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Growth, sex reversal pattern, and reproductive characteristics of Barramundi (*Lates calcarifer*) broodstock candidates reared in floating cages

Che Ismail Che-Zulkifli[a], Mohd Abdul Muin Md Akil[b], Adnan Amin-Safwan[c], Hairul Hafiz Mahsol[d], Muath Q. Al-Ghadi[e], Ayman A. Swelum[f], Mohamed E. Abd El-Hack[g], Vincenzo Tufarelli[h], Marco Ragni[i], and El-Sayed Hemdan Eissa[j]

[a]Crustacean Aquaculture Research Division, Fisheries Research Institute Pulau Sayak, Kota Kuala Muda, Malaysia; [b]Centre of Global Sustainability Studies, Universiti Sains Malaysia, Gelugor, Malaysia; [c]Department of Applied Sciences and Agriculture, Tunku Abdul Rahman University of Management and Technology, Johor Branch, Segamat, Malaysia; [d]Conservation Biology Program, Faculty of Tropical Forestry, Universiti Malaysia Sabah, Jalan UMS, Kota Kinabalu, Malaysia; [e]Department of Zoology, College of Sciences, King Saud University, Riyadh, Saudi Arabia; [f]Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; [g]Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt; [h]Department of Precision and Regenerative Medicine and Jonian Area, University of Bari 'Aldo Moro', Bari, Italy; [i]Department of Soil, Plant and Food Sciences, University of Bari 'Aldo Moro', Bari, Italy; [j]Fish Research Centre, Arish University, Arish, Egypt

ABSTRACT

Sex reversal of male to female is a characteristic of barramundi (*Lates calcarifer*), which is affected by several factors, thereby changing the broodstock population. A study was conducted in floating cages in Langkawi, Malaysia, to determine the weight point at the onset of the sex reversal phenomena. A total of 75 female and 55 male adult individuals (3–4 weeks of age) were sampled from the fish cultured in cages to ascertain their sex at different weights. The water temperature and salinity values were 29.82 °C and 33.12 ppt, respectively. The specimens were classified into twelve bodyweight classes (2.00–8.00 ± 0.5 kg intervals). Female specimen body weight distribution was highest in the 6.01–6.50 kg class (22.6%), followed by the 5.51–6.00 kg and 4.51–5.00 class (13.3%), while male specimen body weight distribution was highest in the 4.51–5.00 kg class (32.1%), followed by the 4.01–4.50 kg class (30.3%). Length-to-weight relationships for females and males of Asian Seabass indicated positive allometric growth. The correlation between body weight and GSI, using Pearson's correlation, for both sexes, for the male and female barramundi, there was a weak correlation between body weight and GSI, which was 37 and 30%, respectively. Based on the present study's findings, it can be concluded that sex reversal from male to female in Barramundi largely occurred at 4.57 kg body weight and 66.8 cm total length.

KEYWORDS
Lates calcarifer; reproductive and growth performance; sex reversal; floating cages

Introduction

Barramundi (*Lates calcarifer*) is a fish species of significant commercial importance; it is fished internationally and raised in aquaculture worldwide, It is also a species that tolerates crowding and shows great physiological tolerance from freshwater to seawater.[1–3] Among the various species of finfish, Barramundi shows improved aquaculture yield, with high survival rates during culture from fry to market size, in both ponds and cages.[4] Barramundi is Malaysia's main marine finfish aquaculture species, and one of the most valuable species in Malaysia and throughout the South East Asia Region.[5] It is in high demand among the middle-class population of Malaysia because of its affordable price. For Malaysia's aquaculture industry, Barramundi ranks as the third-largest contributor to aquaculture production after tiger prawn (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*).[6]

Barramundi is a sequential protandrous hermaphrodite. It first matures as a male and subsequently changes sex to female. Based on visual observation, the female is normally larger than the male for Barramundi. Barramundi is normally cultured in floating cages and brackish water earthen ponds.[7] It is also an important aquaculture species throughout the Asian Pacific Region because of its fast growth and

CONTACT Vincenzo Tufarelli vincenzo.tufarelli@uniba.it Department of Precision and Regenerative Medicine and Jonian Area, University of Bari 'Aldo Moro', Valenzano, Bari, 70010, Italy; El-Sayed Hemdan Eissa sayed_hemd@hotmail.com Fish Research Centre, Arish University, Arish, Egypt

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successful cultivation in net cages and Recirculating Aquaculture Systems (RAS)[8] marine environments.[9] Hatchery production of seed of Barramundi has been well developed, and juveniles are easy to wean to pellets. Seabass grows rapidly, reaching a harvestable size of 600 g in six months.[5]

Studies on length-weight relationships are important to assess the reproductive status in fish, after that establishing a suitable system for reproductive manner.[10,11] In animals, the relationship may indicate taxonomic differences or phases in their life history, such as metamorphosis and maturity stages.[12] These studies are also important in fisheries science for its value in the estimation of the biomass of fish through underwater length observation.[12] Several studies have been completed on the length-weight relationship of Asian Seabass, such as Refs.[4,12–22] The study has a fundamental role in fishery biology and aquaculture of any species.[4]

Sex reversal is common in tropical fishes, including *Labridae*, *Scaridae*, and *Serranidae*.[23] Sex determination in fish is primarily under genetic control but may also be influenced by environmental conditions.[24] Some fish, such as the cuckoo wrasse, *Labrus bimaculatus*, are sequential, protogynous hermaphrodites, spawning initially as females but then reversing to functional males.[25] However, fish, such as the black porgy, *Acanthopagrus schlegeli*, is sequential, protandrous hermaphrodites, spawning initially as males but then reversing to functional sexual females.[26] The selection of a healthy, mature, and productive male broodstock is identified with a successful spawning process. The optimum ratio of male to female at 1:2 may become a constraint due to the high incidence of sex reversal in male seabass as they reach maturity after increasing their body weight to a certain size.

A study on the growth and sex reversal of barramundi was carried out by past researchers.[19,27–35] A close study as a present study was carried out by Guiguen et al.,[36] which emphasizes sex reversal from male to female. The latest study on this topic was carried out by Roberts et al.,[20] indicating that faster juvenile growth promotes earlier sex change in barramundi. Concerning the significance of barramundi aquaculture in Malaysia, more data is needed to establish baseline material on gonadal development, sexual maturation, and sex change of fish raised under local farming situations to notify local hatcheries. Based on our screening in literature, there are no data on sex reversal incidence amongst broodstock candidates of Barramundi reared in floating cages despite the district being accountable for the majority of global production of the species. Thus, the objective of the present study was to (a) assess the association between length and body weight in relation to the growth patterns and (b) determine sex reversal incidence amongst broodstock candidates of Barramundi reared in floating cages.

Materials and methods

Fish origen

The experiment was carried out at the offshore cage culture site in the sheltered area of Pulau Simpang Tiga, Langkawi Island, Kedah, Malaysia (Fig. 1). A local farmer from Terengganu, on the east coast of Peninsular Malaysia, supplied the entire fish sample population. The water temperature was 29.82 °C and the salinity value was 33.12 ppt. Three thousand pieces of fingerlings (an average of 10 cm of body length) were used as initial candidates. Before stocking, the fish were treated by dipping them in fresh water for 30 s to remove the parasites from the body. A unit $6 \times 6 \times 6$ square meters High-Density Polyethylene (HDPE) suspension floating cage was used in this trial. The stocking density was ten pieces in one cubic meter of water column. The initial net mesh was 2 cm, which was changed to a larger 5 cm mesh as the fish grew to 15 cm. The environment inside the floating cages is similar to the outside because the water can pass through the net. The fish were fed with trash fish (small marine fish and sardines) for fingerlings and mature fish to 5% of body weight twice per day. Barramundi was cultured from fingerling (3–4 weeks of age) to adult size for about four years. The net was changed with a clean net every three weeks to avoid fouling or clogging. Freshwater bath treatment for 10 min was carried out every two weeks to remove fish parasites.

Samplings

Monthly sampling was conducted by randomly taking 12 fish pieces at the time of the full moon (June and July). There were 78 females and 58 males barramundi sampled during the study. Clove oil was used as an anesthetic at 40 ppm to reduce the sampled fish activity levels, and sampled fish were fasted for 48 h before sampling. The parameters measured were sex, weight (kg ± 0.1) using an electronic balance (Scotts, Switzerland), and total length (cm) by measuring tape. The fish were sacrificed. Gonadosomatic Index (GSI) was determined with the following formula: GSI = gonad weight/bodyweight × 100. The length-

Figure 1. Google Maps presented the location of the study at Pulau Simpang Tiga, Langkawi Island, Kedah, Malaysia.

weight relationship was determined using the data of body weight and total length. According to the theory of cube law, if the 'm' value in a length-weight relationship is equivalent to 3, the growth of the fish is considered isometric.[37] The growth is considered negative allometric if the value is <3. If the gradient's value is more than 3, the growth is considered positive allometric.[38]

Histological study

A slice of sample gonad was placed into a bottle containing 10% buffered formalin. The gonad sample was shipped to the laboratory to undergo a histology procedure. Histology slides were observed under a compound microscope. The microscope image was captured, and gonad maturity was determined based on the histology image or the size of the oocytes. The Gonad stage for males and females was classified into four stages. The Gonad stage of males and females was provided as Supplementary Data based on the classification of Guiguen et al.[36] and Terence et al.[22]

Statistical analysis

Data were analyzed using SPSS 21 (SPSS, Chicago, IL, USA) to determine the correlation between body weight and GSI and body weight with gonad weight using Pearson's correlation. The probability levels applied were <0.05 and 0.01 ($p < 0.05$ and $p < 0.01$). Other collected data were analyzed using Microsoft Excel (Microsoft, Redmond, WA, USA) to obtain the mean and standard deviation.

Results

Growth model and weight-length relationship

The length-weight relationship of female Asian seabass cultured in marine cages was $y = 3.7808x + 49.338$ (linear), and the coefficient of determination (R^2) was 0.8479 (Fig. 2). Meanwhile, the length-weight relationship of male Barramundi was: $y = 3.5817xs + 51.115$, and the coefficient of determination (R^2) was 0.8052. Based on the linear equation $y = mx + c$, the value of the gradient (m) was 3.7808 in females, while the value of 'm' was 3.5817 in males.

The gradient of the length-weight relationship equation for both females and males was more than 3, which is 3.8006 and 3.5817, respectively. The result shows that the growth pattern for both female and male Barramundi, reared in floating cages in Langkawi, was positive allometric. The coefficient of determination (R^2) for female and male Barramundi was 84.7 and 80.5%, respectively.

Gonado-somatic index

The correlation between body weight and GSI, for both sexes, using Pearson's correlation, for the male and female Barramundi is presented in Table 1. There was a weak correlation between body weight and GSI 0.368, and 0.302 for males and females, respectively

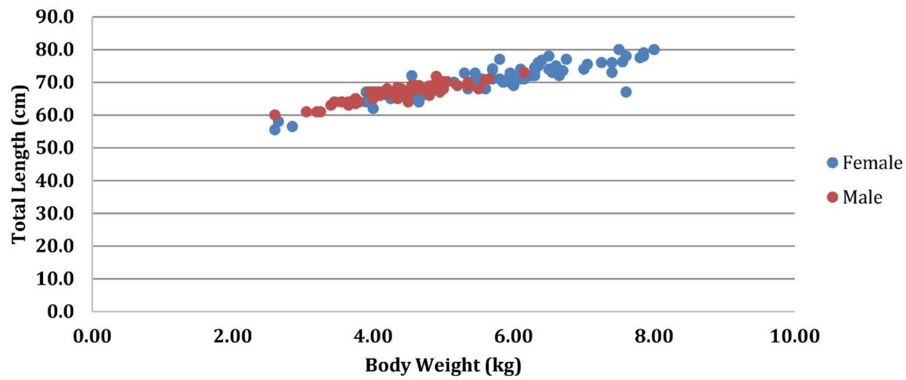

Figure 2. Weight length relationship of Asian Seabass reared in floating cages. The equation of female: $y = 3.7808x + 49.338$ (linear); $R^2 = 0.8479$. The equation of male: $y = 3.5817x + 51.115$; $R^2 = 0.8052$.

Table 1. Pearson's correlation test among body weight, gonadosomatic index (GSI), and gonad weight.

Sex		GSI	Gonad weight
Male	Body weight	0.368*	0.460**
Female	Body weight	0.302*	0.493**

*Correlation significant at 0.05 level (2-tailed).
**Correlation significant at 0.01 level (2-tailed).

(Table 1). Moreover, the correlation between body weight and gonad weight was 0.46 and 0. 49 for males and females, respectively.

The distribution of body weights of female and male Asian Sea bass reared in floating are presented in Table 2, The largest number of female fish (F4) had body weight of 6.15, while the largest number of male fish (M4) had 4.54 kg as reported in Table 2 and Fig. 3. The distribution of length of female and male barramundi is reported in Fig. 4. As shown in Table 2, 21.4% of the Barramundi male fish (M1) had 66.69 cm of total length, while 38.08% of male fish (M2 and M3 stages) had a similar total length of 64.16 cm. Moreover, 40.47% of male fish (M4 stage) had 67.91 cm.

Most of the observed male or female fish had a mature gonad. From the results, 51% of the total females were in the gonad stage of F4, 12.7% in stage 3, 19.1% in stage 2, and 38.3% were in stage 1 (Fig. 4). For male fish, 38.4% in stage 4, and 20.5% in combined stage 3, stage 2, and stage 1 (Fig. 5).

Correlations and growth distribution

The result of logistic regression between sex transition and body weight shows that fifty percent transition from male to female occurred at 4.57 kg (Fig. 6), whereas the result of logistic regression between sex transition and total length shows that 50% transition occurred at 66.8 cm (Fig. 7).

Histological slide observation on sex transition of Barramundi

Based on observation of gonad histology, male gamete (spermatozoa) and female gamete (oocytes) existed during the transition period. Fig. 8 shows the existence of two gamet in the gonad of Barramundi that sampled. Both gametes are in high stage, spermatozoa for male gamete and the early stage of vitelogenic oocytes.

Discussion

Length-weight relationship data of Barramundi in captivity is useful to researchers in further biological and nutritional studies.[11] In captivity, information on the length-weight relationship is useful for predicting the size of the fish based on its length. Still, it also gives information on the growth pattern of the fish.[38] The information obtained from the study can be a basis for the size prediction and study of the growth pattern of Asian sea bass reared in marine cages. The formula of the length-weight relationship obtained from the study $y = 3.7808x + 49.338$, $R^2 = 0.8479$ (female), and $y = 3.5817x + 51.115$, $R^2 = 0.8052$ (male), can be useful references in future studies of Asian Sea bass aquaculture, particularly in marine cages.

As observed in this research, there is a relationship between the size of the fish and the volume of eggs and milt because the value of GSI was related to the size of the gonads (Table 1).

According to Hossain et al.,[39] there is a correlation between the size of the ovary and the fecundity of the fish, which in turn means that larger gonads contain more eggs and milt than smaller ones. This information is important in choosing the size of broodstock to increase the produced volume of eggs and milt. Moreover, providing data like that may be also useful to hatchery operators to calculate how many and what

Table 2. Sex type, body weight, total length, and not all male (GSI) of sampled Barramundi reared in floating cages (means ± standard deviation).

Sex type1	*n*	Body weight, kg	Total length, cm	GSI
M1	9	4.57 ± 1.38 (3.05–7.60)*	66.69 ± 5.21 (61.0–78.0)	0.37 ± 0.27 (0.05–0.80)
M2	8	3.75 ± 0.63 (2.60–4.70)	64.16 ± 2.46 (63.0–67.8)	0.47 ± 0.33 (0.18–0.81)
M3	8	4.19 ± 0.64 (2.60–4.70)	64.16 ± 2.75 (61.0–70.2)	1.42 ± 0.54 (0.28–1.90)
M4	17	4.45 ± 0.47 (3.50–5.50)	67.91 ± 1.97 (64.0–71.0)	2.74 ± 1.05 (0.37–4.58)
F1	17	5.16 ± 1.20 (3.75–7.05)	68.90 ± 4.39 (62.0–77.0)	0.40 ± 0.32 (0.04–1.48)
F2	9	4.59 ± 1.06 (2.6–5.95)	66.60 ± 5.11 (64.0–72.0)	0.91 ± 0.28 (0.55–1.50)
F3	6	5.43 ± 1.70 (2.65–7.55)	68.70 ± 6.84 (68.0–76.3)	3.01 ± 1.46 (1.44–5.59)
F4	25	6.15 ± 0.99 (2.85–7.80)	72.90 ± 4.31 (56.5–80.0)	5.71 ± 2.58 (0.24–11.48)

*In brackets the range values (min–max).

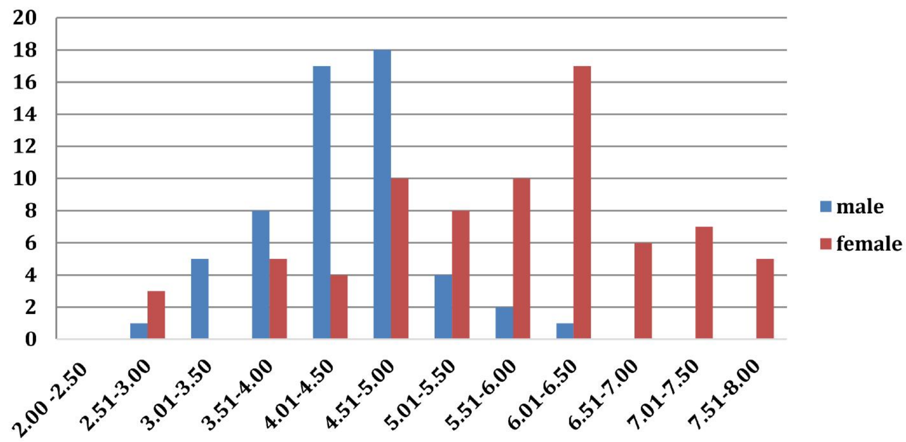

Figure 3. Distribution of body weight of Asian Seabass divided into 12 classes.

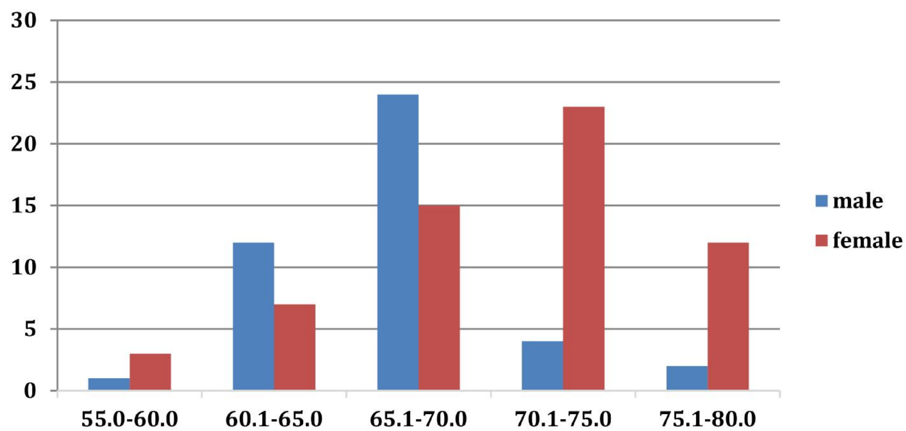

Figure 4. The number of male and female fish under five classes of length.

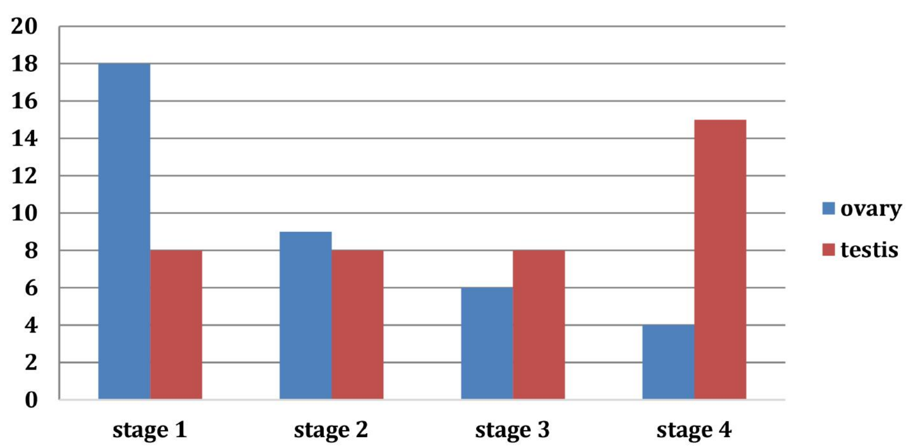

Figure 5. Frequency of gonad stage of females and males.

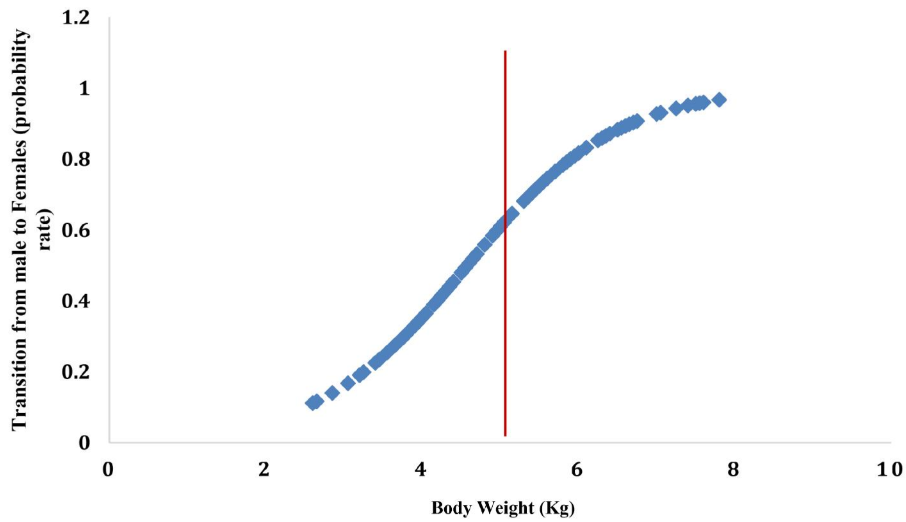

Figure 6. Logistic regression on body weight of *L. calcarifer* at 0.5 the probability, in which 50% of males were transformed into females. The cross line is at 4.5 kg.

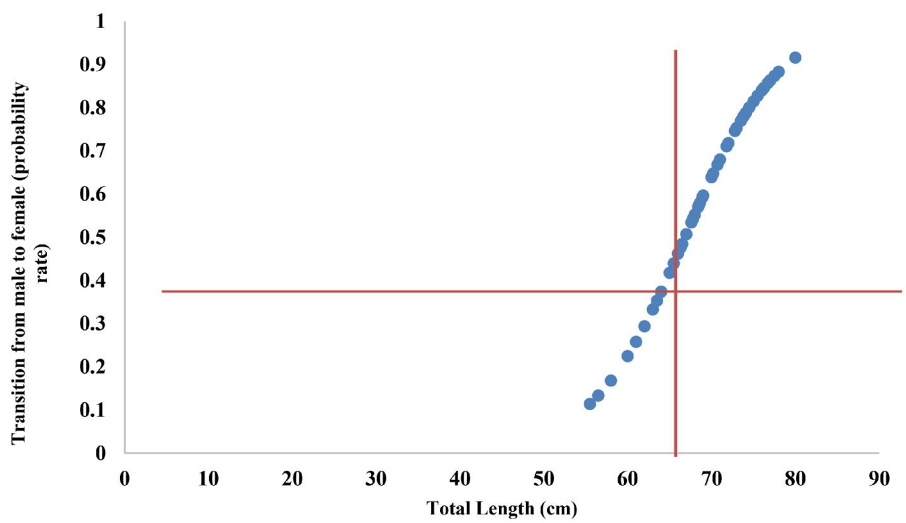

Figure 7. Logistic regression on total length (cm) of *L. calcarifer*. At the probability of 0.5, which means 50% of males were transformed into females. The cross line is at 67 cm.

size of broodstock are needed to reach target seed production. An excessive number of broodstock reared in tanks will result in higher production costs, such as feeding the broodstock. Enhancing our information regarding the sex changes in Barramundi would help hatchery managers predict the maturation size. It may be useful for selecting fish from sea cages as brooders, as well as help identify the driving factors of precocious sex change in the species.[22]

The fish started to change their sex, from male to female, when their body weight reached 4.57 kg and above. Compared to another study, the body weight of fish during the transition time for the present study is not far from the study by Guiguen et al.,[36] which is 5.015 kg. The fish gonads change from testis to ovary, and their function changed from milt production to egg production. However, based on the result, not all female barramundi changed to female after body weight had reached 4.57 kg, but the percentage of exceptions was very small study in Singaporean waters, Terence et al.,[22] found that Barramundi were changed by 70% at 3 kg, while when fish reached 5 kg, a total of 90% of individuals were females.

The maturation of fish can vary from the beginning stage to the mature stage. In the present study, the sampling was conducted during the time of the full moon, which is theoretically the breeding period of the fish. However, even though all of the fish in the cages were matured, the gonads were found to be in different stages, whether female or male. That means that in broodstock tanks that contained many fish, the monthly production of fertilized eggs did not come from the same broodstock. Only females and males with mature gonad stages were ready for spawning

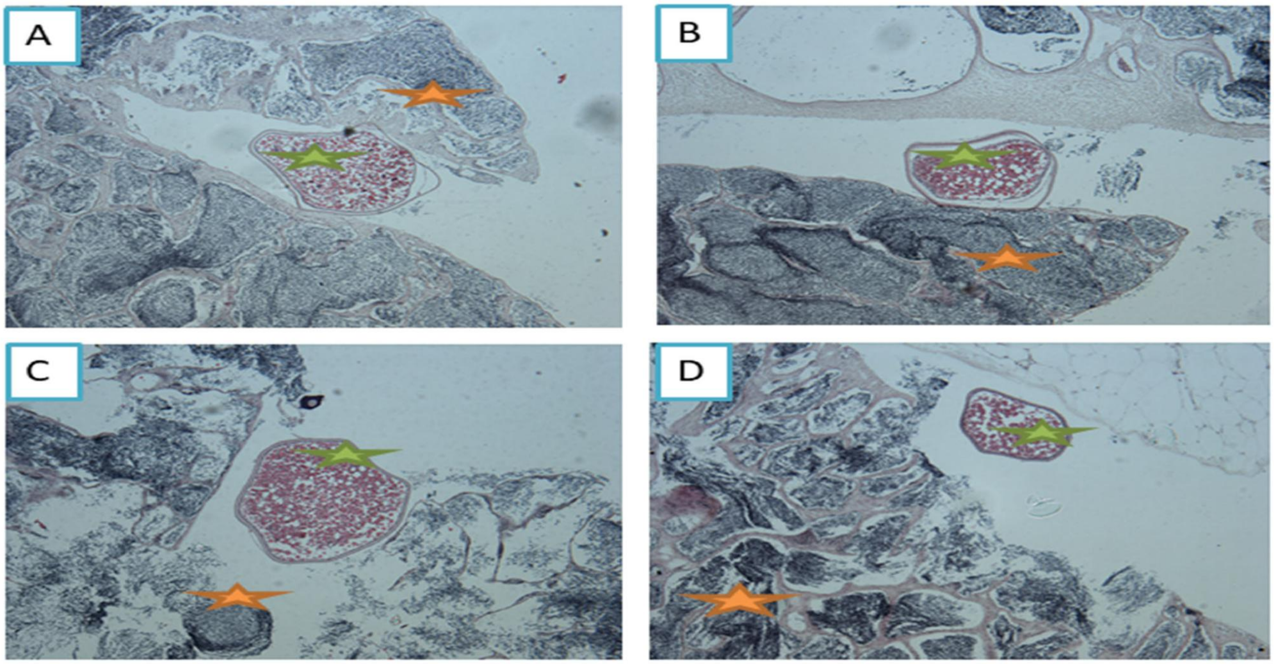

Figure 8. Histological slide observation shows two types of gamete: early-stage vitellogenin (blue star) and spermatozoa (orange star) in male gonad.

then. Regarding the fish egg production business, fertilized eggs can be produced monthly if farmers have the required number of mature broodstock. Recently, Budd et al.,[14] the machinery drivers of the changes in length-at-sex modification in Barramundi between regions are unidentified. However, other reports[20,40] suggested that this might be related to the differences in environmental factors, such as salinity, water pH, and temperature. Moreover, the epigenetic modulating of Barramundi has been reported by several authors.[14,40] They clarified that female-specific DNA methylation rises quickly through sex reverse in Barramundi, rather than gradually with fish growth. DNA methylation is a main epigenetic alteration and a significant regulator of post-transcription manner. Still, the function of DNA methylation during the early sex reverse of Barramundi is imprecise. The main limitations of the current study are a) the relation between the selected trait and other molecular pathways, such as studying the microRNA, non-coding RNA, and other extracellular vesicles. In addition, further omics tools, such as proteomics, metabolomics, or lipidomics change during the sex reverse through various growth stages. All these previous limitations will be taken into consideration in the upcoming explorations.

Conclusion

Based on the study present study's findings, the main significant output was that the growth pattern for both females and males of Barramundi reared in floating cages displayed positive allometric growth. The size of the fish correlated to the gonadosomatic index for both females and males. Sex change from male to female for Barramundi was consistent in captivity, mostly occurring at 4.57 kg and above. Various stages of gonad were found within a group of mature male and female fish at any particular time.

Ethical approval

The research was conducted following the animal ethics procedure of the American Psychological Association APA's 2002 Ethic Code.

Author contributions

All authors equally contributed to writing this research article.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Che Ismail Che-Zulkifli http://orcid.org/0000-0001-5109-8421
Mohd Abdul Muin Md Akil http://orcid.org/0000-0001-6712-265X
Adnan Amin-Safwan http://orcid.org/0000-0001-5510-9159
Marco Ragni http://orcid.org/0000-0002-5117-7960
El-Sayed Hemdan Eissa http://orcid.org/0000-0002-7229-2175

Data availability statement

Data supporting this study's findings are available from the corresponding author upon reasonable request.

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