Jurnal Akuakultur Indonesia 23 (2), 201–211 (2024)

Original article DOI: 10.19027/jai.23.2.201-211

# Sex determination and acclimation response of dwarf snakehead fish *Channa limbata* from West Java

# Determinasi kelamin dan respons aklimasi ikan gabus bogo *Channa limbata* asal Jawa Barat

**Dinar Tri Soelistyowati[1*], Agus Oman Sudrajat[1], Harton Arfah[1], Alimuddin Alimuddin[1], Riva Hafidah[2], Yudha Hanggara[2], Thomas Edison[3]**

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, West Java, Indonesia
[2]Graduate Study Program, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, West Java, Indonesia
[3]Undergraduate Study Program, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, West Java, Indonesia
*Corresponding author: dinar@apps.ipb.ac.id

Received December 8, 2023; Received in revised form December 20, 2023; Accepted February 10, 2024

## ABSTRACT

The *Channa limbata* fish is a type of tropical freshwater fish of the Channidae family which is relatively small as an aquarium ornamental fish with a distinctive color at the tip of its dorsal fin and has a snake-like head (dwarf snakehead). Natural snakehead fishing activities have threatened its sustainability. Breeding *C. limbata* fish through cultivation can increase its potential for sustainable use. This study aims to evaluate the acclimation response of wild-type dwarf snakehead fish in captivity and its sexual characteristics as a basis for domestication and hatchery technology. The fish samples used were natural catches from rivers in West Java measuring <100 mm to >150 mm of body length then individually acclimated indoors in an aquarium (35×20×20 cm) for 14 days. Snakehead fish live in shallow, slow-flowing river waters with a temperature of 20.2-21.3°C, TDS 16-24 mg/L at neutral pH, while the rearing water temperature and TDS are higher (temperature: 24.9-27.6°C; TDS: 88-110 mg/L). The fish mortality rate during acclimation reached 25% in fish measuring >150 mm of length on tenth day, while fish measuring <150 mm more adaptive with 100% survival. The male fish measuring 100-150 mm have 13-15 pectoral fin rays while female fish have fewer (13-14). The gonad development level of male *C. limbata* in nature is slower than female fish measuring 100-150 mm with a gonadosomatic index of ovaries reached 10 times higher than testicular.

Keywords: acclimation, *C. limbata*, gonadosomatic index, ovaries

## ABSTRAK

Ikan *Channa limbata* merupakan jenis ikan air tawar tropis dari famili Channidae yang berukuran relatif kecil sebagai ikan hias akuarium dengan warna yang khas pada ujung sirip punggungnya dan bentuk kepala mirip ular (*dwarf snakehead*). Aktivitas penangkapan ikan gabus alam telah mengancam kelestariannya. Pembibitan ikan *C.limbata* melalui budidaya dapat meningkatkan potensi pemanfaatannya secara berkelanjutan. Penelitian ini bertujuan untuk mengevaluasi respons aklimatisasi ikan gabus alam di dalam penangkaran dan karakterisasi seksualnya sebagai landasan teknologi pembenihan ikan gabus *C. limbata* yang tepat. Sampel ikan yang digunakan merupakan hasil tangkapan alam dari sungai di Jawa Barat berukuran <100 mm hingga >150 mm kemudian diaklimasi indoor di akuarium (35×20×20 cm) selama 14 hari. Ikan gabus hidup di perairan sungai yang dangkal berarus lambat dengan suhu 20,2-21,3°C, TDS 16-24 mg/L dan pH netral, sedangkan suhu air pemeliharaan dan TDS lebih tinggi (suhu: 24,9-27,6°C; TDS: 88-110 mg/L). Angka kematian ikan selama aklimatisasi mencapai 25% pada ikan berukuran >150 mm hari ke 10, sedangkan ikan berukuran <150 mm lebih adaptif dengan sintasan 100%. Ikan jantan *C.limbata* berukuran 100-150 mm memiliki jari-jari sirip pektoral berjumlah 13-15, sedangkan ikan betina lebih sedikit (13-14). Tingkat perkembangan gonad ikan jantan lebih lambat dari pada ikan betina dengan indeks gonadosomatik ovarium mencapai 10 kali lipat lebih tinggi dibandingkan testis.

Kata kunci: aklimatisasi, *Channa limbata*, indeks gonadosomatik, ovarium

## INTRODUCTION

The ornamental snakehead fish *Channa limbata* (Cuvier & Valenciennes, 1831), known locally as the “bogo fish,” is a tropical freshwater species from the Channidae family, relatively small in size with a head resembling that of a snake (dwarf-snakehead). The distribution of this snakehead fish species includes Iran, Afghanistan, Pakistan, India, Nepal, Bangladesh, Indonesia, Vietnam, Korea, and China (Khoomsab & Wannasri, 2017). In Indonesia, the distribution of the snakehead fish species encompasses Sumatra, Java, Kalimantan, Bangka, Madura, Bali, Lombok, Flores, Ambon, and Halmahera (Tarigan *et al*., 2016). This fish is inhabiting shallow waters in low to highlands (>1000 m above sea level) with a pH range of 7-8 and a temperature of 23–27ºC, along with relatively low dissolved oxygen and high CO2 (Kusmini *et al*., 2016). The small-sized dwarf snakehead fish holds economic value as an aquarium ornamental fish due to its attractive color patterns, especially with its fins displaying blue and orange hues (red-tail snakehead).

Furthermore, the snakehead fish contains albumin and potential amino acids for wound healing post-operation, patients with hypoalbuminemia, and children’s development (Rahayu *et al*., 2016). Continuous natural capture activities of snakehead fish have threatened its sustainability, such as in Singapore and China (He *et al*., 2019), in Java and Kalimantan (Kusmini *et al*., 2016). Additionally, environmental damage due to anthropogenic activities, agricultural/ plantation waste, and invasive fish also impact the sustained decline in populations (Gustiano *et al*., 2013). Managing the genetic resources of natural snakehead fish through breeding activities outside their habitats can reduce dependence on natural capture and enhance their potential as a productive, high-quality, and sustainable ornamental fish commodity. For the future of aquaculture primarily could focus on some truly domesticated species, or diversification of native domesticated species.

Acclimatization is the initial stage of domestication to adapt fish from their natural habitat to controlled maintenance outside their habitat and turn them into a cultivation commodity. The success of the initial domestication stage of wild fish adapting to environmental conditions outside their habitat assure better quality generations. The strategies for fish production consist of five levels of ‘domestication’ for being the most domesticated (Teletchea & Fontaine, 2014). The first to third levels representing a transitory form of fish production dependent on the availability of the wild resource.

The process of transferring fish from their habitat to captivity can cause mortality due to capture, transportation, and acclimatization in the early stages of domestication, which generally lasts for 10-14 days, referred to as hauling loss or delay mortality syndrome (Hadiroseyani *et al*., 2016). Maintaining fish in captivity can influence the change of fish behavior, both physically and physiologically (phenotypic plasticity). The ability of fish to respond the condition in the captive environment allows them to complete their entire reproductive cycle naturally. Implementing domestication strategies and generational succession for the development of cultivated ornamental snakehead fish requires a study of its bioecology and an understanding of its sexual system as the basis for appropriate fish breeding techniques.

The continuity of the fish’s reproductive cycle is crucial for preserving the species (Niass & Fall, 2015). The reproductive function development of each fish species in nature exhibits a high level of diversity according to its habitat conditions (Susatyo *et al*., 2022). The differentiation of fish genders occurs in the early stages of development influenced by environmental factors such as changes in water temperature and pH (Budd *et al.*, 2015). Determining the sexual identity of fish can be done based on primary and secondary sexual characteristics observed in measurable macroscopic and microscopic ways.

The regulation system of fish’s physiological functions and endocrine functions is influenced by environmental sensory signals and transduction to the hypothalamus-pituitary-gonad axis for the fish’s reproductive process (Shin *et al*., 2014; Tao *et al*., 2013). Snakehead fish are mouthbreeders, storing fertilized eggs inside the male fish’s mouth until they hatch (Khairanti *et al*., 2023). This research aims to evaluate the acclimation response in the initial domestication of wild *C.limbata* snakehead fish from West Java and determine their gender based on observations of primary and secondary sexual characteristics. The results of this research serve as a foundation for the breeding activities of *C. limbata* snakehead fish outside their habitat to produce high-quality and sustainable cultivated fish commodities.

## MATERIALS AND METHODS

### Fish collection

The collection of *C. limbata* snakehead fish samples was conducted in the watershed area of Cilember, Bogor Regency (West Java). Fish were collected from their natural habitat using fishing equipment and water quality measurements were carried out directly at the fish collection site. The captured fish were individually packed in plastic bags with added oxygen. The fish samples consisted of three size groups, juveniles or small (50-100 mm, S), fingerlings or medium (100-150 mm, M), and breeders or large (>150 mm, L).

### Fish acclimatization

Subsequently, the captured fish were transported to the breeding location via land transportation and acclimatized at the Fish Reproduction and Genetics Laboratory, IPB University. Observations on habitat characteristics included the physicochemical properties of water (temperature, pH, dissolved oxygen, total dissolved solids, total organic matter, and the type of aquatic substrate). Survival observations were conducted during the transportation process and post-transportation, involving a 30-minute initial adaptation period in the holding container, followed by a 14-day acclimatization period. Prior to acclimatization, post-transportation, the fish were transferred by submerging the plastic bags containing the fish into a temporary holding aquarium for 30 minutes.

Subsequently, the plastic bags were opened, allowing the fish to spontaneously released and were individually housed for a 14-day acclimatization period. The fish acclimatization process was conducted indoors at room temperature using individual aquariums measuring 35×20×20 cm with a water depth of 15 cm. The fish were not fed for two days to purge any potential diseases present in their digestive tracts. On the third day, the fish were provided with a diet consisting of yellow mealworms (larvae of *Tenebrio molitor*) or bloodworms.

The amount of food given was adjusted based on the fish's feeding response, gradually increasing the quantity. The ideal amount of feed for juvenile snakehead fish per day is 5% of their biomass weight. Observations of adaptation responses included swimming and feeding behavior, as well as their survival rate. Feeding response assessments were categorized as responsive to feed (++), slow or moderate feeding (+), and unresponsive or no feeding (-) (Herjayanto *et al*., 2020). Survival rates were monitored daily during the acclimatization period. Water quality parameters such as pH, dissolved oxygen (DO), temperature, and total dissolved solids (TDS) were measured daily for maintenance purposes.

### Sex determination

Identification of male and female fish characteristics was conducted based on biometric measurements of the fish's body using Channa genus truss morphometrics (Cuvier & Valenciennes, 1831; Nainggolan *et al*., 2019) and meristic characters (Siregar *et al*., 2020). The truss morphometric characters measurement was taken directly on the specimen prior to acclimation. Morphometric characteristics included 26 truss points along the fish's body and three characteristics related to head shape. Meristic characteristics involved the count of rays in the dorsal fin, pectoral fin, pelvic fin, anal fin, and caudal fin (Figure 1). Observed fish samples were categorized into three length groups: 50-100 mm (small, S), 100-150 mm (medium, M), and larger than 150 mm (large, L). The gender of the samples then inspected for their development of gonad observation as primary characteristics by killing the samples.

### Gonad histology

Primary sexual characteristics of male and female fish were observed macroscopically and microscopically in gonad and liver samples to assess the level of reproductive function development based on the gonadosomatic index (GSI) and hepatosomatic index (HSI). The observed gonad samples included ovaries and testes of all size classes (S, M, L). GSI and HSI values represent the percentage ratio of gonad and liver weight to body weight (Gong *et al*., 2017). Microscopic observation of germ cell development was conducted by analyzing gonadal tissue. Gonad samples were fixed in 10% buffered neutral formalin and then transferred to 70% alcohol, stored at room temperature for 24 hours. Subsequently, gonad samples were embedded in paraffin, sliced using a microtome, stained with Hematoxylin and Eosin (H&E) tissue staining, and observed under a light microscope at 40× magnification to assess germ cell development (Milton *et al*., 2018).

**Data analysis**

Statistical analysis of morphometric and meristic characteristics between fish size groups (S, M, L) and between male and female sexes was performed using Student's t-test with a confidence interval of 95%. The acclimation response of fish in captivity and the macroscopic and microscopic assessment of gonads were analyzed descriptively.

## RESULTS AND DISCUSSION

**Result**

*Truss Morphometrics of C. limbata*

Based on the size groups of the wild snakehead fish *C. limbata* (S, M, L) originating from West Java (Table 1), there is a proportional increase in standard length along with an increase in total length ($p<0.05$). The significant differences obtained were considered to discriminate the fish into three existence morphological groups according to their size. The *C. limbata* fish measuring less than 150 mm (S, M) and the group measuring more than 150 mm (L) exhibit differences in characteristic values in eye diameter (13), dorsal fin height (17), anal fin height (22), as well as the length and height of the caudal fin (24, 26). Morphometric characteristics of the head shape of *C. limbata* snakehead fish in all length groups (S, M, L) do not show significant differences (Table 2).

*Sexual Dimorphism C. limbata*

The morphometric characteristics distinguishing gender in *C. limbata* fish from West Java are identified in the height of the dorsal and pectoral fins. In the group of fish sized 100-150 mm, male fish exhibit longer heights in the dorsal fin (17) and pectoral fin (20) compared to females (Table 3). The meristic characteristics of male and female *C. limbata* fish (Table 4) show

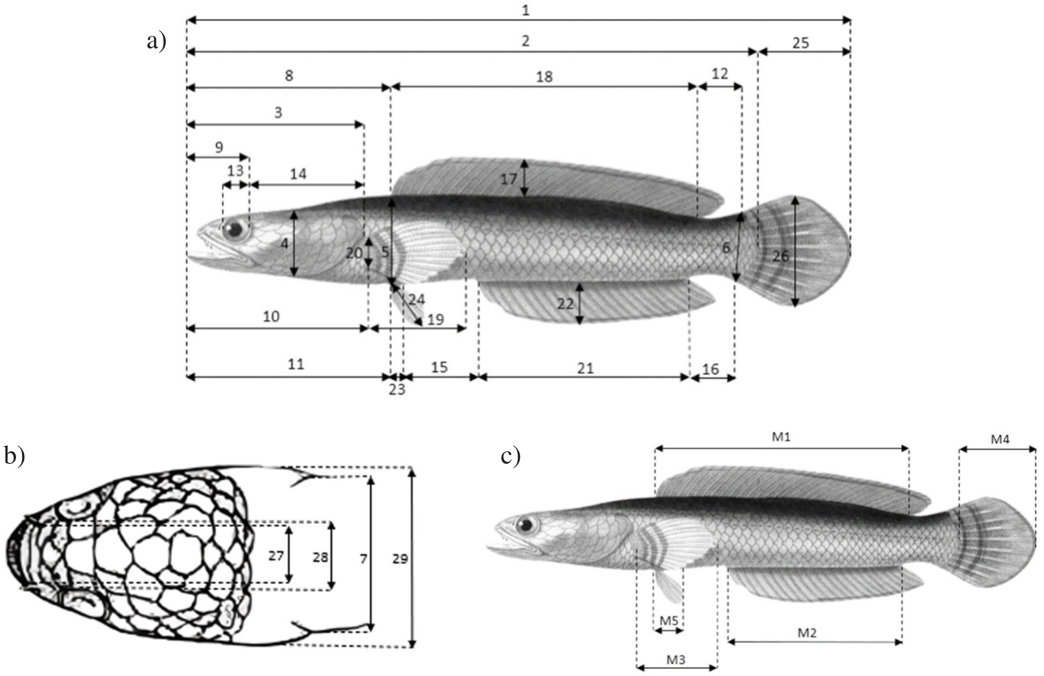


Figure 1. Truss morphometric characteristics of the snakehead fish body (a), head shape of the snakehead fish (b), and meristic characteristics of the snakehead fish (c). (Modified from Cuvier and Valenciennes (1831) Histoire naturelle des poissons).

Note: (1) Total Length; (2) Standard Length; (3) Head Length; (4) Head Height; (5) Body Height; (6) Tail Stem Height; (8) Distance from mouth to fin base; (9) Mouth to Eye Distance; (10) Distance from mouth to pectoral fin base; (11) Distance from mouth to base of pelvic fins; (12) Distance from the dorsal fin to the base of the caudal fin; (13) Eye Diameter; (14) Distance between eyes and gill covers; (15) Distance of Pelvic Fin to Base of Anal Fin; (16) Distance from the anal fin to the base of the caudal fin; (17) Dorsal Fin Height; (18) Length of Dorsal Fin Base; (19) Pectoral Fin Base Length; (20) Pectoral Fin Height; (21) Length of Anal Fin Base; (22) Anal Fin Height; (23) Pelvic Fin Height; (24) Pelvic Fin Base Length; (25) Tail Fin Base Length; (26) Caudal Fin Height; (7) Body Width; 27) Distance Between Tackles; (28) Distance between eyes, (29) Distance between gill covers; (M1) Number of dorsal fin rays; (M2) number of anal fin rays; (M3) pectoral fin rays; (M4) tail fin radius; (M5) pelvic fin rays.

Table 1. Truss Morphometric characteristics of *C.limbata* from West Java.

| Morphometric characteristics | Fish Sample Size | | |
|---|---|---|---|
| | 50-100 mm (S) | 100-150 mm (M) | >150 mm (L) |
| (1) Total Length (mm) | 72.79 ± 10,1385a | 111.67 ± 1.6337b | 155.52 ± 7.65c |
| (2) Standard Length (mm) | 59.61 ± 7.7709a | 92.56 ± 4.3893b | 127.34 ± 7.860c |
| (3) Head Length | 0.27 ± 0.0171 | 0.29 ± 0.0160 | 0.25 ± 0.0550 |
| (4) Head Height | 0.14 ± 0.0091 | 0.14 ± 0.0039 | 0.14 ± 0.0238 |
| (5) Body Height | 0.15 ± 0.0124 | 0.15 ± 0.0041 | 0.15 ± 0.0349 |
| (6) Tail Stem Height | 0.08 ± 0.0101 | 0.11 ± 0.0051 | 0.11 ± 0.0156 |
| (7) Body Width | 0.16 ± 0.0075 | 0.15 ± 0.0104 | 0.08 ± 0.0718 |
| (8) Distance from mouth to fin base | 0.35 ± 0.0221 | 0.37 ± 0.0156 | 0.38 ± 0.0110 |
| (9) Mouth to Eye Distance | 0.08 ± 0.0025 | 0.08 ± 0.0032 | 0.09 ± 0.0012 |
| (10) Distance of mouth-pectoral fin base | 0.29 ± 0.0210 | 0.32 ± 0.0171 | 0.32 ± 0.0105 |
| (11) Distance of mouth-pelvic fins base | 0.33 ± 0.0073 | 0.36 ± 0.0074 | 0.38 ± 0.0036 |
| (12) Distance of dorsal fin-caudal fin base | 0.07 ± 0.0156 | 0.07 ± 0.0060 | 0.05 ± 0.0077 |
| (13) Eye Diameter | 0.05 ± 0.0010 | 0.04 ± 0.0045 | 0.03 ± 0.002c |
| (14) Distance between eyes and gill covers | 0.21 ± 0.0443 | 0.20 ± 0.0100 | 0.22 ± 0.0221 |
| (15) Distance of pelvic fin-anal fin base | 0.19 ± 0.0090 | 0.18 ± 0.0190 | 0.15 ± 0.0045 |
| (16) Distance of anal fin-caudal fin base | 0.10 ± 0.0060 | 0.09 ± 0.0147 | 0.09 ± 0.0029 |
| (17) Dorsal Fin Height | 0.08 ± 0.0054 | 0.08 ± 0.0253 | 0.11 ± 0.001c |
| (18) Length of Dorsal Fin Base | 0.56 ± 0.0136 | 0.55 ± 0.0231 | 0.61 ± 0.0067 |
| (19) Pectoral Fin Base Length | 0.16 ± 0.0103 | 0.17 ± 0.0104 | 0.19 ± 0.0051 |
| (20) Pectoral Fin Height | 0.06 ± 0.0099 | 0.06 ± 0.0034 | 0.06 ± 0.0049 |
| (21) Length of Anal Fin Base | 0.34 ± 0.0251 | 0.36 ± 0.0122 | 0.41 ± 0.0125 |
| (22) Anal Fin Height | 0.06 ± 0.0105 | 0.08 ± 0.0058 | 0.09 ± 0.001c |
| (23) Pelvic Fin Height | 0.07 ± 0.0029 | 0.07 ± 0.0017 | 0.09 ± 0.0014 |
| (24) Pelvic Fin Base Length | 0.01 ± 0.0021 | 0.02 ± 0.0031 | 0.03 ± 0.004c |
| (25) Tail Fin Base Length | 0.26 ± 0.0867 | 0.21 ± 0.0236 | 0.20 ± 0.0433 |
| (26) Caudal Fin Height | 0.15 ± 00016 | 0.18 ± 0.0135 | 0.20 ± 0001c |

Note: The morphometric character of head shape is a comparison of the measured character value with the standard length (2). The t-test at the 95% confidence interval is significantly different marked with the letters: a (S<M), b (M<L), c (S<L).

Table 2. Morphometric characteristics of head shape *C. limbata* from West Java.

| Morphometric characteristics | Fish Sample Size | | |
|---|---|---|---|
| | 50-100 mm (S) | 100-150 mm (M) | >150 mm (L) |
| (2) Standard length (mm) | 59.61 ± 7.7709a | 92.56 ± 4.3893b | 127.34 ± 7.860c |
| (7) Body width | 0.16 ± 0.0075 | 0.15 ± 0.0104 | 0.08 ± 0.0718 |
| (27) Distance between tackles | 0.05 ± 0.0012 | 0.06 ± 0.0087 | 0.02 ± 0.0005 |
| (28) Distance between eyes | 0.09 ± 0.0037 | 0.0 ± 0.0055 | 0.03 ± 0.0018 |
| (29) Distance between gill covers | 0.18 ± 0.0035 | 0.19 ± 0.0075 | 0.06 ± 0.0017 |

Note: The morphometric character of head shape is a comparison of the measured character value with the standard length (2). The t-test at the 95% confidence interval is significantly different marked with the letters: a (S<M), b (M<L), c (S<L).

Table 3. Morphometric characters of secondary sexual characteristics of snakehead fish *C. limbata* from West Java.

| **Morphometric characters** | **100-150 mm (male)** | | | **100-150 mm (female)** | | |
|---|---|---|---|---|---|---|
| | x̄ | ±SD | %KK | x̄ | ±SD | %KK |
| (1) Total length (mm) | 111.67 | 2.00 | 1.79 | 118.79 | 16.22 | 13.65 |
| (2) Standard length (mm) | 92.56 | 5.38 | 5.81 | 99.89 | 16.83 | 16.85 |
| (3) Head length | 29.44 | 1.96 | 6.66 | 28.19 | 1.28 | 29.44 |
| (4) Head height | 13.78 | 0.48 | 3.49 | 13.61 | 1.08 | 13.78 |
| (5) Body height | 14.59 | 0.50 | 3.45 | 14.84 | 0.92 | 14.59 |
| (6) Tail stem height | 10.56 | 0.62 | 5.87 | 10.30 | 0.52 | 10.56 |
| (7) Body width | 15.42 | 1.28 | 8.28 | 16.67 | 1.59 | 15.42 |
| (8) Distance from mouth to fin base | 36.57 | 1.91 | 5.21 | 36.01 | 1.62 | 36.57 |
| (9) Mouth to eye distance | 8.42 | 0.40 | 4.70 | 7.41 | 1.14 | 8.42 |
| (10) Distance mouth-pectoral fin base | 31.63 | 2.09 | 6.60 | 30.85 | 0.71 | 31.63 |
| (11) Distance mouth-pelvic fins base | 35.63 | 0.91 | 2.55 | 33.23 | 2.13 | 35.63 |
| (12) Distance dorsal fin-caudal fin | 6.78 | 0.73 | 10.78 | 7.43 | 0.45 | 6.78 |
| (13) Eye Diameter | 3.79 | 0.55 | 14.40 | 3.80 | 0.24 | 3.79 |
| (14) Distance eyes and gill covers | 20.16 | 1.22 | 6.07 | 19.40 | 0.55 | 20.16 |
| (15) Distance pelvic fin-anal fin base | 17.73 | 2.33 | 13.15 | 16.68 | 0.48 | 17.73 |
| (16) Distance anal fin-caudal fin base | 8.96 | 1.80 | 20.09 | 8.20 | 0.79 | 8.96 |
| (17) Dorsal fin height | 8.38a | 3.10 | 36.98 | 7.58b | 0.91 | 8.38a |
| (18) Length of dorsal fin base | 54.64 | 2.83 | 5.18 | 54.86 | 1.15 | 54.64 |
| (19) Pectoral fin base length | 17.04 | 1.28 | 7.49 | 16.51 | 1.44 | 17.04 |
| (20) Pectoral fin height | 5.91a | 0.42 | 7.12 | 5.30b | 0.24 | 5.91a |
| (21) Length of anal fin base | 36.32 | 1.49 | 4.11 | 36.46 | 1.71 | 36.32 |
| (22) Anal fin height | 8.31 | 0.71 | 8.58 | 8.29 | 1.15 | 8.31 |
| (23) Pelvic fin height | 6.83 | 0.21 | 3.00 | 6.89 | 0.35 | 6.83 |
| (24) Pelvic fin base length | 2.01 | 0.38 | 18.73 | 2.29 | 0.21 | 2.01 |
| (25) Tail fin base length | 21.23 | 2.90 | 13.64 | 20.50 | 2.86 | 21.23 |
| (26) Caudal fin height | 17.99 | 1.65 | 9.17 | 20.88 | 1.64 | 17.99 |
| (27) Distance between tackles | 6.47 | 1.06 | 16.37 | 5.63 | 0.49 | 6.47 |
| (28) Distance between eyes | 9.07 | 0.67 | 7.39 | 9.44 | 0.57 | 9.07 |
| (29) Distance between gill covers | 18.82 | 0.92 | 4.90 | 18.22 | 1.05 | 18.82 |

Note: Morphometric characters (3-29) are measured character values divided by standard length. The t-test at the 95% confidence interval is significantly different marked with a different letter in the mean value.

variations in the number of soft-rayed pectoral fin spines, ranging from 13-15 (male) and 13-14 (female). The dorsal fin rays range from 30-33 with a blue-orange strip at the tip; the base of the dorsal fin is positioned behind the base of the pectoral fin, and its end extends beyond the anal fin. Meanwhile, the ventral fin is located in front of the base of the dorsal fin and is half the length of the pectoral fin.

*Gonad development level*

The gender of fish is influenced by environmental factors that affect gonadogenesis. The gonad development in male and female snakehead fish within the size group of 100-150 mm exhibits different maturity levels (Table 5, Figure 2). Value of gonadosomatic index (GSI) and hepatosomatic index (HSI) in female fish sized 100-150 mm (M) are higher (1.31 ± 0.40% and 1.00 ± 0.28%) compared to males across all fish size groups. In Channa species, the ovarian development generally shows faster progression than testicular development in fish of the same size (Irmawati *et al*., 2019). The generative cell development in wild-caught male snakehead fish shows spermatocyte (SC) and spermatid (ST) phases in fish sized 50-100 mm (S), the spermatid and spermatozoa (SZ) in fish sized 100-150 mm (M) and >150 mm (L). In the ovaries of fish sized 100-150 mm (M), there are different egg cells present such as oogonia (OG), early perinuclear oocytes (EPO), late perinuclear oocytes (LPO), and yolk vesicles (VY).

*Acclimatization response*

Wild snakehead fish *C. limbata* in the nature (river) along sample collection inhabit shallow waters with a depth of 30-50 cm, sandy and muddy substrates, slow currents, with a temperature range of 20.2-29.5°C, pH 5.87–8.66,

Table 4. Meristic characteristics of natural snakehead fish *C. limbata* from West Java.

| Kode | Meristic characteristics | Male (n=12) | Female (n=3) |
|---|---|---|---|
| M1 | Number of dorsal fin rays | 30-33 | 30-33 |
| M2 | Number of anal fin rays | 20-23 | 20-23 |
| M3 | Number of pectoral fin rays | 13-15 | 13-14 |
| M4 | Number of tail fin radius | 13-15 | 13-15 |
| M5 | Number of pelvic fin rays | 6 | 6 |

Table 5. Level of sexual development of natural snakehead fish *C.limbata* from West Java.

| Total length (mm) | Body weight (g) | Gonad weight (mg) | Liver weight (mg) | GSI (%) | HSI (%) |
|---|---|---|---|---|---|
| 100-150 (male) | 12.7 ± 3.34 | 10.6 ± 3.65 | 79.57 ± 30.52 | 0.08 ± 0.02 | 0.63 ± 0.24 |
| 100-150 (female) | 14.79 ± 4.88 | 194.5 ± 77.17 | 143.97 ± 52.00 | 1.31 ± 0.40 | 1.00 ± 0.28 |

GSI: gonadosomatic index, HSI: hepatosomatic index.

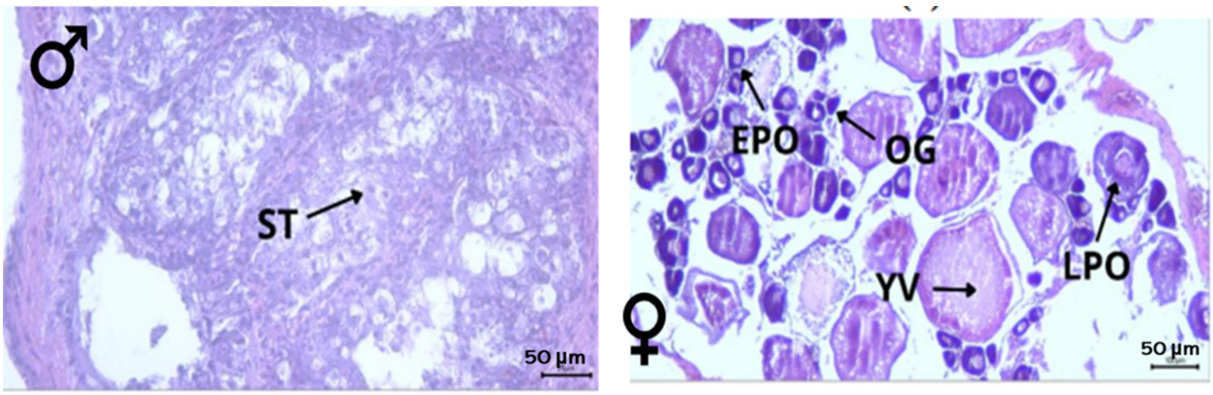


Figure 2. Gonad development of dwarf snakehead fish *C. limbata* from West Java measuring 100-150 mm long male (left) and female (right). Spermatids (ST), oogonia (OG), early perinuclear oocytes (EPO), late perinuclear oocytes (LPO), and yolk vesicles (VY).

and relatively low dissolved oxygen (DO) levels (7.2-8.6 mg/L), CO2 3.99-20 mg/L, TDS 16-267 mg/L, total organic matter (TOM) 15-51.7 mg $KMnO_4$/L. While in the indoor maintenance water quality during acclimation has a higher temperature range of 24.9-27.6°C, pH 6.23-6.86, DO 3.8-5.6 mg/L, TDS 88-110 mg/L. The acclimatization response of wild snakehead fish *C. limbata* in captivity indicates slow swimming movements and decreased feeding activity (Table 6). Fish sized less than 100 mm (S) adapted quicker to the captive environment and show feeding responsiveness starting on the third day compared to larger fish over 100 mm (M, L). Fish sized over 150 mm (L) exhibit passive movement and do not respond to food until the 10$^{th}$ day, showing adaptive behavior. The mortality rate of fish during acclimatization is 25% for fish larger than 150 mm starting from the 10th day, while no mortality is observed in fish less than 100 mm (S) and 100-150 mm (M) (Figure 3).

**Discussion**

There are still very few studies on the biological aspects of the *Channa limbata* fish as a reference in sustainable management and cultivation (Hoomsab & Wannasri, 2017). This research is in the first stage of initial domestication with adaptation to the limited environment in the cultivation container. Water quality in natural

Table 6. The acclimation response of dwarf snakehead fish *C. limbata* of different sizes (S, M, L) during the rearing period in captivity (indoor) for 14 days.

| Day | Movement respone | | | Eating Response | | |
|---|---|---|---|---|---|---|
| | 50-100 mm | 100-150 mm | >150 mm | 50-100 mm | 100-150 mm | >150 mm |
| 1 | passive | passive | passive | - | - | - |
| 2 | passive | passive | passive | - | - | - |
| 3 | passive | passive | passive | + | - | - |
| 4 | passive | passive | passive | + | + | - |
| 5 | passive | passive | passive | + | + | - |
| 6 | passive | passive | passive | + | + | - |
| 7 | passive | passive | passive | + | + | - |
| 8 | passive | passive | passive | + | + | - |
| 9 | passive | passive | passive | + | + | - |
| 10 | passive | passive | passive | + | + | + |
| 11 | active | passive | passive | ++ | + | + |
| 12 | active | passive | passive | ++ | + | + |
| 13 | active | passive | passive | ++ | + | + |
| 14 | active | passive | passive | ++ | + | + |

Note: passive: the fish stays still at the bottom of the aquarium; active: the fish swims actively; '-': food is not consumed by fish; '+': food is consumed after 1 minute; '++': fish consumed food before 1 minute. S (50-100 mm), M (100-150 mm), L (>150 mm).

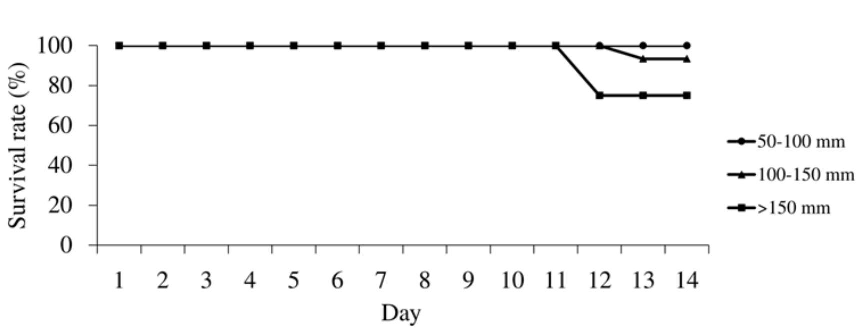


Figure 3. Survival rate of dwarf snakehead fish *C. limbata* during 14 days of acclimation in captivity.

habitats of *C. limbata* is at lower temperature and TDS compared to the water temperature and TDS conditions during the rearing period. The habitat of Channa fish can be found in mountain river areas with cold water and low dissolved content values. Feed response and movement response during rearing show that there is a decrease in response as size increases (Herjayanto *et al*., 2020).

The results of acclimatization for 14 days showed a decrease in movement response, feeding response and survival as the size of fish increased during rearing. The range of survival rate values during 14 days of maintenance shows the highest value of 100% in fish measuring under 100 mm and the lowest value of 75% in fish measuring over 150 mm. The increase in water temperature in captivity compared to the natural habitat of *C. limbata* is suspected to cause stress and fish mortality. Differences in environmental conditions between the natural habitat and captive conditions can influence the fish's physiological regulatory system (Tao *et al*., 2013). Larger fish will attempt anaerobic metabolism to prolong their survival in hypoxic conditions (Urbina & Glover, 2013).

As fish grow larger, their metabolic rate tends to decrease. The larger fishes may have a longer satiety period than the small group so that the large group tend to less responsive to eat and movement for more efficient in utilizing energy maintenance. Sexual dimorphism is an important aspect in the domestication stage, namely continuous reproduction (Teletchea & Fontaine, 2014). Morphometric and meristic characters were tested between sizes of male fish and between males and females at sizes 100-150 mm. Sexual dimorphism in other snakehead fish species is identified through differences in body coloration between male and female *C. gachua* (Jearranaiprepame, 2017) and variations in the length of the anal fin and eye diameter in *C. striata* (Arma *et al*., 2014).

Male snakehead fish commonly have longer dorsal fins than females, with a more dominant red coloration on their bodies (Gustiano *et al*., 2019; Gustiano *et al.*, 2020), and they show a comparison between body weight and length known as allometric (Vodounnou *et al*., 2017). The characteristics of tail fin height between male fish samples measuring 50-100 mm and 100-150 mm were significantly different. The morphometric characters that are significantly different between male fish measuring 50-100 mm and male fish measuring >150 are the length of the pelvic fin base, eye diameter, pectoral fin height, anal fin height and tail fin height. This difference value can be used as a reference for changes in the morphometric characters of male *C. limbata* fish towards adulthood.

Studies related to sexual dimorphism in body morphometrics of fish in *Channa gachua* found that there were no differences in morphometric characters between the sexes except for the characterization of body color between males and females (Jearranaiprepame, 2017). The plasticity to adapt to the environment factors possibly has an impact on the different of specific morphological on fish population at different habitat (Muslimin *et al*., 2020). The morphometric difference between the sexes of snakehead fish is in the length of the anal fin and eye diameter (Arma *et al*. 2014). Snakehead fish have a cylindrical body shape towards the head and tapering towards the tail, and their eye diameter is one-fifth to one-eighth of the length of their head (Gustiano *et al*., 2013).

Local adaptation responded to environmental conditions can yield morphologically distinct populations. The ecological diversification, especially flow regime, was considerably be involved with the different variations of the whole fish body (Jearranaiprepame, 2017). The GSI value of female bogo fish has a higher value than the GSI value of male bogo fish, but the HSI value does not differ between all sizes of fish and gender of bogo fish. The histology results of fish testicles measuring 50-100 mm show that there are spermatocytes that are round in shape and the cell membrane is clearly visible and spermatids that are smaller in size are darker in color (Al Mahmud *et al*., 2016), whereas in fish measuring 100-150 mm there are spermatids and spermatozoa.

Spermatozoa are the final stage of sperm differentiation with a dark color and are the smallest cells (Al Mahmud *et al.* 2016) and in the ovaries there are oogonia (OG), early perinuclear oocytes (EPO), late perinuclear oocytes (LPO), and yolk vesicles (VY). Sperm differentiation includes a series of morphological changes that occur in the seminiferous epithelium (Teves *et al*., 2020). The development of germ cells in large male snakehead fish (100-150 mm) which have differentiated into spermatozoa shows that they are ready for the fertilization process.

## CONCLUSION

The secondary sexual characteristic of snakehead fish *C.limbata* measuring 100-150 mm in length were distinguished by the characters of dorsal fin height and pectoral fins height with the ovarian development being ten times higher compared to testicular development. The nature habitat of *C. limbata* is shallow water river with slow currents at a temperature range of 20.2-21.3°C while in the rearing conditions was higher (24.9-27.6°C) however the fish measuring 50-150 mm showed a good acclimation response with the survival rate reached 100%.

## ACKNOWLEDGMENT

This research was funded by the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology in accordance with the Implementation Contract of the Research Program in 2023, Number: 102/E5/PG.02.00PL/2023 dated June 19, 2023. The author would like to thanks to all researcher of undergraduate and graduate students Department of Aquaculture have contributed to fish samples collection, Laboratory of Reproduction and Genetics of Aquatic Organisms (dry and wet), Faculty of Fisheries and Marine Sciences (FPIK), IPB University, Bogor for facilities.

## REFERENCES

Arma NR, Illijas MI, Irmawatf, Mappanyiwi A. 2014. Morphometric and Meristic Characteristics of Snakehead Fish for Hatchery Production. Conference: International and National Seminar on Fisheries and Marine: *Pekanbaru Riau Indonesia.*

Al Mahmud N, Rahman HMH, Mostakim GM, Khan MGQ, Shahjahan M, Lucky NS, Islam MS. 2016. Cyclic variations of gonad development of an air-breathing fish, Channa striata in the lentic and lotic environments. Fisheries and Aquatic Sciences 19: 5.

Budd A, Banh Q, Domingos J, Jerry D. 2015. Sex control in fish: Approaches, challenges and opportunities for aquaculture. Journal Marine Science Engineering 3: 329–355.

Cuvier G, Valenciennes A. 1831. Histoire naturelle des poissons, Tome septième, Livre septième, Des Squamipennes, Livre huitième, Des poissons à pharyngiens labyrinthiformes, F, G, Levrault, Paris. Pls. 170–208.

Gong X, Wang D, Bao B, Zhang Q, Jiang X, Liu L. 2017. Gonadal development and silvering of the Japanese Eel (*Anguilla japonica*) in the Yangtze River during downstream migration. Aquaculture and Fisheries 2:173–178.

Gustiano R, Oktaviani T, Soelistyowati DT, Kusmini II, Wahyutomo, Huwoyon GH. 2013. Analysis of genotype variation and truss morphometric of three populations of snakehead fish [*Channa striata* (Bloch, 1973)]. Berita Biologi 12: 325–333.

Gustiano R, At-Thar AMF, Kusmini II. 2019. Diversity, Biology Reproduction and Broodstock Management of *Channa striata*. Indonesia, Bogor: IPB Press.

Gustiano R, Kurniawan K, Kusmini II. 2020. Bioresources and diversity of snakehead. Channa striata (Bloch 1793): a proposed model for optimal and sustainable utilization of freshwater fish. IOP Conf. Series: Earth and Environmental Science 762: 012012.

Hadiroseyani Y, Sukenda, Surawidjaja, Enang H, Utomo Nur BP, Affandi R. 2016. Survival rate of transported ricefield eels, *Monopterus albus* (synbranchidae) in open and close system at water salinity level of 0 and 9 g/L. AACL Bioflux 9: 759–767.

He B, Liu X, Huan Y, Che X, Yan T, Yan J, Long Z, Li B, Wen ZY. *et al*. 2019. Complete mitochondrial genome of the snakehead (*Channa gachua*) and its phylogeny. Mitochondrial DNA Part B Resources 4: 4174–4175.

Herjayanto M, Syamsunarno MB, Prasetyo NA, Mauliddina AM, Agung LA, Widiyawan ER, Rahmayanti N, Irianingrum N, Nurkhotimah E, Gani A, Salsabila VN. 2020. preliminary study of closed system transportation, rearing and observation of the eggs *Oryzias javanicus* (Bleeker 1854) ftom Tunda Island. Jurnal Iktiologi Indonesia 20: 93–103.

Irmawati, Tresnati J, Nadiarti, Fachruddin L. 2019. Sex Differentiation and Gonadal Development of striped snakehead (*Channa striata* Bloch, 1793). IOP Conference Series: Earth and Environmental Science 253: 012007.

Jearranaiprepame P. 2017. Morphological differentiation among isolated populations of dwarf snakehead fish *Channa gachua* (Hamilton. 1822) using truss network analysis. Acta Biologica Szegediensis 61: 119–128.

Khairanti D, Adiesty Z, Nasution MW, Miftahuddin. 2023. Pengamatan keanekaragaman jenis ikan genus Channa daerah kecamatan Medan Tembung kabupaten Deli Serdang. Journal on Education 5: 6768–6779. (In Indonesia).

Khoomsab K, Wannasri S. 2017. Biological aspects of *Channa limbata* (Cuvier. 1831) in Ta Bo – Huai Yai Wildlife Sanctuary, Phetchabun Province Thailand. Sains Malaysiana 46: 851–858.

Kusmini II, Gustiano R, Prakoso VA, dan Ath-thar MHF. 2016. Budidaya ikan gabus. Indonesia, Jakarta: Penebar Swadaya.

Milton J, Bhat AA, Haniffa MA, Hussain SA, Rather IA, Anazi KM, Hailan WAQ, Farah MA. 2018. Ovarian development and histological observations of thretened dwarf snakehead fish. *Channa gachua* (Hamilton. 1822). Saudi Journal of Biological Sciences 25: 149–153.

Muslimin B, Rustadi R, Hardaningsih H, Retnoaji B. 2020. Morphometric variation of Cork fish (Channa striata Bloch, 1793) from nine populations in Sumatra Island, Indonesia. Iranian Journal Ichthyology 7: 209–221.

Nainggolan WO, Efizon D, Putra RM. 2019. Morphometric, meristic, and growth patterns of snakehead fish (Channa striata Bloch, 1793) in Sei Paku Reservoir, Sub-District Kampar Kiri, Kampar District, Riau Province. Jurnal Online Mahasiswa Fakultas Perikanan dan Kelautan Universitas Riau 6: 1–9.

Niass F, Fall S. 2015. Reproduction and larval rearing of the freshwater prawn, *Macrobrachium vollenhovenii* in Senegal. International Journal of Biological and Chemical Sciences 9: 2523–2534.

Rahayu P, Marcelline F, Sulistyaningrum E, Suhartono MT, Tjandrawinata RR. 2016. Potential effect of Striatin (DLBS0333), a bioactive protein fraction isolated from *Channa striata* for wound treatment. Asian Pacific Journal of Tropical Biomedicine 6: 1001–1007.

Siregar EY, Putra RM, Eddiwan. 2020. Morfometrik. meristik dan pola pertumbuhan ikan toman (*Channa micropeltes* Cuvier. 1831) di waduk PLTA Koto Panjang Desa Pulau Gadang Kecamatan XII Koto Kampar Kabupaten Kampar Provinsi Riau. Jurnal Online Mahasiswa. 7: 1–12. (In Indonesia).

Susatyo P, Lestari W, Sugiharto, Chasanah T. 2022. Reproductive aspects of javaen barb fish, *Systomus orphoides* in the initial domestication program. Biodiversitas 23: 1511–1519.

Shin HS, Habibi HR, Choi CY. 2014. The environmental regulation of maturation in goldfish, *Carassius auratus*: Effects of various LED light spectra. Comparative Biochemistry and Physiology A: Molecular & Integrative Physiology 168 17–24.

Tao W, Cheng Y, Liu Z, Yan S, Long X. 2013. Effects of light intensity on growth, immune response, plasma cortisol and fatty acid composition of juvenile *Epinephelus coioides* reared in artificial seawater. Aquaculture 414–415: 135–139.

Tarigan RR, Widodo MS, Kilawa Y. 2016. Physiological studies of snakehead fish (*Channa gachua*) maintained in controlled containers. International Journal of Chem Tech Research 9: 404–411.

Teletchea F, Fontaine P. 2014. Levels of domestication in fish: implications for the sustainable future of aquaculture. Fish and Fisheries 15: 181–195.

Teves ME, Roldan ERS, Krapf D, Strauss JF, Bhagat V, Sapao P. 2020. Sperm Differentiation: The Role of Trafficking of Proteins (Review). International Journal of Molecular Sciences 21: 3702.

Urbina MA, Glover CN. 2013. Relationship between Fish size and metabolic rate in the oxyconforming inanga *Galaxias maculatus* reveals size-dependent strategies to withstand hypoxia. Physiological and Biochemical Zoology 86: 740–749.

Vodounnou DSJV, Kpogue DNS, AKPO Y, Lagnika M, Fiogbe ED. 2017. Determination of sexual dimorphism of African snakehead (*Parachanna obscura*): morphometric and meristic parameters, weight-length relationship and condition factor. International Journal Biology Chemical Sciences 11: 1742–1752.