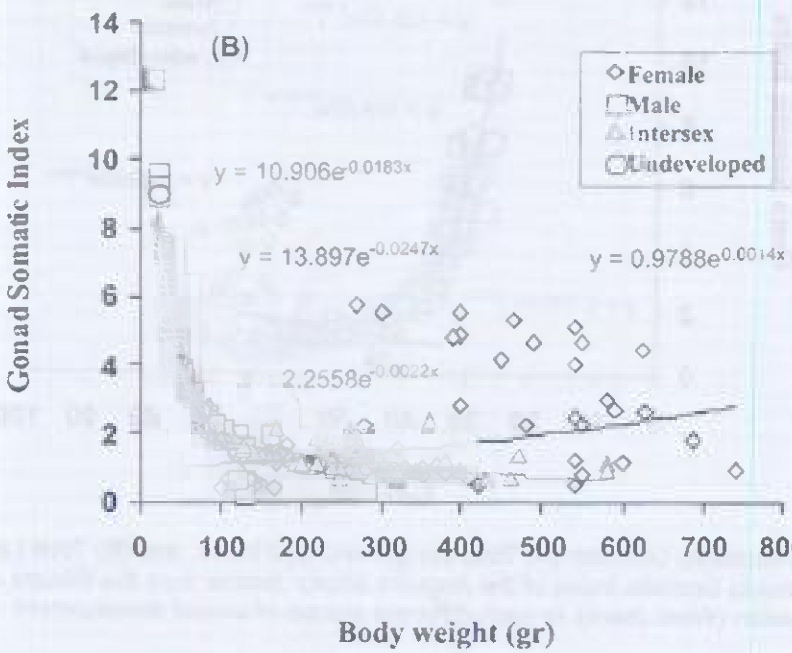


Fig. 3. Frequency distribution of the Gonad Somatic Index (GSI) and the Eye Index (EI) of four groups of sexual development of the *Anguilla bicolor bicolor* from the Waters of Segara Anakan (West Jawa)

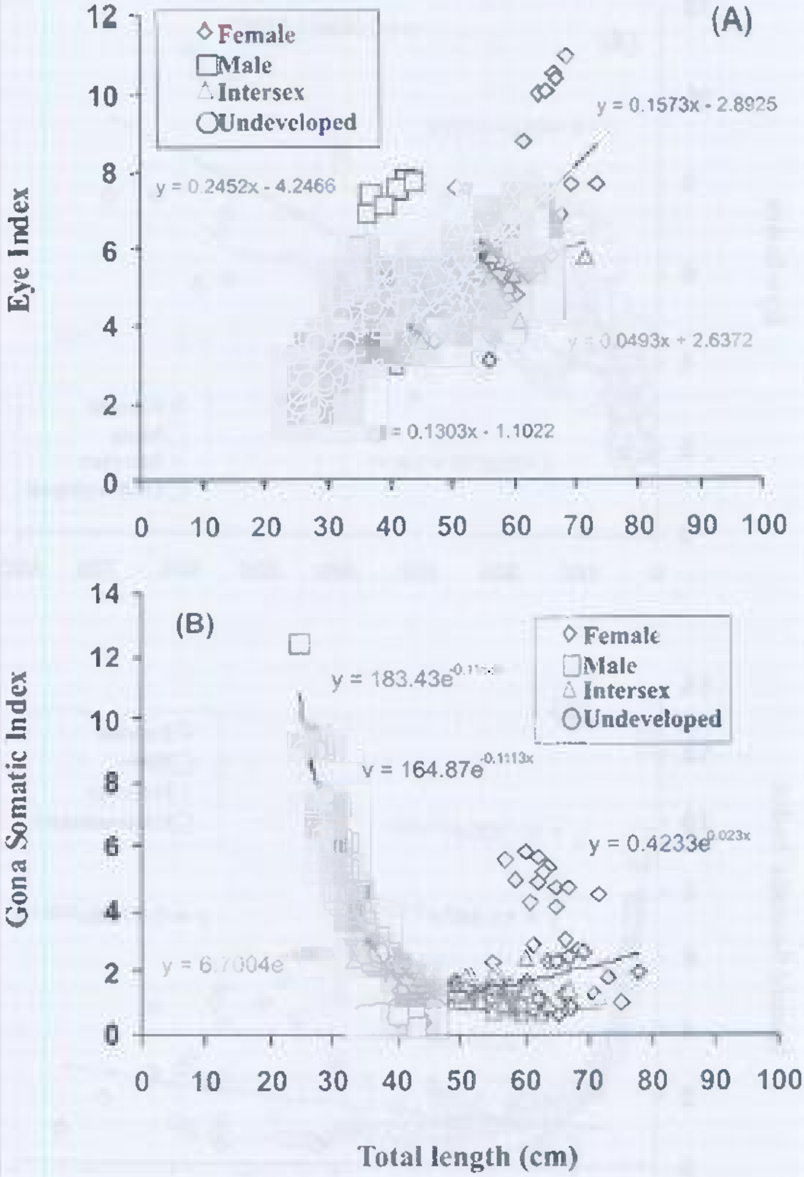


Fig. 6. Relationship between (A) Total Length and Eye Index, and (B) Total Length and Gonado Somatic Index of the *Anguilla bicolor bicolor* from the Waters of Segara Anakan (West Jawa), in each different groups of sexual development

Table 1. Morphology characters of *Anguilla bicolor bicolor* in each sexual development

Characters	Female			Male			Intersex			Undeveloped		
	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean
BW (g)	79	55-900	310.76	149	15-200	45.77	94	60-580	219.95	36	10-70	30.03
TL (cm)	79	33.5-78.0	55.23	149	24.5-54.5	31.7	94	32.5-71.5	50.97	36	16.0-37.0	28.81
PDL (cm)	79	13.0-33.0	23.00	149	9.5-25.5	12.85	94	13.5-31.5	21.12	36	9.0-22.2	11.90
PAL (cm)	79	14.0-33.5	24.16	149	9.5-26.5	13.71	94	14.5-32.5	22.45	36	9.5-22.7	12.76
BD (mm)	79	16.2-51.7	30.59	149	9.7-30.0	14.42	94	15.4-39.3	25.95	36	9.2-16.5	12.44
HD (mm)	79	15.5-50.8	28.99	149	8.4-26.4	14.09	94	15.3-38.3	26.29	36	9.4-36.0	12.50
HW (mm)	79	12-41	26.85	149	5.6-25.1	19.19	94	12.9-32.5	22.16	36	7.0-15.4	10.25
HL (mm)	79	44.5-105.2	77.42	149	20.7-64.2	42.21	94	43.8-106.2	72.46	36	29.0-51.7	38.70
LG (mm)	79	14.5-36.4	25.43	149	3.2-18.9	12.13	94	6.4-38.3	22.68	36	7.9-17.5	11.63
IOW (mm)	60	9.0-25.8	16.97	145	5.7-21.1	9.08	94	8.0-25.9	15.47	36	5.2-11.4	8.52
Pf-L (mm)	79	13.7-14.8	27.35	149	7.4-28.0	12.28	94	12.3-38.8	22.04	36	6.5-15.3	10.14
Gr-L (mm)	30	7.5-20.0	12.95	107	2.4-18.5	7.01	91	6.0-19.0	12.63	31	4.0-8.5	6.42
Gl-L (mm)	30	8.0-27.0	14.09	107	4.4-13.0	7.66	91	6.2-21.0	13.71	31	3.6-9.0	7.05