La mer 57 : 89–107, 2019, https://doi.org/10.32211/lamer.57.3-4_0
Société franco-japonaise d'océanographie, Tokyo

Morphological changes in silvering stages of *Anguilla bicolor bicolor* collected from Segara Anakan, Central Java, Indonesia

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Abstract: To understand the morphological changes during the silvering stages of *Anguilla bicolor bicolor*, 68 males and 39 females were collected from Segara Anakan in Cilacap, Central Java, Indonesia during December 2015 - September 2016, May 2017 and June 2018. Specimens were categorized into 5 stages based on body and pectoral fin coloration: Y1, Y2, S1, S2 and S3. Total length of silver males ranged from 342 mm to 501 mm, with mean ± SD 414.83 ± 40.38 mm and were notably smaller than silver females which ranged from 674 mm to 937 mm (786.11 ± 68.98 mm). Silver females were present in catches throughout the year, with peak collection during the dry months (May and June). This corresponded to the only period when silver males were caught. Locomotion indices such as, tail, dorsal fin, anal fin, pectoral fin and eye increased with progression in silvering stages, while feeding behavior indices such as, both upper and lower jaw in males showed increasing, lower lip depth in females showed decreasing and upper lip depth in females showed decreasing and snout remained constant. The increase in locomotion indices suggested that *A. bicolor bicolor* from Segara Anakan underwent morphological changes in preparation for spawning migration similar to those of temperate species, but increasing upper and lower jaw in males together with all samples caught using baited traps, suggested that these tropical eels remained as feeding individuals even at late stage silver eels.

Keywords : *Anguilla bicolor bicolor, silvering, morphological change, Segara Anakan*

1. Introduction

The life cycle of anguillid eels is divided into 5 principle stages which are leptocephalus larvae, glass eel, elver, yellow eel and silver eel stages (BERTIN, 1956). Leptocephalus larvae of tropical eel *Anguilla bicolor bicolor* migrate passively and drift following ocean currents in the eastern Indian Ocean (AOYAMA *et al.*, 2007). They then metamorphose into glass eels at continental shelves and enter coastal waters and actively migrate into estuaries and rivers (TESCH, 1980). Glass eels then become elvers which are distinguished through morphological and behavioral changes such as shortening of body length, deposition of guanine in intra-abdominal membrane

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and a benthic migration (TESCH, 2003; FUKUDA *et al.*, 2013). The growth phase in their areas of distribution is termed the yellow eel stage where they may spend many years until they reach sexual maturity and develop into migrating silver eels. The silver stage eels display changes in body coloration which are due to physiological and environmental conditions (TESCH, 2003; DURIF *et al.*, 2005; HAGIHARA *et al.*, 2012). Metamorphosis of yellow eels to silver eels is difficult to distinguish and therefore, identifying silver eels which are on the verge of migration is determined through skin color (DURIF *et al.*, 2005).

OKAMURA *et al.* (2007) described the silvering stages for *A. japonica* using two characteristics which were the coloration of the ventral skin and pectoral fins. In this description, eels were divided into two yellow eel stages YI and Y2 and two silver eel stages S1 and S2. Y1, an early yellow eel stage was described as yellow eels without metallic hue at the base of pectoral fins, while Y2 was late stage yellow eels and described as having a metallic hue at the base of pectoral fins and without melanization at the tip of pectoral fins. S1 was early stage silver eels with complete melanization at the tip of pectoral fins but without a fully pigmented belly usually with a black or dark brown coloration while, S2 was late stage silver eels with black or dark brown coloration on the belly.

Further studies on the morphological changes during the silvering/migration process of *A. australis* in the Makara Stream, Lake Onoke, and Lake Ellesmere in New Zealand showed that migrant eels had dorsally flattened heads and the snout had a slightly chiseled appearance. Furthermore, lips had thinned and the pectoral fins were slightly elongated with a black fringe. Dorsally the skin was black/brown, bronze, or green with a lateral metallic bronze coloration. Coloration on the ventral surface was generally metallic silver while the belly was bright silver (TODD, 1981).

Several other studies also focused on the morphological and physiological changes within the silvering process of anguillid eels. These included the changes of skin color and enlargement of eyes in *A. australis, A. dieffenbachii, A. anguilla, A. japonica, A. marmorata* and *A. celebesensis* (TODD 1981; DURIF *et al.*, 2005; OKAMURA *et al.*, 2007; HAGIHARA *et al.*, 2012), lengthening of pectoral fins in *A. anguilla* (DURIF *et al.*, 2005), degeneration of the gut in *A. anguilla* and *A. japonica* (DURIF *et al.*, 2005; OKAMURA *et al.*, 2007), increase of retinal sensitivity in *A. anguilla* (BOWMAKER *et al.*, 2008), changes of fat contents in *A. rostrata* and *A. anguilla* (LARSSON *et al.*, 1990; SVEDANG and WICKSTROM 1997), increase of fatty acid contents in the skeletal muscles in *A. anguilla* (EGGINTON 1986), and modification of swim bladder in rete mirable, gas gland and submucosa in *A. japonica* (YAMADA *et al.*, 2001).

Although there have been several studies on the silvering processes of anguillid eels, majority of these have been on temperate species, while information on tropical eels are still lagging. Considering that 13 species/sub-species of the 19 anguillids currently known are determined to be tropical species with nine species found around Indonesian waters (SUGEHA *et al.*, 2008), it is important to efficiently identify the various silvering stages of anguillid eels using relatively clear and simple characteristics to facilitate easy monitoring studies. Morphological changes of silvering eels in Indonesia was first studied by HAGIHARA *et al.* (2012), which was conducted in Central Sulawesi, Lake Poso and described the silvering stages of *A. marmorata* and *A. celebesensis*, but failed to provide information on *A. bicolor*. Therefore, the present study was aimed at assessing the morphological changes within the silvering process of female and male *A. bicolor*

bicolor from Segara Anakan, Indonesia.

2. Materials and methods

Fish catchment sampling location and species identification

A total of 107 specimens of *A. bicolor bicolor* were used in the present study. Specimens were identified through morphological characters as described by Ege (1939). As all specimens were morphologically identical, 8 specimens with mean, minimum and maximum morphological characters were further subjected to genetic identification using a partial sequence of mitochondrial DNA 16S rRNA region (Minegishi *et al.*, 2005; Tawa *et al.*, 2012). Genetic identification was conducted to discriminate *A. bicolor bicolor* and *A. bicolor pacifica* as morphological identification between these species has been considerably difficult. DNA sequences were submitted to DDBJ (DNA Data Bank Japan) under the accession numbers LC433757–LC433764. Specimens were stored at the Kyushu University Museum (KYUM-PI 5382–5389).

Specimens were obtained from fishermen who collected eels in the Segara Anakan in Cilacap, Central Java, Indonesia (Fig.1), during December 2015–September 2016, May 2017 and June 2018.

These fishermen employed traditional Indonesian baited traps 'wuwu' and fishing gear with hooks 'ureg-ureg', and 'opyok' together with crabs, earthworms and frogs as bait. 'Wuwu' baited traps used by the fishermen were set in the evening during 5–6 pm, and were then retrieved the next morning during 3–5 am. 'Ureg-ureg' was used in the day during ebb tide, while 'opyok' was used during the night with flood tides. Specimens collected for this study were only available if fishermen had retrieved eels the previous day. Therefore shortcomings such as quantifying the frequency and effort at which fishermen collected eels is unknown.

Silvering stages and sex determination

The silvering stages were characterized based on the percentage cover of black coloration of pectoral fins and percentage cover of metallic hue at the base of pectoral fins measured along the anterioposterio axis of the pectoral fin. Black color coverage of pectoral fins = 100 (BPC PFL^{-1}) where BPC is the length of black color coverage of pectoral fins and PFL is the pectoral fin length. Metallic hue at the base of pectoral fins = 100 (MBP BPF^{-1}), where MBP is the length of metallic hue at base of pectoral fin and BPF is the length of base of pectoral fin along the anteriorposterio axis. The percentage cover of silver color and metallic hue on the belly of specimens = 100 (MBC VL^{-1}), where MBC is silver color and metallic hue on belly and VL is the length from the lateral midline to the ventral line and was measured along the dorsoventral axis (Fig. 2). These external morphological color changes appear to be similar for tropical eels as with temperate eels such as *A. japonica* (Okamura *et al.*, 2007; Hagihara *et al.*, 2012) and therefore used as a criterion for differentiating silver eel stages in the present study. Sex was determined through visual inspection of external morphology. Specimens with lobed gonads were considered as males, described by Bertin (1956), while specimens having gonads resembling frilled ribbons of tissue were considered as females as described by Todd (1981).

Morphological measurement

Specimens were transported to the laboratory and anaesthetized with 50 ppm Tricaine Mathanesulfonate (MS222) before being examined and measured for morphological characteristics (Fig. 3) using a digital vernier caliper to the nearest 0.01 mm. Body weight of specimens were meas-

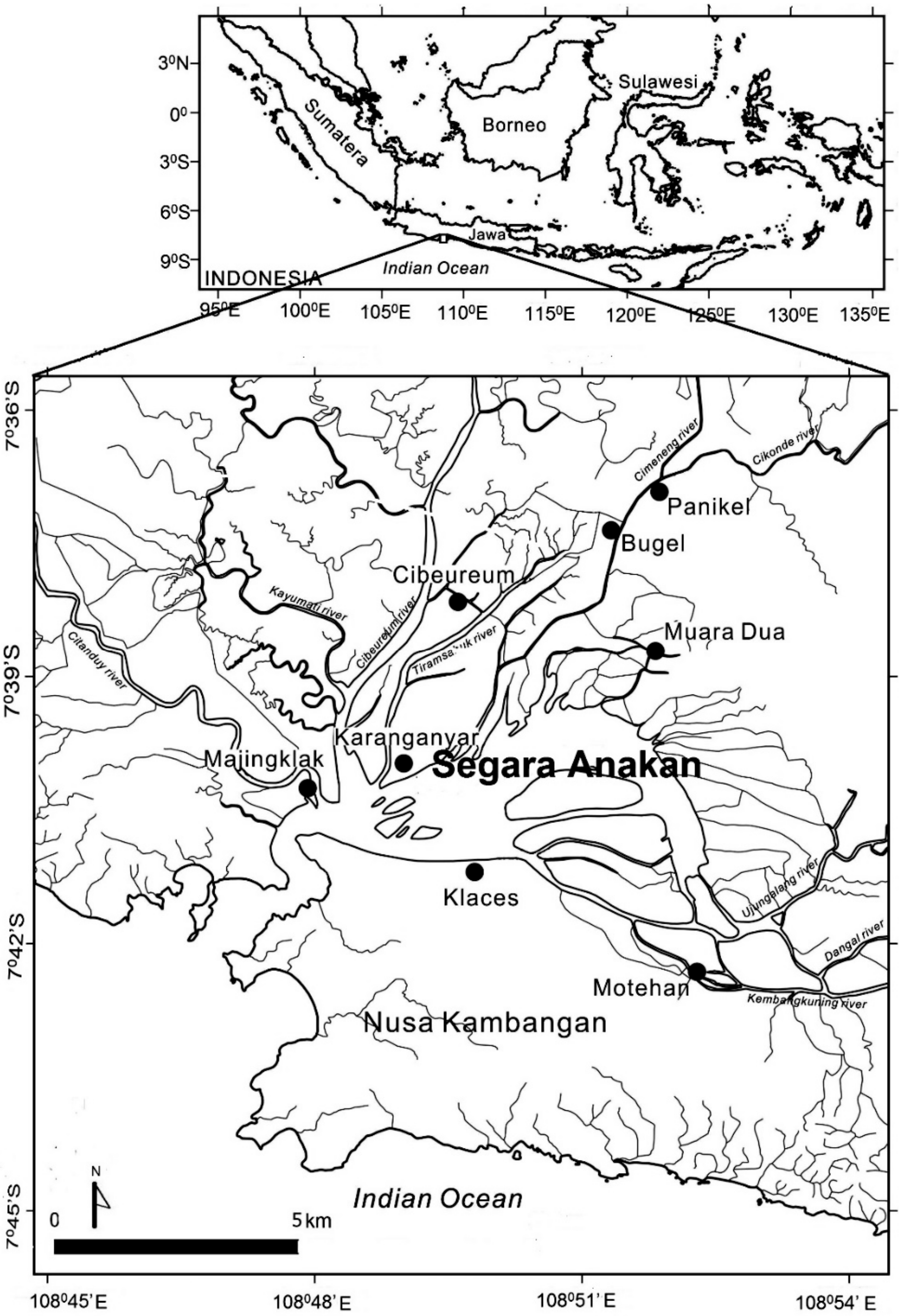

Fig. 1 Locations where *A. bicolor bicolor* were collected by fishermen. Map showing the location of Segara Anakan in Java Island, Indonesia near Indian Ocean.

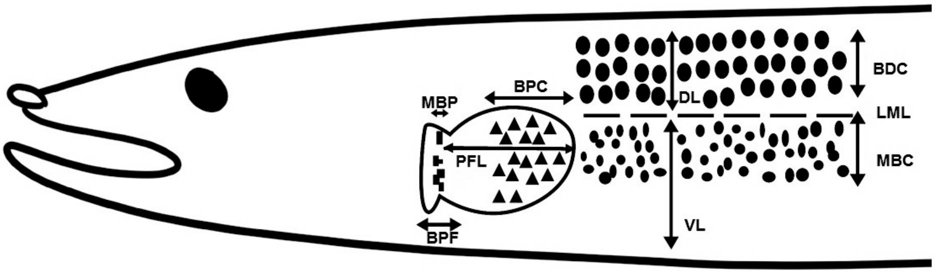

Fig. 2 Diagram showing measurements used for determining silvering stages of *A. bicolor bicolor* from Segara Anakan, Indonesia.
Base pectoral fin (BPF), black color coverage of pectoral fin (BPC:▲), black dorsal coloration (BDC:●), dorsal line (DL), lateral midline (LML), metallic belly coloration (MBC: •) (metallic hue at the base of pectoral fin (MBP:■), pectoral fin length (PFL), ventral line (VL)

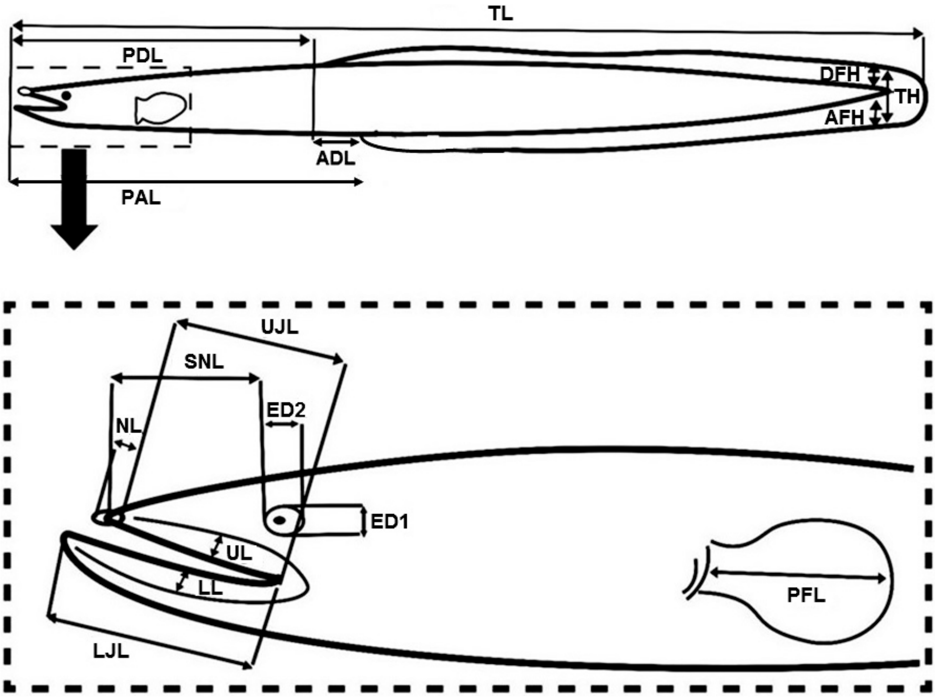

Fig. 3 Diagram showing morphological measurements of *Anguilla bicolor bicolor* from Segara Anakan, Indonesia.
Ano-dorsal length (ADL), Anal fin height (AFH), dorsal fin height (DFH), eye diameter, horizontal (ED2), eye diameter vertical (ED1), lower jaw length (LJL), lower lip depth (LL), nostril length (NL), pectoral fin length (PFL), pre-anal fin length (PAL), pre-dorsal fin length (PDL), snout length (SNL), tail height (TH), upper jaw length (UJL), upper lip depth (UL) of *A. bicolor bicolor* from Segara Anakan in Cilacap, Central Java, Indonesia.

ured using analytical scales. Specimens with a body weight of < 510 g were measured to the nearest 0. 01 g, while specimens with a body weight of > 510 g was measured to the nearest 1 g.

Fifteen morphometric measurements were incorporated into the present study and these were; ano-dorsal length (ADL), anal fin height (AFH), dorsal fin height (DFH), eye diameter vertical (ED1), eye diameter horizontal (ED2), lower jaw length (LJL), lower lip depth (LL), nostril length (NL), pre-anal fin length (PAL), pre-dorsal fin length (PDL), pectoral fin length (PFL), snout length (SNL), tail height (TH), total length (TL), upper jaw length (UJL) and upper lip depth (UL).

ADL was measured the distance between the origins of the dorsal and anus. TH, DFH and AFH were measured at 98% of TL and these were used to calculate: tail height to total length ratio (TI), TI = 100 TH TL^{-1}, dorsal fin height to total length ratio (DFI), DFI = 100 DFH TL^{-1}, anal fin height to total length ratio (AFI), AFI = 100 AFH TL^{-1}. Measurements of PFL, SNL, NL, UL, LL, UJL, LJL were taken from the left side of specimens and were used to calculate pectoral fin length to total length ratio (PFI), PFI = 100 PFL TL^{-1}, snout length to total length ratio (SNI), SNI = I00 SNL TL^{-1}, nostril length to total length ratio (NI), NI = 100 NL TL^{-1}, upper lip depth to total length ratio (UDI), UDI = 100 UJL TL^{-1}, lower lip depth to total length ratio (LDI), LDI = 100 LJL $TL\text{-}^{1}$, upper jaw length to total length ratio (UJI), UJI = 100 UL TL^{-1}, lower jaw length to total length ratio (LJI), LJI = 100 UL TL^{-1}. Measurements of ED1 and ED2 were used to calculate eye index (EI), EI = 100 π TL^{-1} $[0.25(ED1 + ED2)]^2$ (Pankhurst, 1982). Fulton's condition factor (K) was calculated using the BW and TL and used to describe the condition of specimens, K = 10^6 BW TL^{-3}. Gonad somatic index (GSI) was calculated as a percentage of body weight (GW), GSI = 100 GW BW^{-1}.

Statistical analysis

Statistical analyses were performed on the data to compare variance of indices using Kruskal-Wallis test followed by Pairwise test using Benferroni correction to determine the level of significance between stages. Correlation between indices and developmental stages were analyzed using Kendall correlation. All statistical analyses were conducted using SPSS software version 24.0 (SPSS Inc., Chicago, IL, USA).

We further categorized morphological changes into three indices which were; development index, locomotion index and feeding behavior index. The development index included GSI and K, meant to indicate readiness to migrate to spawning locations. Locomotion index included TI, DFI, AFI, PFI and EI, meant to indicate changes to locomotory appendages as yellow eels which are benthic dwellers metamophose to silver eels which are pelagic or mesopelagic swimmers and require optimal swimming capabilities to reach distant spawning locations. Feeding behavior index included NI, UJI, LJI, UDI, LDI and SNI, indicates changes to feeding appendages which gradually occur as silver eels stop feeding prior to migratory behavior.

3. Results

Observation of body color

From the 107 specimens in total, there were 68 males and 39 females. The present study was able to distinguish five developmental stages based on external morphological characters for both males and females (Fig. 4) and these were designated as: Y1, Y2, S1, S2 and S3. Morphological differences can be practically used in the field to distinguish between stages (Fig 5).

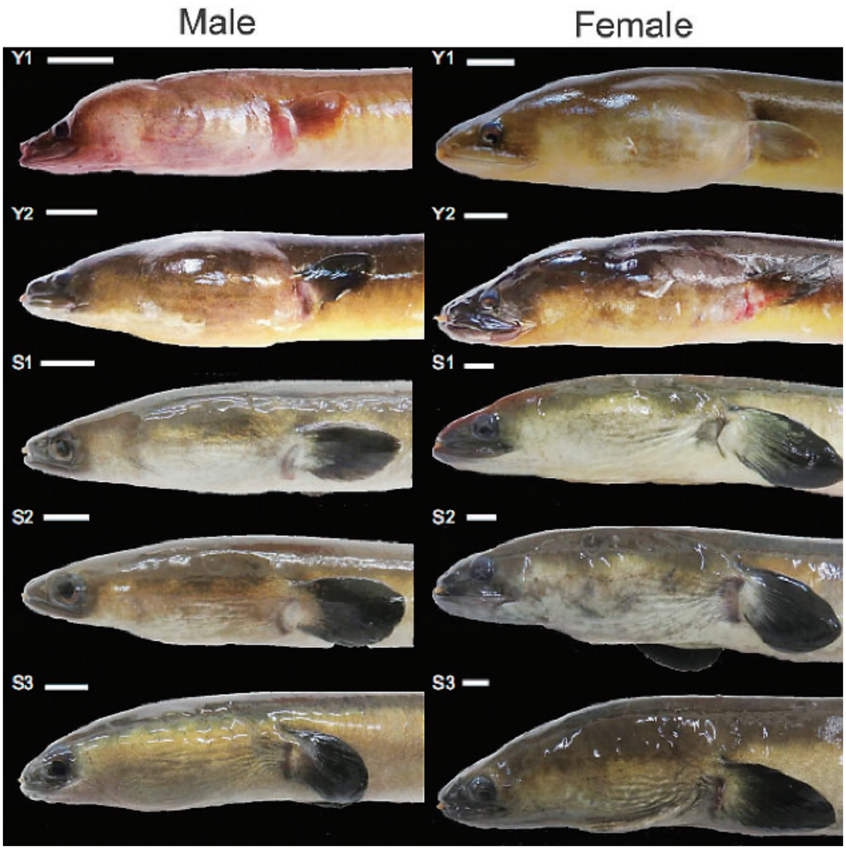

Fig. 4 Morphological characteristics of silvering stages based on body and pectoral fin coloration in males and females of *Anguilla bicolor bicolor* from Segara Anakan. For Y1, Y2, S1, S2 and S3, see text. Bar length 10 mm.

Y1 has a yellow colored belly, without metallic pigmentation. Pectoral fins without black coloration and the base of pectoral fins without metallic hue.

Y2 has similar coloration on belly and base of pectoral fins as Y1, but with black coloration on pectoral fins.

S1 has < 60% of belly covered with metallic pigmentation. Black color coverage on pectoral fins are ≤ 80% and with < 50% metallic hue at the base of pectoral fins.

S2 has ≤ 80% but ≥ 60% of belly covered with metallic pigmentation and black color coverage of pectoral fins ≥ 50% but ≤ 70%. Metallic hue at the base of pectoral fins is similar to that of S1, < 50%.

S3 has > 80% metallic pigmentation on the belly with > 80% black color coverage of pectoral fins and > 70% metallic hue color at the base of pectoral fins.

There is an increasing trend in the percentage of metallic pigmentation (silver belly coloration), black color coverage of pectoral fins and metallic hue coverage at the base of pectoral fins with increase in silvering stages (Fig 6).

Y1 had a total of 30 specimens, with TL of males (n = 19) ranging from 265–384 mm and females (n = 11) from 390–513 mm, (Fig 6). Y2

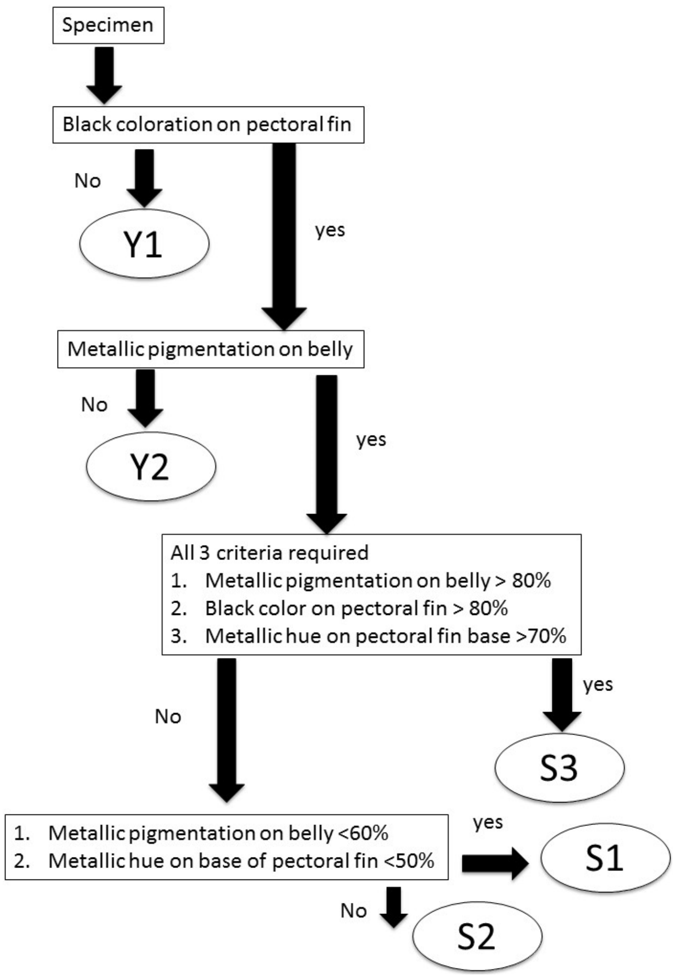

Fig. 5 Diagram showing morphological differences of color to distinguish between stages of females and males *Anguilla bicolor bicolor* from Segara Anakan, Indonesia

had 24 specimens with TL of males (n = 14) ranging from 327–429 mm, while females (n = 10) from 441–780 mm. S1 had 12 specimens with TL of males (n = 6) ranging from 350–442 mm and females (n = 6) from 674–799 mm. S2 had 20 specimens with TL of males (n = 14) ranging from 342–501 mm and females (n = 6) from 702–829 mm. Finally S3 had 21 specimens with TL of males (n = 15) ranging from 409–496 mm and females (n = 6) from 785–937 mm.

Morphological measurement

Based on Kruskal Wallis test, there were significant differences of the following indices between the various silvering stages in males and females ($P < 0.01$); K, GSI, TI, DFI, AFI, PFI, EI, and NI, in males only ($P < 0.01$); UJI and LJI and in females only ($P < 0.05$); LDI. While there were no significant differences of UDI and SNI. TL of males were notably smaller than females and ranged from 342 mm–501 mm with mean ±

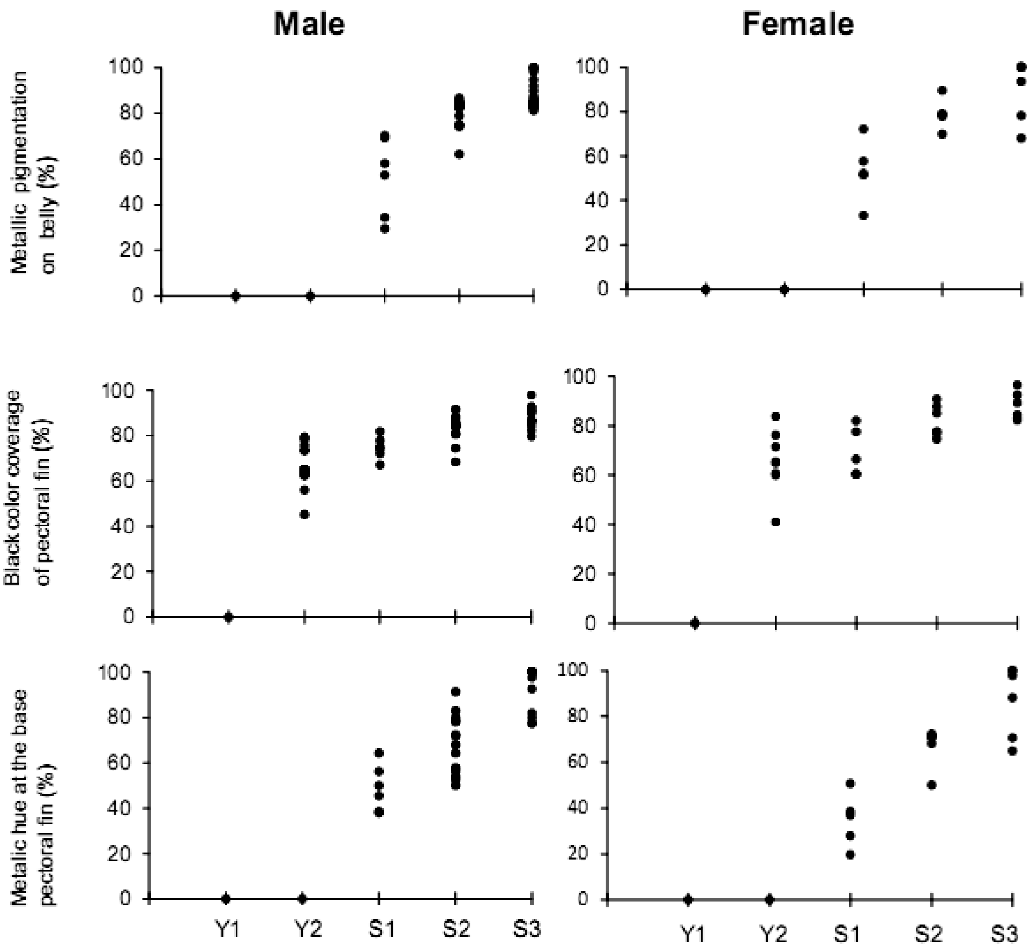

Fig. 6 Percentage of silver belly coloration, black color coverage of pectoral fin, and metallic hue at the base of pectoral fin in silvering stages of male and female *Anguilla bicolor bicolor* from Segara Anakan, Indonesia.

SD of 414.83 ± 40.38 mm, while that of females ranged from 674 mm–937 mm (786.11 ± 68.98 mm).

Development index

For both male and female *A. bicolor bicolor* there was a trend of a gradual increase in K and GSI with progression of silvering stages (Fig. 7). In male silver stages (S1–S3) displayed sgificantly higher K and GSI compared to Y1 stages. Y2 was observed to have significantly lower GSI compared to S2 and S3. For both male and female there was no significantly differences K an GSI between three silver stages (S1–S3) ($P < 0.05$). Based on Kendall correlation, K and GSI showed strong positive correlations between silvering stages in both males and females ($P < 0.01$).

Locomotion index

There was a general increase of locomotion indices (TI, DFI, AFI, PFI, and EI) with incre-

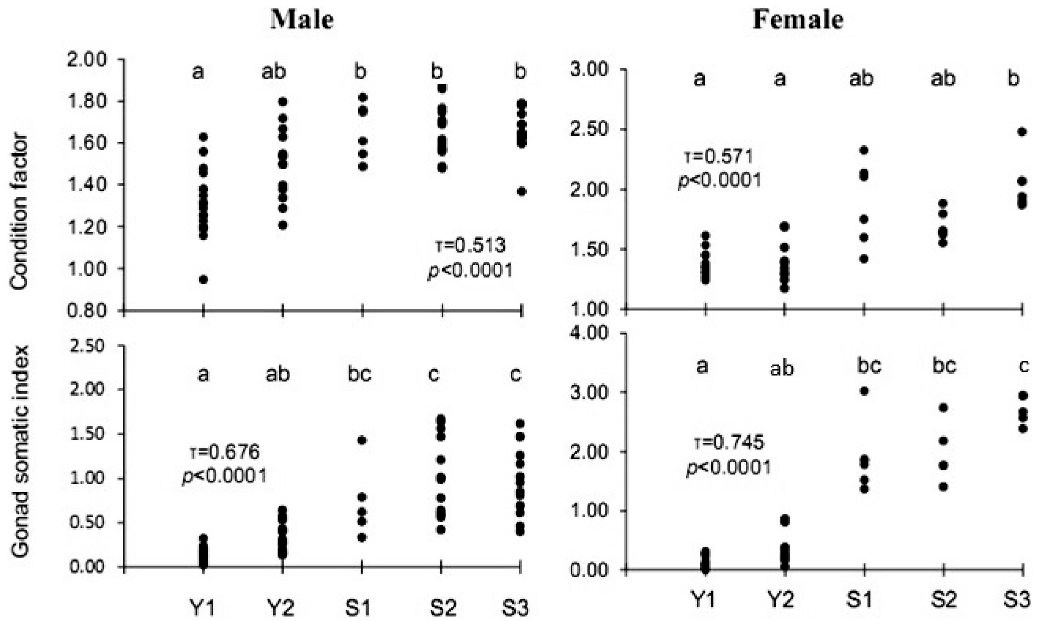

Fig. 7 The development indices in silvering stages of male and female *Anguilla bicolor bicolor* from Segara Anakan, Indonesia.

ment of stages in both males and females (Fig. 8). In males all five locomotion indices (TI, DFI, AFI, PFI and EI) within silver stages (S1–S3) were significantly higher than those of Y1. All indices of Y2 were significantly lower than those of S2 and S3 ($P < 0.05$).

In females all five locomotion indices of Y1 had significantly lower than those of S1 and S2. S3 was observed to have significantly higher AFI, PFI and EI compared to Y1. Y2 was significantly lower TI, DFI, AFI than those of S1 and S2. S3 was observed to have significantly higher TI, AFI, EI compared to Y2 ($P < 0.05$). Based on Kendall correlation, both males and females showed positive correlation between locomotion indices and developmental stages ($P < 0.01$).

Feeding behavior Index

In males the UJI and LJI of S3 were significantly higher than those of Y1. NI of Y1was significantly higher than that of Y2 and S2. Y2 displayed significantly higher NI compared to S2 ($P < 0.05$). Based on Kendall correlation, UJI and LJI showed positive correlation with developmental stages, while negative correlation between NI and developmental stages ($P < 0.01$) (Fig. 9).

In females S3 was significantly lower NI and LDI than those of Y1. NI of Y2 was significantly higher than that of S3. Based on Kendall correlation, NI and LDI showed negative correlation with developmental stages ($P < 0.01$) (Fig. 9).

Seasonal occurrence

Silvering females were caught during most months of the year (Table 1), while silvering males were only caught during May 2017 and May-June 2018. Silvering females were also observed in both the wet months of the year (December to March) and dry months (May to June) in Segara Anakan with corresponding peak season for females the same as males which was in

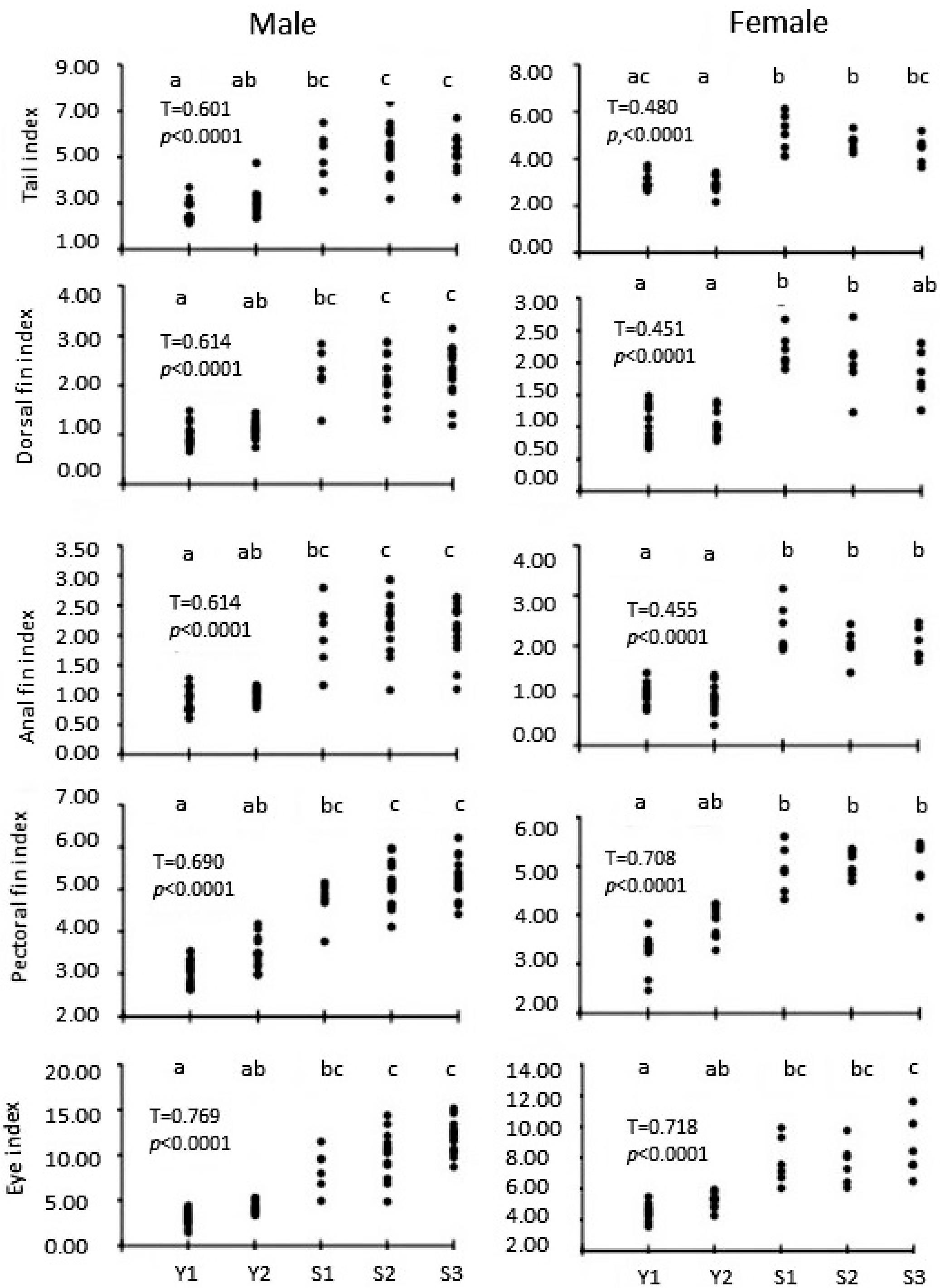

Fig. 8 The locomotion indices in silvering stages of male and female *Anguilla bicolor bicolor* from Segara Anakan, Indonesia.

Table 1. Catch date, total length and body weight of silver phase *Anguilla bicolor bicolor* used in present study

Date	Sex	Sample	Total length (mm)		Body weight (g)		Season
		size (n)	range	mean ± SD	range	mean ± SD	
Dec 31th, 2015	F	1	818		905		R
Jan 29th, 2016	F	1	937		1565		R
Feb 1st, 2016	F	1	908		1400		R
May 18th, 2016	F	1	810		1100		D
Aug 27th, 2016	F	1	785		1200		D
Sep 19th, 2016	F	1	745		870		D
May 4th, 2017	F	3	702–829	754.67 ± 66.21	533–935	801.33 ± 215.27	D
May 5th, 2017	M	29	342–501	430.10 ± 39.50	83–199	134.50 ± 35.17	D
May 10th, 2017	F	9	674–866	764.78 ± 54.94	537–1339	815.70 ± 226.79	D
May 23th, 2018	M	5	350–456	430.10 ± 134.50	78–160	134.50 ± 35.17	D
June 2nd, 2018	M	1	450		125		D

R: Rainy season, D: Dry season

May 2017 and 2018, during the dry months.

4. Discussion

Body and pectoral fin coloration in silvering stages

The present study is the first to identify the difference in size of silvering female and male *A. bicolor bicolor* and categorize the silvering stages of both sexes collected from Segara Anakan in Indonesia based on body and pectoral fin coloration into 5 stages: Y1, Y2, S1, S2 and S3. When comparing the 5 silvering stages of *A. bicolor bicolor* of the present study to similar studies, Okamura *et al.* (2007) and Hagihara *et al.* (2012) demarcated silvering stages of *A. japonica* in Mikawa Bay, Japan and *A. celebesensis*, in Poso Lake, Sulawesi Indonesia respectively into 4 stages: Y1, Y2, S1 and S2. Furthermore Hagihara *et al.* (2012) also described the silvering stages of *A. marmorata* from the same area which were grouped into 3 stages: Y1, Y2, and S1. The silvering stages of female *A. bicolor bicolor* from Segara Anakan, Indonesia reported by Arai *et al.* (2016) and female *A. bicolor bicolor* and *A. bengalensis bengalensis* from Penang, Malaysia (arai and Abdul Kadir, 2017) were grouped into 5 stages but these were based on the development of GSI and gonad histology. Additionally, male *A. bicolor bicolor* from Penang, Malaysia was grouped in to 3 stages and male *A. bengalensis bengalensis* into 2 stages by Arai and Abdul Kadir (2017). Discrimination of silvering eels employing gonadal histology is invasive, therefore a more practical method utilizing external morphological characters such as body and pectoral fin coloration presented in the present study will enable easy identification for monitoring studies within the South-east Asian region.

The synchronous development of silver coloration on the body and pectoral fins together with the maturation of gonads which were observed for tropical eel *A. bicolor bicolor* of the present study have also been observed in several species of temperate eels: *A. japonica* (Han *et al.*, 2003; Okamura *et al.*, 2007), *A. anguilla* (Durif *et al.*,

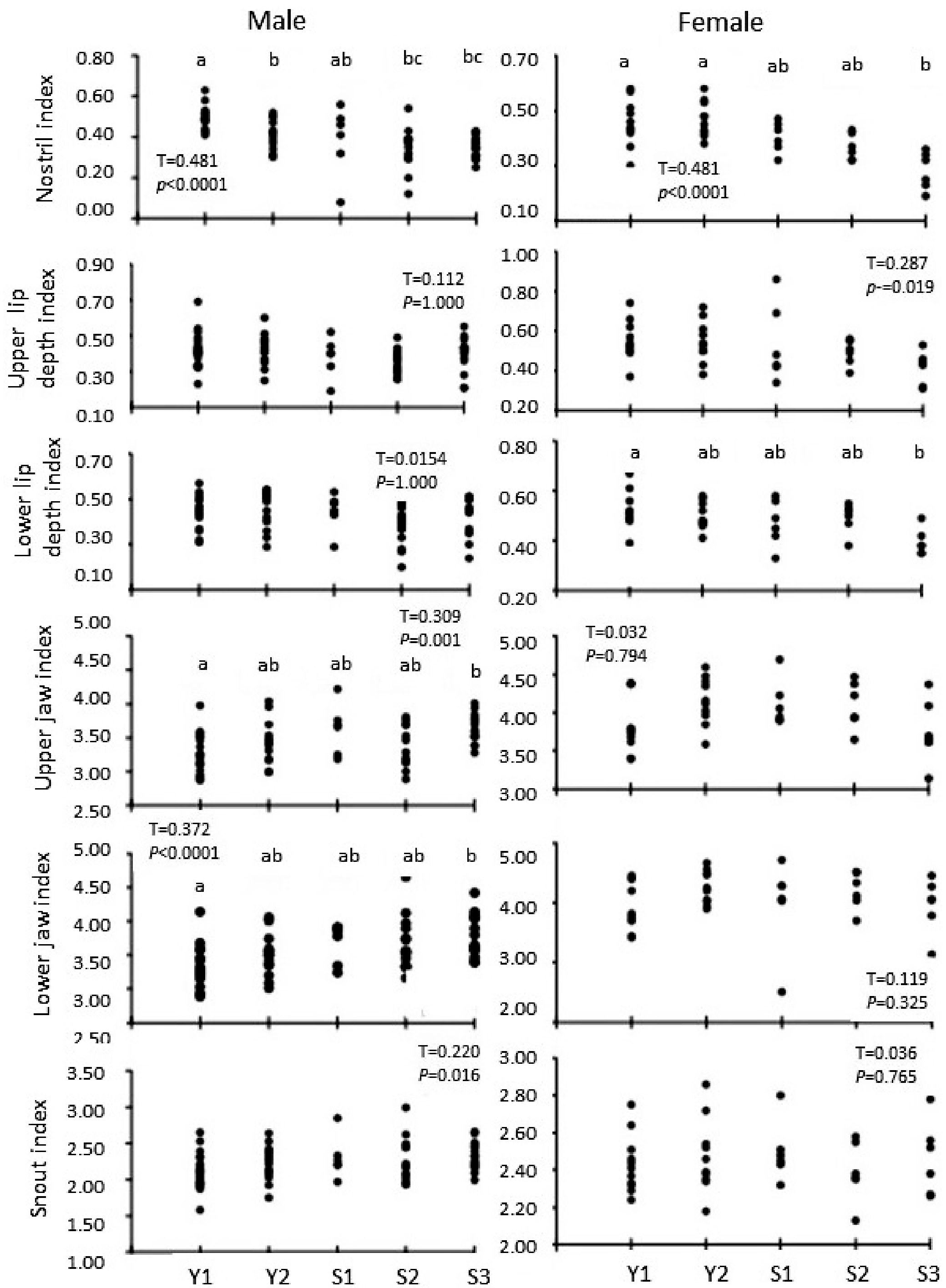

Fig. 9 The feeding behavior indices in silvering stages of male and female *Auguilla bicolor bicolor* from Segara Anakan, Indonesia.

2005), *A. dieffenbachii*, *A. australis* (TODD, 1981) and other tropical eels: *A. bicolor bicolor*, (ROBINET and FEUNTEUN, 2002), *A. celebesensis* and *A. marmorata* (HAGIHARA *et al*., 2012). Both temperate and tropical anguillid eels displayed similar morphological development during the silvering process with dorsal regions developing black coloration while ventral regions a metallic silver coloration. The pectoral fins also developed darker coloration until the distal portion of the base of the pectoral fin which displayed golden or silver coloration. (TODD, 1981; ROBINET and FEUNTEUN, 2002; DURIF *et al*., 2005; OKAMURA *et al*., 2007; HAGIHARA *et al*., 2012). HAN *et al*. (2003) suggested that these changes in skin coloration were highly correlated with gonadal development in Japanese eels and therefore the external morphological changes observed in the present study for *A. bicolor bicolor* supports that suggestion.

Morphological and physiological changes

Firstly the development indices of the present study, K and GSI were observed to increase with the increase of silvering stages (Fig. 7). DURIF *et al*. (2005) reported similar results for *A. anguilla* and suggested that with increasing sexual maturity, anguillid eels tend to increase in size and K. In most species of anguillid eels it has been reported that female eels tend to maximize their size, i. e. K, in order to maximize fecundity (WENNER and MUSICK, 1974; VOLLESTAD and JONSSON, 1986; HELFMAN *et al*., 1987). For large *A. anguilla*, DURIF *et al*. (2006) observed that gonad weight (GSI) increased exponentially with size at maturation and that larger silver eels developed proportionally more gonads as compared to smaller silver eels, suggesting that an increase in K would facilitate greater reproductive success. DURIF *et al*. (2006) further reported that K or fat content of female eels directly impacts the capacity for egg production and also provides energy reserves for spawning migrations. Although there was no significant increase of GSI expressed in the present study, there is a noticeable increasing trend of GSI with sexual maturity. This is similar to those of several studies (TODD, 1981; HAN *et al*., 2003; DURIF *et al*., 2006). In *A. anguilla*, DURIF *et al*., (2006) reported that maturation was characterized by the weight of gonads which in turn reflected fecundity. TODD (1981) further reported that higher GSI values in *A. australis* and *A. dieffenbachii* displayed greater oocyte diameter while ARAI and KADIR (2017) stated that tropical eels, *A. bengalensis* and *A. bicolor bicolor* both displayed greater development of the gonad structure with increase in GSI. Therefore it is clear that increasing development indices with increasing sexual maturity observed for *A. bicolor bicolor* in the present study correlates to an adaptive feature which would enable greater reproductive success and migration success and is typical for most anguillid eels.

Secondly, locomotion indices of the present study (Fig. 8) displayed a general increasing trend of all indices from early yellow stages to late silver stages. The increase in PFI is an important adaptation for anguillid eels during their spawning migrations as pectoral fins act as stabilizers and aids in predator avoidance (HAGIHARA *et al*., 2012). The present study is also the first to provide information on DFI, AFI and TI for anguillid eels which displayed increasing trends with increase in silvering stages. Increase in all aspects of the caudal fin region i.e. DFI, AFI and TI will improve swimming ability during spawning migration (MULLER *et al*., 2001). Furthermore the EI of *A. bicolor bicolor* in our study also displayed an increasing trend of 3.82-folds in males and 1.78-folds in females with increasing sexual maturity from yellow to silver stages (Fig. 7). The increase in size of the eyes (EI) in the present study may also function in aiding of predator detection during oceanic migrations to spawning locations. This has also been observed for several other species of anguillid eels such as *A. australis, A. dieffenbachii* (TODD, 1981) and *A. anguilla* (NOWOSAD *et al.*, 2014). The present study was also the first to provide silvering indices for males of *A. bicolor bicolor* from Indonesia. The EI of late silver males in this study (11.92 ± 1.75) was also found to be considerable higher than other anguillid eels, *A. bengalensis bengalensis* and *A. bicolor bicolor* from Malaysia (6.09 ± 1.08 and 7.65 ± 2.49, respectively) (ARAI and ABDUL KADIR, 2017), *A. japonica* (5.7 ± 0.6) (YOKOUCHI *et al.*, 2009) and *A. anguilla* (9.9 ± 1.6) (DURIF *et al.*, 2005).

The feeding behavior index, i.e. UDI, and SNI of *A. bicolor bicolor* of both sexes in this study did not display degression with progression of silvering stages from Y1 to S3. UJI and LJI in males showed the increasing trend with progression of silvering stages, while LDI in females showed the decreasing trend with progression of silvering stages. NI in both males and females showed the decreasing trend with progression of silvering stages (Fig. 9). Furthermore all samples for this study including late stage silver eels were lured and collected using baited traps and hooks. These results suggest that silver eels of *A. bicolor bicolor* from Segara Anakan were feeding individuals even at late stage (S3) silver eels. This is in contrast to temperate eels such as *A. anguilla, A. rostrata* and *A. japonica.* DURIF *et al.* (2005) reported that *A. anguilla* ceased feeding at the onset of silver eel maturation, which resulted in degeneration of the gut. PANKHURST and SORENSEN (1984), LIONETTO *et al.* (1996) and OKAMURA *et al.* (2007) also observed similar degeneration of the elementary tract in silver eels of *A. anguilla, A. rostrata* and *A. japonica* respectively. This degeneration may also be a result of cessation in feeding. We suggest that *A. bicolor bicolor* may be feeding individuals right up until they begin oceanic migrations. Furthermore it is possible that *A. bicolor bicolor* cease feeding as soon as oceanic migration commences as evident through the decrease in NI. Nostrils of anguillid eels are connected to the olfactory organ which plays an important role in the location of feed (ATTA, 2013). Therefore a decrease in NI with sexual maturity suggests that although late stage silver eels feed within continental waters they may cease feeding at the onset of oceanic migration. With very few studies on silvering stages of tropical eels and even fewer on comparison of feeding behavior indices of tropical silver eels it is important that similar studies are conducted on other tropical anguillid species to verify if this hypothesis is common for other tropical species.

Although silver eel collections during the present study were conducted intermittently by local fishermen, a seasonal trend could be observed for both males and females. Female silver eels were observed relatively throughout the year in low numbers with peak collection periods during the dry season in May 2017 and 2018. This is typical for tropical anguillid eels which have been suggested to spawn throughout the year (KUROKI *et al.*, 2009). Males however were only collected during the dry months of the year, in May 2017 and May-June 2018, of 35 samples which also corresponds to peak collection seasons for females (Table 1). As there may have been shortcomings with the frequency and effort of eel collection by fishermen, it is entirely possible that males were also present during the year but were simply not collected. Additionally it may be fairly assumed that peak silver eel migration periods of *A. bicolor bicolor* in the Segara Anakan are during the dry seasons around May. Temperate anguilld eels *A. japonica* (MATSUI, 1957), *A. rostrata* (JESSOP, 1987), *A. anguilla* (VOLLESTAD *et al*., 1986), *A. australis* and *A. diefffenbachii* (TODD, 1981; SLOANE, 1984; JELLYMAN, 1987) all make their spawning migrations either during fall or winter seasons. VOLLESTAD *et al*. (1986) further reported that high water discharge and low temperature were the main factors influencing the onset of *A. anguilla* silver eel runs. Therefore peak silver eel runs for *A. bicolor bicolor* from Segara Anakan seem to be triggered by low temperatures rather than rainfall, as dry seasons are also relatively cold seasons in the Java region and this is when majority of silver eels were captured in the present study. Average temperatures and rainfall per month during May-August 2017 was 30 ℃ and 122 mm respectively, compared to 32 ℃ and 375 mm respectively for September 2017–April 2018 (Indonesian Central Bureau of Statistics, 2019a, b). Contraction of habitat and reduction of resource availability brought on by dry seasons may trigger eels in Segara Anakan to commence spawning migrations.

The present study was able to provide information on the morphological differences of silvering stages in *A. bicolor bicolor* of both sexes from the Segara Anakan Estuary. Two yellow eel and three silver eel stages were differentiated using clear and easily distinguishable external morphological characters. An identification key using external morphological features presented in this study will enable fisheries agencies to quickly and conveniently distinguish the various silver eel stages. This will abate in the monitoring of silver eel migration patterns in the region and assist in conservation programs aimed at effectively utilizing this species. The present study was only able to collect samples from fishermen when they were available and a more systematic approach is required to obtain a more robust understanding of the abundance and seasonality of *A. bicolor bicolor* silver eel migrations in the Segara Anakan Estuary. Currently this species is listed as 'near threatened' by the IUCN Red List of Threatened Species (JACOBY *et al*., 2014) and further life history studies are required for the promulgation of effective conservation programs in the future.

Acknowledgements

The authors would like thank all the SIDAT team namely Adhi Prasetyo, Ariel Hanaya, Christin Ratucoreh, Juniari Saraswati, Oa Etty Kerans and Rika Trianasari in Animal Structure and Development Laboratory, Biology Faculty of Universitas Gadjah Mada who helped us collect the samples. Bpptnbh Biology Faculty, Universitas Gadjah Mada which supported this research through funding.

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Received: May 24, 2019
Accepted: October 10, 2019