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Research Article

Genetic Relationship Analysis of Genus *Nomorhamphus* from Lindu Lake, Central Sulawesi and Adaptive Responses to Exposure Different Light Wavelengths

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Abstract

Genus *Nomorhamphus* is an endemic fish that can be found in Sulawesi, Indonesia. This fish belongs to the halfbeak group and has diverse colors and morphology. It has economic value as an export commodity in the ornamental fish trade. Exploration of the genetic relationship of genus *Nomorhamphus* in Central Sulawesi is still limited. Nomorhamphus's fulfillment of export demand still comes from wild catches. This study aimed to identify the genetic relationship of genus *Nomorhamphus* in the inlet rivers of Lake Lindu, Central Sulawesi, and evaluate the adaptation response of genus *Nomorhamphus* to different wavelengths of light exposure. Caudal fin of six fish from the three inlet rivers of Lake Lindu were preserved in 95% ethanol for DNA extraction purposes. This research used a completely randomized design with three treatment, namely rearing under white (400 nm), green (525 nm), and red (625 nm) light for 60 days. Each treatment had three replications in the form of aquariums, with each aquarium being filled with four fish. A total of 42 fish were utilized in the study. CO1 sequence was amplified with universal primers of FISH-F2 and FISH-R2. The PCR amplification products were then sequenced and performed with phylogenetic tree analysis. The genetic diversity analysis suggests that the genus *Nomorhamphus* of all three rivers, Lindu Lake, Central Sulawesi is one species as *Nomorhamphus* sp.. Male and female growth length and gonad maturation were developed faster under green light exposure, while survival rate, blood glucose level, and color quality were not significantly different under different light. Exposure of wild fish to green light *Nomorhamphus* sp. can accelerate gonad maturation and growth to accelerate domestication.

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1. Introduction

Sulawesi Island, Indonesia is the habitat center for an endemic fish called genus *Nomorhamphus*. This fish can only be found in Sulawesi, Indonesia (13 species) and the Philippines (seven species) (Lawelle *et al.*, 2021). Genus *Nomorhamphus* or in local name known as anasa fish has a unique and distinctive morphology, namely a mouth shape in the form of a long lower jaw (halfbeak), different color of fins and body for each species (Huylebrouck *et al.*, 2012) such as red, orange, yellow, even black, so that it has economic export value in the ornamental fish trade (Kusumah *et al.*, 2016). In Indonesia, genus *Nomorhamphus* are often captured from the wild due to their high market value, ranging from Rp12.000,00 to Rp60.000,00 per fish depending on the species. Some species of genus *Nomorhamphus* (Sulawesi) are exported abroad as ornamental fish, such as *N. celebensis* (Poso Lake, Central Sulawesi), *N. ebradtii* (Southeast Sulawesi), *N. liemi* (South Sulawesi), *N. Rex* (South Sulawesi), *N. towoetii* (South Sulawesi) (Kusumah *et al.*, 2016). The diversity and economic value of genus *Nomorhamphus* fish keep scientists exploring their genetics across Sulawesi, although research in Central Sulawesi primarily focuses on Lake Poso (Kraemer *et al.*, 2019), so there is a need for genetic exploration of genus *Nomorhamphus* in Lake Lindu, Central Sulawesi. In Indonesia, the fulfillment of export demands still on wild catches, which threatens their survival in the wild. Therefore, efforts to domesticate this fish are necessary.

The validation of genus *Nomorhamphus* in parts of Central Sulawesi (Kraemer *et al.*, 2019) can be done using the DNA barcoding method on sequence target cytochrome C oxidase subunit 1 (CO1). CO1 is found in the mitochondria of eukaryotic cells (Wei *et al.*, 2023). According to (Roesma *et al.*, 2022), CO1 genes have short nucleotide bases that are widely conserved and undergo slight variation, deletion, insertion, or substitution (Mahrus *et al.*, 2022), thus are stable as a marker on species-level phylogeny analysis (Astuti *et al.*, 2022). CO1 markers effectively identified the diversity of *Osteochilus* spp. in Riau (Asiah *et al.*, 2020), *Oryzias sarasinorum* from Lake Lindu, Central Sulawesi (Zainal *et al.*, 2022), and *Dermogenys* spp. in Southeast Asia (Farhana *et al.*, 2018). Species validation is required for scientific name data as one of the conditions for fish export.

The development of the potential of genus *Nomorhamphus* as an ornamental fish commodity through the domestication stage faces various challenges of fish physiological responses (Teletchea, 2021), because changes in natural and artificial environmental factors significantly affect the fish regulatory system (Tao *et al.*, 2013). The process of domestication has five stages; stage one is that fish can acclimatize in the aquaculture environment, stage two is that part of the fish cycle develops in the aquaculture environment, Stage three is the entire fish cycle developing in the aquaculture environment but there is still input from nature, stage four is the entire life cycle of fish developing in the aquaculture environment without any input from nature, and stage five is the breeding program (Cucherousset and Olden, 2020; Teletchea, 2021). In principle, the success of domestication is that wild fish can carry out life cycles in aquaculture environments (Teletchea, 2019).

Somatic growth and gonad maturation are part of fish life cycle that are influenced by environmental signals (Shin *et al.*, 2014). Light is an environmental factor that possesses different characteristics such as intensity, photoperiod, and wavelength (Akhtar *et al.*, 2022). Depending on the depth, the long wavelength of light can penetrate water because water molecules absorb short wavelengths faster than long wavelengths (Carleton *et al.*, 2020; Frau *et al.*, 2022). Light wavelengths can affect the physiological systems of fish (Zou *et al.*, 2022). Fish have two light photoreceptors: the retina and the pineal organ. In endocrinology, the fish retina receives light with two photoreceptors: rod and cone cells. In rod cells, there are visual opsins, namely rhodopsin (RH1), while in cone cells, there are visual opsins that are sensitive to specific wavelengths such as SWS1 (blue / UV), SWS2 (blue), RH2 (green), and LWS (red) (Septriani *et al.*, 2021). Furthermore, ganglion cells transduct light signals from the retina to become nerve signals, which are sent to the preoptic area (POA), suprachiasmatic nucleus (SCN), and then forward signals to the pituitary, which affects endocrinology growth, reproduction, pigmentation, stress, and immunity (Falcon *et al.*, 2020). The pineal organ receives light depending on the circadian system through the dark-light photoperiod, which will produce nerve messages, and melatonin production, which further affects the endocrine organs (Falcon *et al.*, 2020). The role of both light photoreceptors greatly influences the endocrinological response in fish. Adaptation response of genus *Nomorhamphus* fish reared at different wavelengths of light needs to be investigated to support successful domestication.

The sensitivity of the retina to wavelengths will be different in each species. Light-emitting diodes (LEDs) are energy-efficient and long-service lamps that emit specific wavelengths that can specifically affect the manipulation of the light spectrum on fish (Shin *et al.*, 2013, 2014). Exposure to wavelengths of red light can accelerate the development of *Chrysiptera cyanea* ovaries (Bapary *et al.*, 2011), and improved the color quality of *Chromobotia macracanthus* (Virgiawan *et al.*, 2020). Similarly, exposure to green wavelength can accelerate the maturation of *Chrysiptera parasema* (Shin *et al.*, 2013), maturation oocyte of *Carassius auratus* (Choi and Choi, 2018; Shin *et al.*, 2014), and boost the immune system of golden mahseer fish (Akhtar *et al.*, 2022). However, the goal of every prior study on genus *Nomorhamphus* has been to identify new species using morphometric identification and the use of the mitochondrial DNA sequence cytochrome-b (Cyt-b). A more comprehensive analysis of the sequences of DNA is necessary for verifying the scientific nomenclature. According to reports, very few genus *Nomorhamphus* identification based on DNA CO1 sequence. Remain a major challenge to domesticate genus *Nomorhamphus,* no studies have been made to domesticate genus *Nomorhamphus* through environmental engineering involving exposure to different wavelengths of light, which is a strategy to accelerate the domestication process.

This study aims to analyze the genetic relationship of genus *Nomorhamphu*s endemic Lindu Lake, Central Sulawesi by DNA Barcoding CO1 method and evaluate the application of wavelengths of white, green, and red light to the growth response, survival, reproduction, and color quality of fish.

2. Materials and Methods

2.1 Material

Genus *Nomorhamphus* samples were collected from three locations of the inlet rivers of Lindu Lake, Central Sulawesi (Figure 1), namely Temper River (STM) in lat: -1,368406; long: 120,037684, Pekalotia River (SPK) in lat: -1,373895; long: 120,027329, and Palolo River (SPL) in lat:-1,204219; long: 120,163079. Fish samples were collected in January 2023. The fish caught were an average size of 2-2.5 cm. The number of fish that could be found in the STM was 12 fish, in the SPL was 18 fish, and in the SPK was 54 fish. Fish were packaged in a closed system using plastic polyethylene (12 x 25 cm) containing 4-5 fish size 2-2.5 cm per plastic. Plastic was placed in a styrofoam box and sent to Bogor via airplane cargo. Fish were acclimatized for 15 minutes, then adapted to an aquarium (30 x 25 x 25 cm) containing four fish size 2-2.5 cm per aquarium with a temperature of 25°C. Fish were satiated for one day, then given *Chironomus* feed on the second day. Fish were reared under exposure to light wavelengths for 60 days from January-March 2023.

2.1.1 Ethical approval

This research was performed in compliance with the animal ethics regulations in approval number 255-2024 IPB, based on approval by the animal code of ethics, IPB research and innovation directorate.

2.2 Method

2.2.1 CO1 target species validation

Molecular testing used fish preserved with 99% ethanol. The research was conducted at the Laboratory of Reproduction and Genetics of Aquatic Organisms (dry), Faculty of Fisheries and Marine Sciences (FPIK), Bogor Agricultural Institute (IPB). DNA was extracted from caudal fin of fish 10-20 mg using a DNA extraction and purification kit (Qiagen, Germany). DNA amplification was performed with 20 µl containing 2 µl of DNA, 10 µl of MyTaq Red Mix (Bioline, US), 0,8 µl of Fish-F2 primer, 0,8 µl of Fish-R2 primer, and 6,4 µl of NFW on a 680 base pair fragment target. Amplifications were conducted using a Peqlab Thermocycler, starting with an initial denaturation at 95 °C, followed by 40 cycles under the following conditions: 30 seconds at 95°C, 30 seconds at 57°C, and 30 seconds at 72°C. Visualization of PCR results was carried out with an agarose gel concentration of 1%, then sequencing results were sent to a DNA sequencing services by Genetika Science company, Indonesian. Sequence alignment was performed using MEGA X (Molecular Evolutionary Genetic Analysis) software (Kumar *et al.*, 2018) with sequence matching on data in GenBank NCBI to construct species phylogenetic trees.

2.2.2 Exposure of different light wavelength

The rearing under exposure of different wavelength was conducted at Laboratory of Reproduction and Genetics of Aquatic Organisms (wet), Faculty of Fisheries and Marine Sciences (FPIK), Bogor Agricultural Institute (IPB) in January-March 2023. Fish were kept using a 30 x 25 x 25 cm aquarium with a capacity of four fish, equipped with foam filters. The application of light photoperiod was 12 lights: 12 dark. The LED lights used are 10 watt RGB Bluetooth smart bulbs (Avaro, China). The lights are installed 10 cm above the water surface in each aquarium, with an intensity setting of 550 lux above the water surface. Each aquarium is coated with black plastic (Novita *et al.*, 2019). Water temperature was 25°C with exposure to different wavelengths of light for 60 days. During the experiment, fish were fed *Chironomus* feed daily at satiation with a frequency of twice a day, and water change (50%) twice a week.

2.2.3 Experimental design

This research used a completely randomized design with three different wavelengths carried out using white (400 nm), green (525 nm), and red (625 nm) LED lights. The experiment consisted of three aquarium replications for each light exposure, containing four fish (2 - 2.5 cm) for each aquarium.

2.3 Data Collection

2.3.1 Growth performance

The weight of the fish was calculated by analytical balance (0.0001 g), and the length of the fish measured using millimeter blocks (0.1 mm). The growth performance of each fish was calculated on day-0 and day-60 of rearing. Specific weight growth rate (SWGR, % day^{-1}) was calculated by referring to Weatherley and Gill (1987), with the formula:

$$SWGR\ \%\ day^{-1} = [(LnWt - LnWo) / t \times 100]$$

where: SWGR: Specific weight growth rate (%/day); Wt: final body weight (g); Wo: initial body weight (g); t: Duration of rearing (60 days).

Growth Length (GL) was calculated by referring to the NRC (1983), with the formula:

$$GL = Lt - Lo$$

where: GL: Growth length (cm); Lt: final length (cm); Lo: initial length (cm).

2.3.2 Survival Rate

Survival rate (SR) was calculated on the final day of maintenance (D-60), referring to Effendie (1997), with the formula:

$$SR = [(No - Nt) / No] \times 100$$

where: Nt: final number of fish; No: initial number of fish.

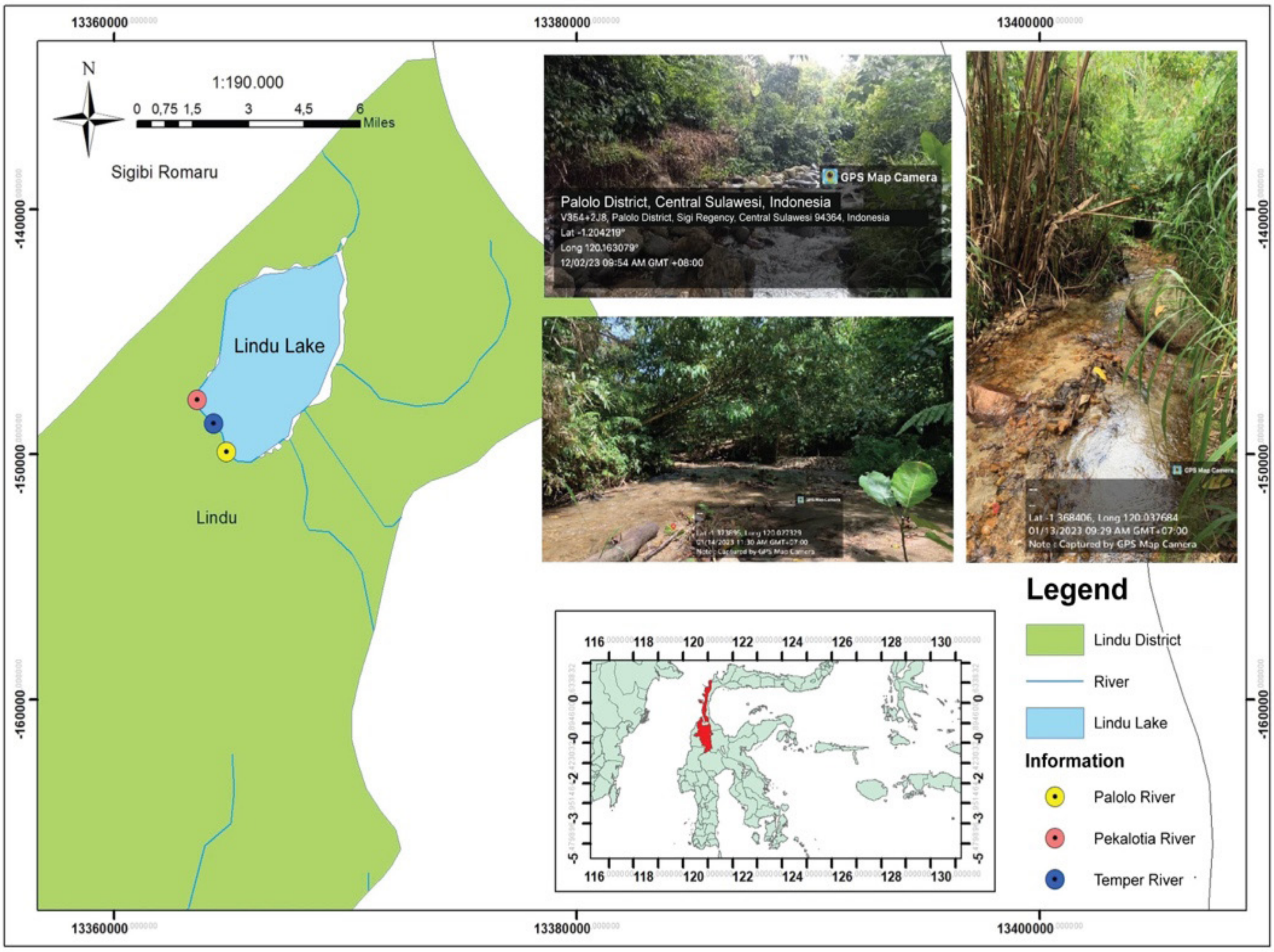

Figure 1. Map of the location *Nomorhamphus* collection in three inlet rivers, Lake Lindu, Central Sulawesi

2.3.3 Reproduction performance

Fish were dissected for gonadal organ harvesting at final day (D-60) of rearing as many as three males and three females at each treatment. The gonadosomatic index (GSI) was calculated on the final day (D-60). The initial size of the fish ranged from 2-2.5 cm. At this size, the fish gonads had not yet differentiated. Histology and gonadosomatic index were not conducted prior to the treatment. GSI was calculated by referring to Effendie (1997) formula:

GSI (%) = (gonad weight/body weight) x 100

Histology was performed using the H&E staining method. Microscopic gonadal development in histology was evaluated, referring to the viviparous group *Dermogenys pusilla* (Senarat *et al.*, 2019).

2.3.4 Color quality of fish

The fish were photographed solitary against a black background. The photos were analyzed using the ImageJ 1.53 (University of Wisconsin, US) application. The color of the pelvic fins was marked with a rectangular area region of interest (ROI) of about 300-430 pixels, and then the intensity measured on the measuring tool.

2.3.5 Body glucose analysis

Three fish before and after each treatment were taken for body glucose testing. Body serum preparation was done by turning off first by being pierced in the brain, then crushed, and homogenized in phosphate buffer saline (PBS) solution with a ratio of 1: 2. Blood glucose was calculated by referring to

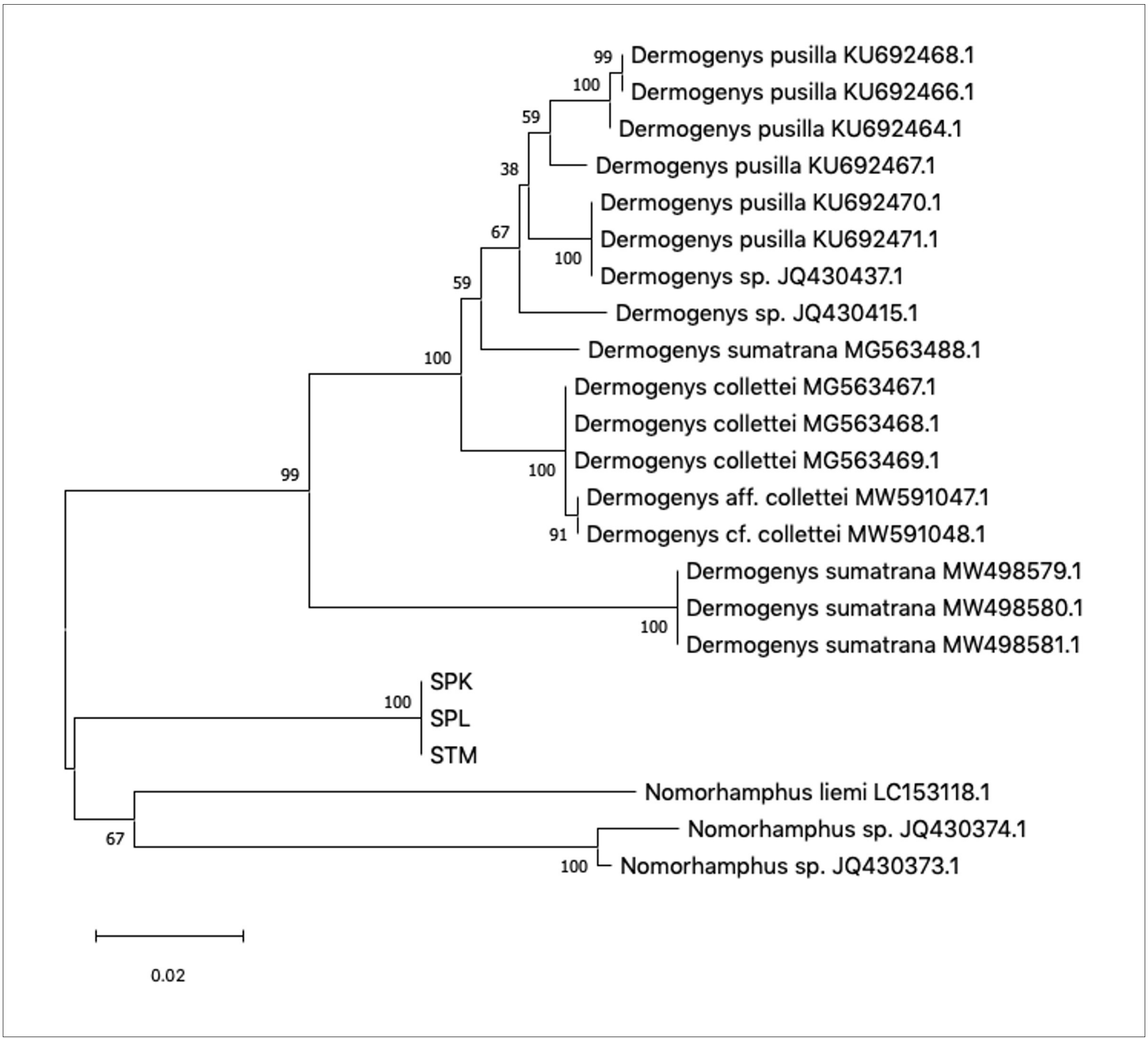

Figure 2. Phylogenetic tree of *Nomorhamphus* from three inlet rivers of Lindu Lake with CO1 gene markers, neighbor-joining (NJ) method and bootstrap 1000x. Description: SPK: Pekalotia River, SPL: Palolo River, STM: Temper River. Outgroup *Nomorhamphus* (GenBank) and outgroup *Dermogenys* (Genbank).

the method by Wedemeyer and Yasutake (1977), blood glucose content was measured with a spectrophotometer at a wavelength of 635 nm, and calculated by the following formula:

Glucose (mg/100 µm) = absorbance sample x concentration sample)/standard absorbance.

2.4 Analysis Data

DNA sequencing and gonadal histology results were analyzed descriptively. In contrast, the growth performance, reproduction performance, color quality, SR, and glucose responses were analyzed by ANOVA and tested further by Duncan with a 95% confidence interval using SPSS software version 26.

3. Results and Discussion

3.1 Genetic Relationship of Genus Nomorhamphus

Identification of genus *Nomorhamphus* with CO1 sequence targets gave rise to DNA bands at a size of 680 bp and based on phylogenetic tree construction using *Nomorhamphus* outgroup (GenBank) and the *Dermogenys* outgroup (Genbank) confirmed the SPK, SPL, and STM fish samples from the Lindu Lake including the *Nomorhamphus* group species (Figure 2). The phylogenetic tree showed that there were two clusters, namely the *Dermogenys* cluster and the *Nomorhamphus* cluster. STM, SPK, and SPL were in the same cluster as *Nomorhamphus* and had a closer relationship with the cluster of *Nomorhamphus* based on phylogenetic tree.

Nomorhamphus fish from three inlet rivers of Lake Lindu, Central Sulawesi were identified at a size of 680 bp. FISH-F2 and FISH-R2 primers also succeeded in raising bands at 678-705 bp of *Ompok hypophthalmus* (Kasayev and Arisuryanti, 2022), 700 bp of *Oryzias sarasinorum* fish (Zainal *et al*., 2022) and 651 bp of Dermogenys spp. fish (Farhana *et al*., 2018). The results of DNA sequence alignment using BLAST NCBI showed that three fish samples from the inlet rivers Lindu Lake (STM, SPK, SPL) had a bootstrap value of 100% in the Nomorhamphus group (JQ430374) and were different from the species *Dermogenys pusilla* (KU692468.1) by 10.34% based on percent identity from NCBI genebank. CO1 is effectively used as a genetic marker because it is easy to amplify, has a high mutation rate, and exhibits intra- and interspecific polymorphisms (Nuryanto *et al*., 2019; Wei *et al*., 2023).

3.2 Growth Response

The growth response in the group of fish exposed to green light wavelength (525 nm) showed

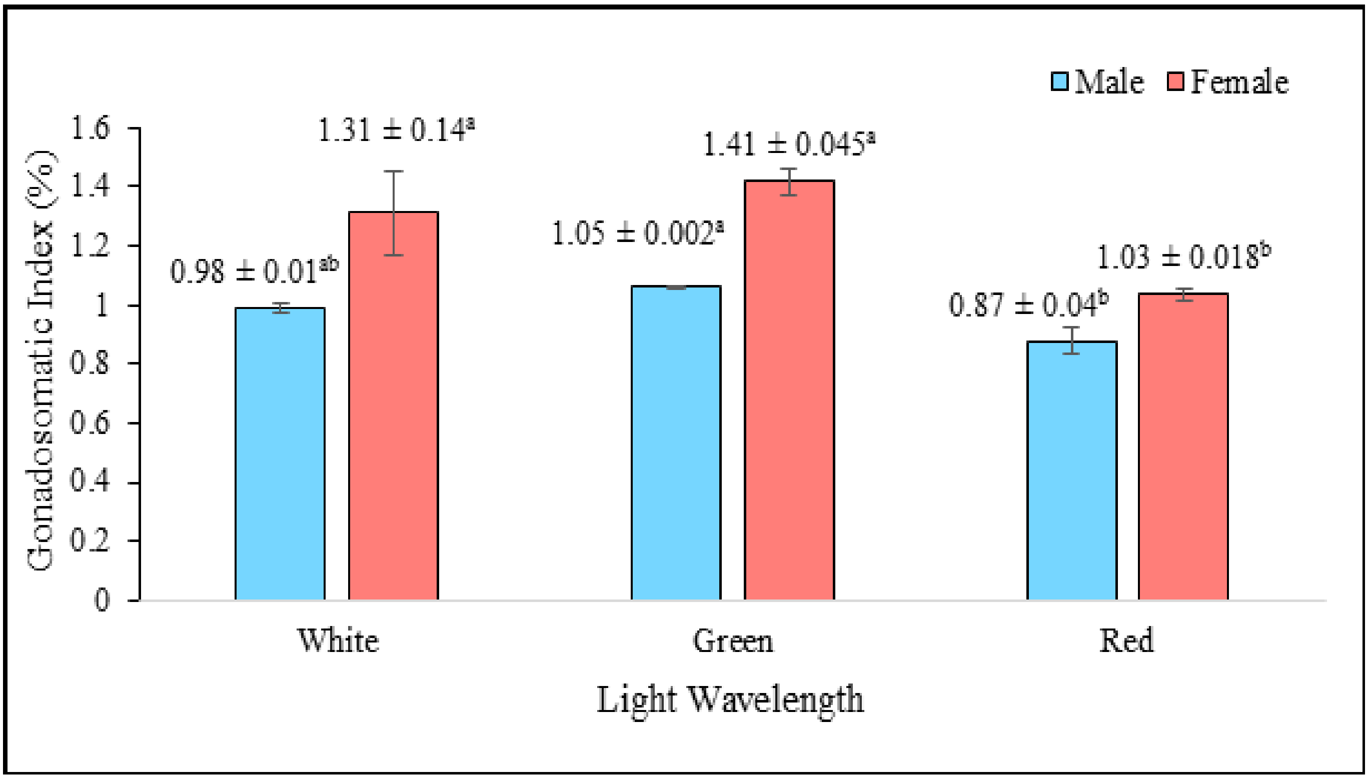

Figure 3. Gonadosomatic index of male and female *Nomorhamphus* day 60 after exposure of different light wavelengths. Remarks: the value listed is the average ± standard deviation, different superscript letters indicate a significant difference ($p<0.05$).

the highest growth length (GL) up to 2 cm ($p<0.05$). The highest GL in male is at green wavelength (1.93 ± 0.03 cm), followed by red wavelength (1.81 ± 0.03 cm), and white wavelength (1.73 ± 0.12 cm). The highest GL in female is at green wavelength (2.11 ± 0.03 cm), followed by red wavelength (2.01 ± 0.03 cm), and white wavelength (1.97 ± 0.03 cm). There was no significant difference in specific weight gain rate (SWGR) among the different wavelength exposure, ranging from 2-3%/day ($p>0.05$) (Table 1). The highest SWGR in male is at green wavelength (2.65 ± 0.15 cm), followed by white wavelength (2.53 ± 0.78 cm), and red wavelength (2.28 ± 0.17 cm). The highest SWGR in female is at green wavelength (2.95 ± 0.33 cm), followed by white wavelength (2.76 ± 0.68 cm), and red wavelength (2.61 ± 0.18 cm).

The life cycle of fish is also called the reproductive life cycle, where the life cycle of genus *Nomorhamphus* consists of eggs, larvae, juveniles, and adult fish (Herjayanto *et al.*, 2023). In other words, it includes brood rearing, gonad maturation, spawning, live-bearing, larval rearing, juvenile, prospective brood, until the brood stadia return. This study has succeeded in accelerating the process of growth and reproduction from juvenile stadia to prospective brood by manipulating the light wavelength.

Sensitivity to exposure of specific wavelengths will affect the photoreceptors of the retinal and pineal organs to which the fish respond physiologically (Villamizar *et al.*, 2011). Exposure to green light results in faster length growth in both male and female fish than in other light wavelength. Faster growth performance on green light exposure was also found in *Gadus morhua* fish, *Scopthalmus maximus* (Sierra-Flores *et al.*, 2016), *Chrysiptera parasema* (Shin *et al.*, 2013). Some fish species raised with red wavelength can increase feed consumption but not impact growth because more energy is used for swimming activities (Sánchez-Vázquez *et al.*, 2019). Growth is influenced by growth hormone (GH), which is released by the anterior pituitary and received by growth hormone receptors (GH-R) and stimulates the synthesis of insulin-like growth factor 1 (IGF-1) in the liver. Research by Zou *et al.* (2022) showed that GH and IGF-1 expression in *Paralichthys olivaceus* fish reared at green light exposure was higher than rearing under white light. Mechanisms involving GH and IGF-1 in fish *Paralichthys olivaceus* are thought to also occur in the *Nomorhamphus* sp. Different wavelength lighting on the maintenance of fish does not affect survival. It is also found in other species, including *Chromobotia macracanthus* (Aras *et al.*, 2015), *Amphiprion percula* (Novita *et al.*, 2019), and *Paralichthys olivaceus* (Zou *et al.*, 2022).

3.3 Survival Rate

The fish survival rate (SR) ranges from 83-100% ($p>0.05$) (Table 1). The highest SR is at green wavelength (100 ± 0 cm), followed by red wavelength (92 ±14.4 cm), and white wavelength (83 ±14.4 cm). Different light wavelengths exposed during the maintenance of *Nomorhamphus* sp. do not affect survival rates. This has also been found in other species, including *Chromobotia macracanthus* (Aras *et al.*, 2015), *Amphiprion percula* (Novita *et al.*, 2019), and *Paralichthys olivaceus* (Zou *et al.*, 2022).

3.3.1 Reproduction response

Measurements of the gonadosomatic index (GSI) of male and female fish (Figure 3) showed that exposure to wavelengths of red light (625 nm) inhibited the maturation of the gonads of male and female fish ($p<0.05$). The highest GSI in male is at green wavelength (1.05 ± 0.002%), followed by white wavelength (0.98 ± 0.01%), and red wavelength (0.87 ± 0.04%). The highest GSI in female is at green wavelength (1.41 ± 0.045%), followed by white wavelength (1.31 ± 0.14%), and red wavelength (1.03 ± 0.018%). The histological observations of male and female gonads (Figure 4)

Table 1. Growth response and survival rate of *Nomorhamphus* sp. fish to exposure to different wavelength of light for 60 days

Wavelength	SWGR (%/day)[1]		GL (cm)[1]		SR (%)[1]
	Male	Female	Male	Female	
White	2.53 ± 0.78[a]	2.76 ± 0.68[a]	1.73 ± 0.12[b]	1.97 ± 0.03[b]	83 ±14.4[a]
Green	2.65 ± 0.15[a]	2.95 ± 0.33[a]	1.93 ± 0.03[a]	2.11 ± 0.03[a]	100 ± 0[a]
Red	2.28 ± 0.17[a]	2.61 ± 0.18[a]	1.81 ± 0.03[ab]	2.01 ± 0.03[b]	92 ±14.4[a]

[1]Specific weight growth rate (SWGR), Growth Length (GL), Survival rate (SR)
[2]Different superscript letters in the same column indicate a significant difference ($p<0.05$), the value listed is the average ± standard deviation

day 60 after exposure to green light wavelengths (525 nm) showed the fastest development namely the gonad reached tertiary yolk step (TYS), and the spermatozoa cells had differentiated to form the head and tail of the sperm. Red light wavelength showed female gonad reached secondary yolk stage (SYS) and male gonad reached secondary spermatocytes (SS). White light wavelength showed the initial development, namely the female gonad reached peri nucleus stage (PNS) dan male gonad reached secondary spermatocytes (SS). The differences of gonad stages development between all treatment showed the green light wavelength more adaptive for *Nomorhamphus* sp.

Green wavelength lighting (525 nm) for 60 days accelerated the maturation of male gamete cells reaching the spermatozoa (Sz) stage with an oval-shaped sperm head and an elongated sperm tail (Senarat *et al*., 2019), similarly in female fish spurring oocyte maturation to reach the tertiary yolk step (TYS) stage. This is in line with Shin *et al*. (2013) studying

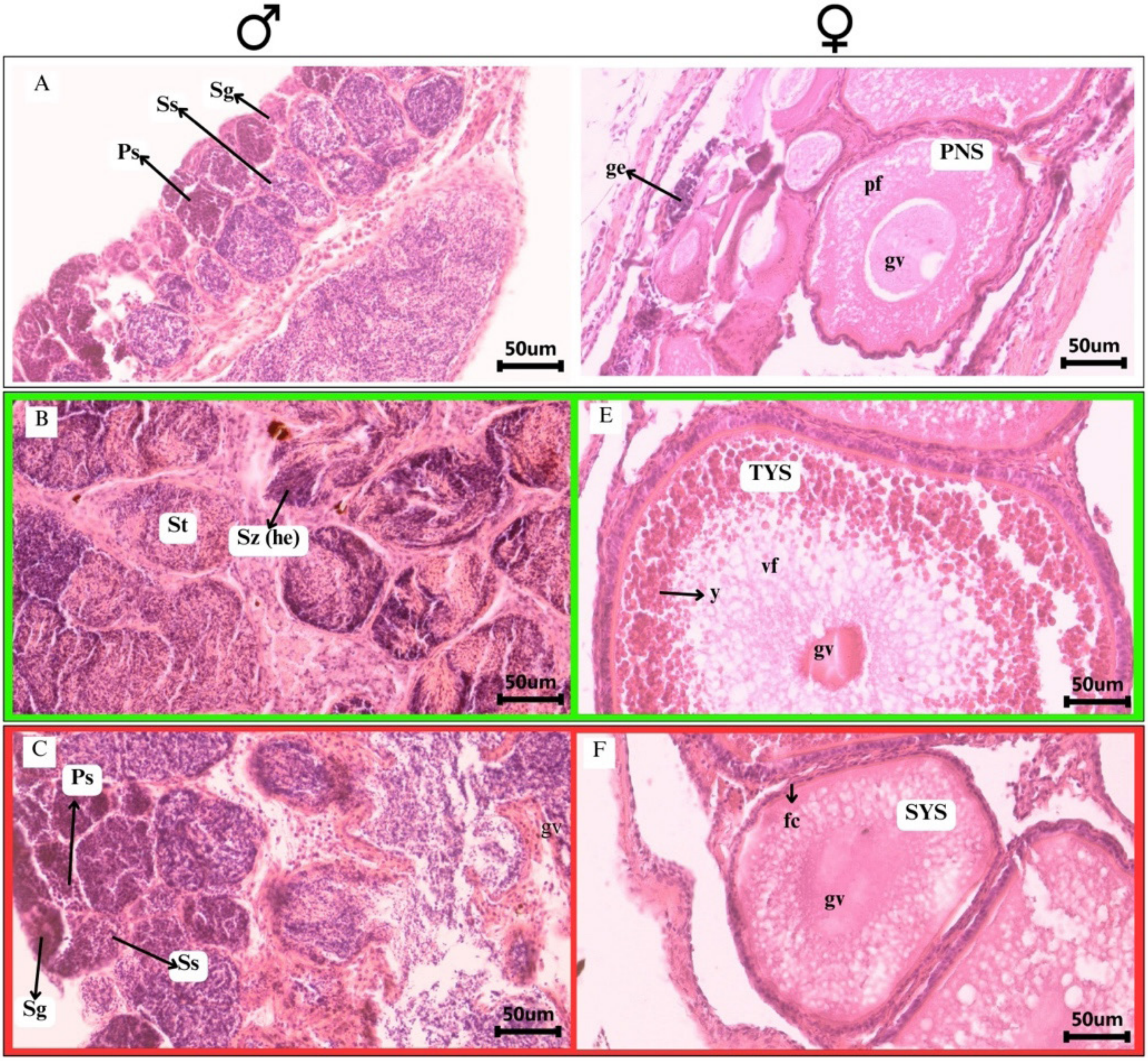

Figure 4. Gonad histology of male (left) and female (right) fish *Nomorhamphus* day-60 after exposure of different light wavelengths. Magnification 40X. Description: A (White, 400 nm), B (Green, 525 nm), C (Red, 625 nm), Spermatogonia (Sg), Primary Spermatocytes (Sp), Secondary Spermatocytes (Ss), Spermatids (St), Spermatozoa (Sz), Head (He). Ovarian histology: Peri-nucleolus stage (PNS), Secondary yolk stage (SYS), Tertiary yolk step (TYS), germinal epithelium (ge), previtellogenic follicles (pf), germinal vesicle (gv), vitellogenesis (vf), yolk globules (y), Follicular cells (fc).

Chrysiptera parasema fish that showed maturation at the TYS stage in green light treatment compared to red light, as well as in carp (Shin *et al*., 2014). Light wavelengths can stimulate melatonin from the pineal organs and retina to induce maturation and mating in fish (Choi *et al*., 2023). Plasma FSH and LH *Carassius auratus* were significantly higher in green than red light (Yun *et al*., 2015). Lh β subunit gene expression in the pituitary, melatonin receptor mt1 and mt2 genes in the hypothalamus significantly appeared in Paralichthys olivaceus maintained at green light, and lhβ stimulated testicular development (Zou *et al*., 2022). The retina transmits light into nervecells, which are sent via the retinohypothalamic tract (RHT) to kisspeptin neurons and then signal to secrete gonadotropin-releasing hormone (GnRH) (Mondal *et al*., 2019; Oakley *et al*., 2009) in the anterior pituitary, which signals gonadotropic cells to release gonadotropin hormone (GTH), then GTH-I will release follicle-stimulating hormone (FSH) for gametogenesis and early development of gonads, while GTH-II releases luteinizing hormone (LH) for the final stage of gonadal maturation. FSH and LH will induce sex hormones in the gonads, namely estradiol and testosterone (Carleton *et al*., 2020; Bairwa *et al*., 2013).

3.3.2 Body glucose level

Body glucose value before exposure was higher than after exposure (Figure 5), and white light (400 nm) showed lower values ($p<0.05$). The highest body glucose level is at before treatment (19.32 ± 0.16 mg/100 ml), followed by red wavelength (18.30 ±0.21 mg/100 ml), green wavelength (18.14 ±1.27 mg/100

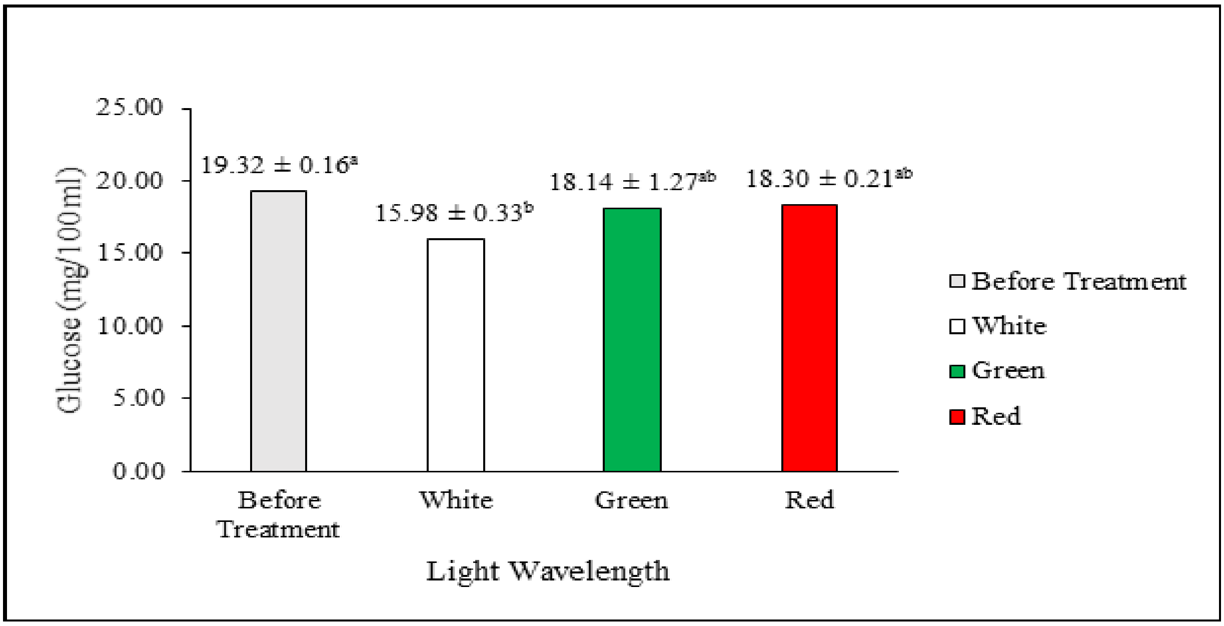

Figure 5. Body glucose values of *Nomorhamphus* at different wavelength exposures over 60 days. Remarks: the value listed is the average ± standard deviation, different superscript letters indicate a significant difference ($p<0.05$).

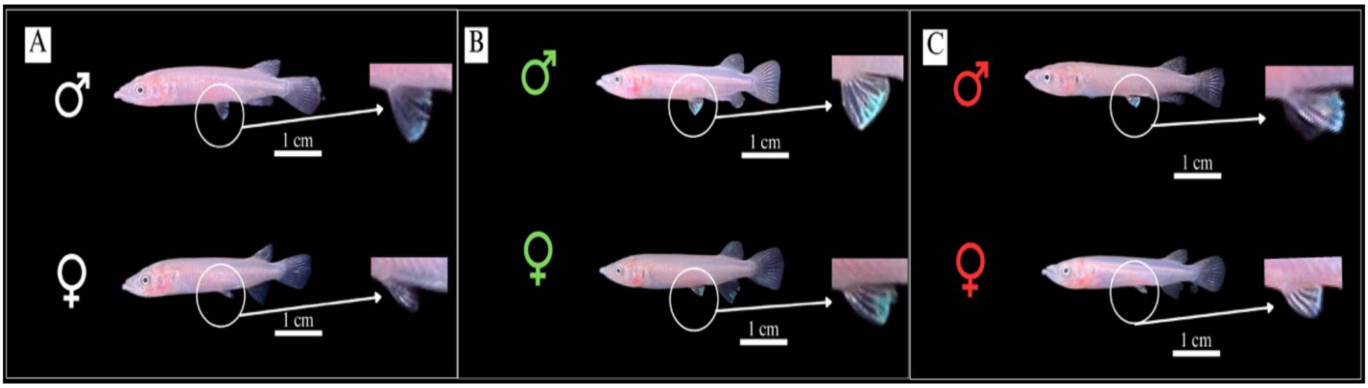

Figure 6. Color performance of male and female pelvic fins *Nomorhamphus* at different wavelength exposures over 60 days. Description: A (White, 400 nm), B (Green, 525 nm), C (Red, 625 nm)

ml), and the lowest is at white wavelength (15.98 ±0.33 mg/100 ml). Based on measurements of body glucose levels related to the amount of carbohydrates that induce metabolic stress in fish (Choi *et al.*, 2023), wavelength lighting exposure does not cause stress in *Nomorhamphus* sp. fish. Cortisol concentrations in green light exposure were significantly higher than white light but did not decrease growth and reproductive performance in *Paralichthys olivaceus* fish (Zou *et al.*, 2022).

Table 2. Color intensity on pelvic fins of male and female *Nomorhamphus* sp. fish to exposure to different wavelengths of light for 60 days

Wave-length	Color intensity of pelvic fins	
	Male	Female
White	127.9 ± 33.8[a]	109.9 ± 7.2[a]
Green	164 ± 33.6[a]	114.7 ± 17.7[a]
Red	139.9 ± 8.3[a]	125 ± 23.9[a]

3.3.2 Color quality of fish

The results of color intensity measurements on the pelvic fins (Table 2) showed no significant difference between male and female fish at different light wavelength exposure for 60 days ($p > 0.05$) and color performance of pelvic fins (Figure 6). The highest color intensity of pelvic fins in male is at green wavelength (1.64 ± 33.6), followed by red wavelength (139.9 ± 8.3), and white wavelength (127.9 ± 33.8). The highest color intensity of pelvic fins in female is at red wavelength (125 ± 23.9), followed by green wavelength (114.7 ± 17.7), and white wavelength (109.9 ± 7.2). The maintenance of fish with exposure to different wavelengths of light is expected to increase the potential of *Nomorhamphus* sp. as an ornamental fish commodity. In this case, applying different light colors does not show a noticeable difference in color intensity on the pelvic fins. Several studies on the application of red light were able to improve the best color quality in *Chromobotia macracanthus* fish (Aras *et al.*, 2015), *Osphronemus gouramy* (Gunawan *et al.*, 2022), and *Cromileptes altivelis* (Nirmala *et al.*, 2022). In endocrinology, color pigmentation in fish is influenced by melanin-concentrating hormone (MCH) and -melanophore-stimulating hormone (-MSH). The hypothalamus synthesizes MCH, which can turn skin pigmentation pale by combining pigments. Proopiomelanocortin (POMC) in the pituitary is a precursor to -MSH, where -MSH can darken skin pigmentation by causing pigment. Fish retinal photoreceptors receive light from the environment and mediate light for changes in skin pigmentation, which can affect MCH gene expression in the hypothalamus and POMC expression in the pituitary. Photoreceptors of the pineal organ also affect POMC expression. Light wavelengths influence skin pigmentation regulation by expressing MCH, POMC, and -MSH genes in goldfish (Kasagi *et al.*, 2020).

The species status of genus *Nomorhamphus* from three rivers inlet Lake Lindu is the same species, namely *Nomorhamphus* sp. Using green light can be an alternative in accelerating the domestication of *Nomorhamphus* sp. fish.

4. Conclusion

The genetic relationship status of genus *Nomorhamphus* from three inlet rivers Pekalotia, Temper, and Palolo, Lindu Lake is one species, namely *Nomorhamphus* sp. Exposure to the green light spectrum (525 nm) can promote the length growth and maturation of fish gonads and does not affect the color quality and survival rate of fish, as well as accelerate the domestication stage of fish *Nomorhamphus* sp. which reaches stage 2 (part of the life cycle is already in the aquaculture environment). The application of green light wavelengths can be used for further maintenance from the candidate broodstock phase to the spawning phase of *Nomorhamphus* sp. fish to optimize reproductive performance and enhance the quality of the fish's color.

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Authors' Contributions

The contributions of each author are follows, Rh; created idea, conducted the research, collected and analyzed data, written the entire manuscript. Dts; created idea, research grant funding, created methodology, corrected draft. Aos; created idea, created methodology, corrected draft. A: data corrected, proofreading and corrected drafts. All authors have discussed and contributed to the final manuscript.

Conflict of Interest

All authors declare that they have no competing interests upon the publication of this research.

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