Comparative analysis of biological differences between seawater- and freshwater-cultured *Lates calcarifer* (Bloch, 1790)

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Abstract. The ability to adapt to their environments has allowed barramundi, *Lates calcarifer* (Bloch, 1790) to survive in both fresh and seawater. This study aimed to evaluate the growth, osmoregulatory function, blood parameters, and meat composition of *L. calcarifer* specimens cultivated in different environmental conditions. This study utilized 4,000 test fish with a total length of 12.50±0.42 and 11.81±0.42 cm. Each 2,000 fish received treatment A (seawater: 34 ppt) and treatment B (freshwater: 0 ppt) in two separate ponds (150 m^3). The fish were cultivated for four months using commercial feeding of 1-2 percent biomass once daily. Monthly examinations were conducted of fish growth, osmoregulation, blood profiles (including hemoglobin, erythrocytes, leukocytes, and hematocrit), and the proximate composition of the dried meat consumed. The data collected was subjected to descriptive analysis. The results of the study, *L. calcarifer* cultivated in a seawater pond (A) had higher daily growth rates (length and weight: 2.37% and 4.64%), blood profiles (hematocrit, hemoglobin, total leukocytes, and erythrocytes: 36.44±3.85%, 11.96 g dL^{-1}, 17.32x10^3 cells mm^{-3}, and 2.84x10^6 cells mm^{-3}), and osmoregulation value (653.2 mOsm L^{-1} H$_2$O) than *L. calcarifer* cultured in a freshwater pond (B). However, the protein and fat content of dried meat from seawater *L. calcarifer* (82.14 and 9.11%) was slightly lower than that of freshwater *L. calcarifer* (84.62 and 10.55%). The findings of this study indicate that barramundi cultivated in seawater ponds perform better biologically than *L. calcarifer* cultivated in freshwater ponds. Gradual adaptation is required when switching from high to low salinity.

Keywords: barramundi, growth performance, hematology, osmoregulation.

Introduction. Barramundi, *Lates calcarifer* (Bloch, 1790) has a higher economic value than other fish species. Approximately 70% of the fish consumed in Australia is imported from Thailand and Vietnam due to the great demand for *L. calcarifer* (Allegrucci et al 1994). The Backyard Hatchery System at the Gondol area in north Bali, Indonesia, has received widespread recognition for its success in producing fingerlings. As of present, the majority of grouper species have extremely low survival rates in hatcheries. The success of *L. calcarifer* aquaculture will be supported by the availability of high-quality fry in adequate quantities.

L. calcarifer, a euryhaline teleost, exhibits remarkable osmoregulatory plasticity, allowing its cultivation in diverse salinity environments ranging from freshwater to full-strength seawater (Rasmussen 1991). *L. calcarifer* are susceptible to viral and bacterial infections since they live in open waters (Islam et al 2024). Therefore, maintaining and reducing the salinity level is one way to prevent infections (Allegrucci et al 1994; Fotedar 2016). Understanding these complex adaptive reactions is critical for optimizing culture conditions, increasing growth performance, and assuring the long-term viability of *L. calcarifer* farming operations (Johari et al 2024). Salinity differences can have a substantial influence on fish energy allocation, hormone synthesis, and physiological performance, with certain species prospering in low salinity and others being repressed (Liu et al 2023).

The equilibrium of water and ion concentrations in the fish body may be affected by the change from saltwater to freshwater (Edwards & Marshall 2012). Freshwater fish exhibit this difference due to ion loss and passive osmotic water intake. Given the significant differences in osmoregulatory systems between freshwater and saltwater, fish face significant challenges when salinity changes occur. Additionally, induced osmotic pressure can impact cellular processes and physiological balance (Edwards & Marshall 2012; Bonzi et al 2021). Therefore, two actions that can be taken to avoid disease are lowering salinity and keeping it lower. Certain biological markers have been identified as crucial instruments for tracking biologists' physiological responses to environmental change when comparing fish species that live in freshwater and saltwater or in different habitats (Leonard & McCormick 1999). This study aimed to evaluate several biological traits of *L. calcarifer* cultivated in different environmental conditions.

Material and Method

Test animal preparation. A total of 4,000 fish were used in the experiment. 2,000 fish each pond, with total lengths of 12.50±0.42 and 11.81±0.42 cm, were transferred to a 150 m³ fish pond. The salinity contrast was examined using seawater at 34 ppt (A) and 0 ppt (A). Commercial feeding with 2% biomass once daily was used to cultivate the fish for 4 months. At the end of the experiment, proximate meat, osmoregulation, and blood profiles. For laboratory analysis, every fish sample was shipped to the National Research and Innovation Agency (NRIA)-Gondol, Bali, Indonesia.

Growth and color pattern. Fish growth was observed monthly, and at the end of the study, fish growth was determined using the Effendi formula (Effendi 2002). Daily growth rate was calculated using the Octarina formula (Oktarina 2009). Color testing was carried out on each of the 5 fish using the Toca Color Finder method. The Toca Color Finder color was closest to the fish's body color.

Blood profile. Twenty fish were anesthetized with 50 ppm 2-phenoxy ethanol for each treatment. A 3 mL syringe filled with 0.1 mL heparin was used to draw blood samples in order to prevent blood clotting. Following the caudal transaction, 1 mL of blood was transferred to a 1.5 mL tube. The plasma and serum were separated by centrifugation at 5,000 rpm for 15 minutes to assess the hematocrit. A technique for measuring the hemoglobin concentration was described by Al-Abood & Al-Hassan (1988). Meanwhile, the Lestari et al (2017) approach was used to examine erythrocytes and leukocytes using a hemocytometer.

Osmoregulation. The K7400S semi-micro osmometer was used to determine the osmolality. Osmolality, which is the ratio of blood to medium osmotic concentrations, is used to compute the osmotic action rate. The cultivation media and measurements of fish blood's osmolarity up to 0.01 mL are subsequently transferred to the microtube.

Proximate meat. Proximate analysis is used to determine the amounts of protein, fat, and ash. As described in SNI 2354.2:2015, the moisture content is ascertained using the gravimetric method. To measure lipid content, Bligh & Dyer (1959) created extraction and gravimetry techniques. Protein levels can be evaluated using the Kjeldahl procedure.

Data analysis. The following parameters were measured: osmometer, blood content, body weight, total length, and proximate concentration, which was calculated using the formula (Bligh & Dyer 1959; Hassan et al 2022). Following a descriptive analysis, the data collected were shown as graphs, tables, and pictures.

Results and Discussion

Growth and survival. The results showed that the growth rate of daily length and daily weight of *L. calcarifer* in treatment A was higher (2.37%; 4.64%) compared to treatment B (2.11%; 4.41%). Treatment B had a lower survival rate (41.25%) than treatment A (88.27%). At the beginning of the experiment, the daily growth rate of fish in freshwater media had decreased since they remained in the adaptation process (Figure 1). These findings indicate that energy consumption seems to be affected by this novel osmoregulation strategy. Meanwhile, fish continue to be agitated and respond less to food throughout the first week. Both the concentration of divalent ions (Ca_2C and Mg_2C) and the total concentration of dissolved solids are associated with the influence of salinity on fish growth due to their effects on permeable membranes and osmoregulation. A lot of energy is required for osmoregulation, which is the process of balancing body fluids with ambient fluids.

The osmoregulation mechanism thus receives the energy used for growth, which leads to a limited development rate. Because more energy is used for further growth when salinity conditions are iso-osmotic, growth can perform at its most effective (Evans et al 2005; Kultz 2015).

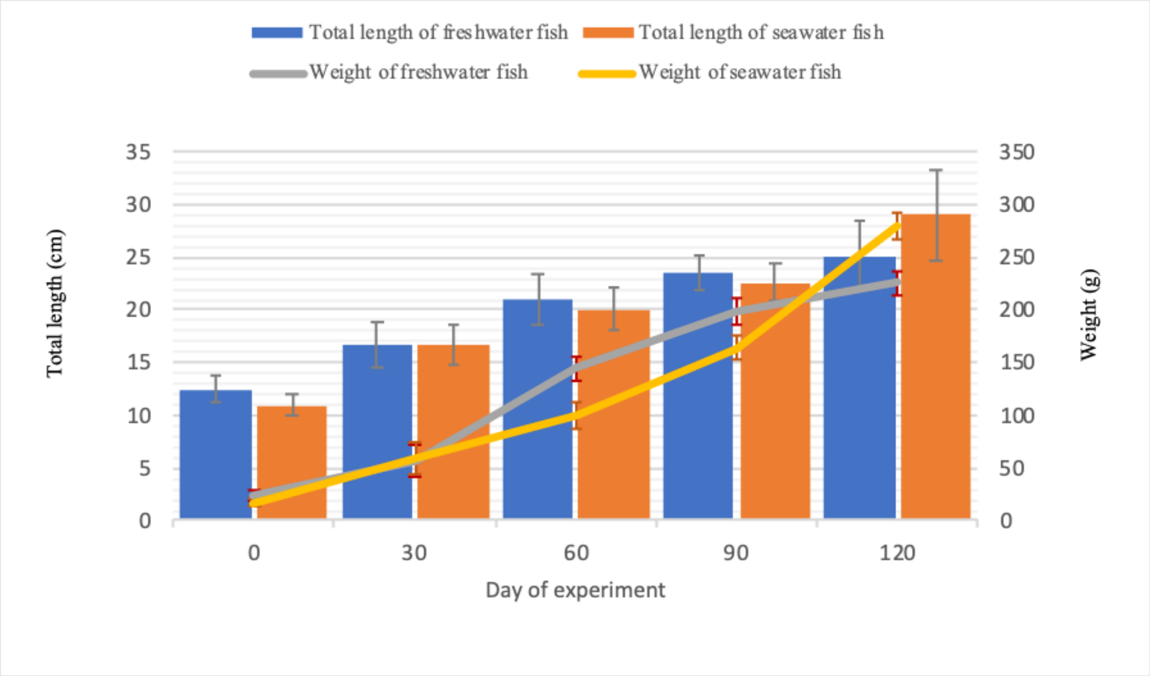

Figure 1. Total length and weight of *Lates calcarifer* rearing in freshwater (A) and seawater (B) during the experiment.

Asian seabass had the greatest growth and survival at 22 ppt salinity when compared to the other salinity treatments. Simillar trend were reported by Hassan et al (2022), a salinity of 20 to 36 ppt is advised for the commercial cultivation of Asian seabass in a closed aquaculture system. The growth of the euryhaline species was adversely influenced by changes in salinity. Fish grown in environments with high salinity may experience appetite suppression. Previous research revealed a correlation between decreasing food consumption and reduced growth under elevated salinity (Augley et al 2008; Partridge & Lymbery 2008).

Color pattern. The color of the *L. calcarifer* rearing in freshwater is generally silver-brown (TC-6002), and rearing in seawater is generally brown-black (TC-5216). The environment and the type of feed consumed both influence the color of the body (Nebel et al 2005). *L. calcarifer* cultured in freshwater (A) and seawater (B), and the morphological performance of body color is shown in Figure 2.

Figure 2. Morphological performance of *Lates calcarifer* rearing in freshwater (A) and seawater (B) at the end of the experiment.

Blood profile. Hematocrit, hemoglobin, total leukocytes, and erythrocytes were all higher in treatment A (36.44±3.85%, 11.96 g dL^{-1}, 17.32 x 10^3 cells mm^{-3}, and 3.69 x 10^6 cells mm^{-3}) than in treatment B (26.1±6.16%, 9.58 g dL^{-1}, 5.55 x 10^3 cells mm^{-3}, and 2.84 x 10^6 cells mm^{-3}). Table 1 shows the blood profile data for *L. calcarifer* reared in freshwater and saltwater.

Table 1

Blood profile of *Lates calcarifer* rearing in seawater and freshwater

Treatment	*Salinity(ppt)*	*Red blood cells (10^6 cells mm^{-3})*	*White blood cells (10^3 cells mm^{-3})*	*Hematokrit (%)*	*Hemoglobin (g dL^{-1})*
(A) seawater	34 ppt	3.69	17.32	36.4	11.96
(B) freshwater	0 pt	2.84	5.55	26.1	9.58

Haematocrit was higher in 34 ppt (36.4%) and lower in salinity 0 (26.1%). Some marine fish species have a haematocrit of approximately 52.05% (Jawad et al 2004). It has been recognized that the correlation between haematocrit and haematological indicators is a useful tool for fish health monitoring. Through their effects on hemoglobin and osmoregulation, many authors have demonstrated how varying salinity directly affects various blood parameters, including hematocrit (Fashihuddin & Kumari 1990; Jawad et al 2004; Gebretsadkan et al 2015). According to the study's findings, there is a correlation between blood haematocrit and body length in *Cyprinus carpio*, with the longer the fish, the greater the hematocrit (Verdegem et al 2008). The hematocrit also rose with fish length. *Clarius batrachus* showed a comparable result (Barton et al 1980).

Osmoregulasi. *L. calcarifer* in treatment A had a greater average blood osmolarity and osmolarity media value (653.2 mOsm L^{-1} H_2O) than *L. calcarifer* in treatment B (324.4 mOsm L^{-1} H_2O). At 0 ppt salinity, the lowest osmolarity rate was 40.33 mOsm L^{-1}, and the greatest osmotic work rate was 163.67 mOsm L^{-1} (Table 2). According to Lignot et al (2000), the difference in osmolarity between bodily fluids and external media represents the level of osmotic effort. The body's osmotic pressure is impacted by the salinity of the media (di Prisco & Tamburrini 1992). The osmotic environment differs considerably from the osmotic pressure of physiological fluids.

The osmotic medium will be a burden for aquatic species, needing a large amount of energy to maintain the body's osmosis in order to maintain a suitable salinity level that will impact osmoregulation (Lignot et al 2000; Greenwell et al 2003; Romano & Zeng 2012). The high energetic demand is intrinsically associated with ion transport processes and the activity of specialized osmoregulatory cells. As emphasized by Romano & Zeng

(2012), osmoregulation entails complex physiological adjustments that can profoundly affect aquaculture productivity. Fish that are unable to regulate their osmoregulation will experience stress and eventually die. The fish osmoregulation mechanism, which maintains plasma osmolality and the media in equilibrium with the environment, will be activated by changes in the salinity environment. This study demonstrates that osmoregulation-related energy expenditure can be decreased in fish raised in a hyperosmotic medium. Despite Jobling (1981), if organisms are maintained in an isoosmotic medium, they will drink excessively in the water to make up for the water lost through osmosis, which will lower osmoregulation energy expenditure. Passive salt consumption and the mitochondria of the gill epithelium, or ionocytes, which secrete surplus ions, can be active at the same time (Boyd & Lichtkoppler 1979).

Table 2

Media osmolarity, blood osmolarity, and osmotic action rate

Code	*Salinity (ppt)*	*Media osmolarity (mOsmL^{-1} H_2O)*	*Blood osmolarity (mOsm L^{-1} H_2O)*	*Osmotic ~~a~~action rate (mOsm L^{-1} H_2O)*
(A) Seawater	34 ppt	991	331	660
		983	348	635
		983	331	652
		991	340	651
		991	328	663
Average		987.8	335.6	652.2
(B) Freshwater	0 pt	0	366	366
		0.004	315	315
		0.003	304	304
		0	297	297
		0.012	340	340
Average		0.003	324.4	324.4

Proximate analysis. Variations in protein and fat were found in a proximate analysis of dried meat (A. 82.14; 9.11 % and B. 84.62; 10.55 %). Table 3 shows that the protein, fat, and ash content of fresh *L. calcarifer* fillets are comparable. The dried meat protein content of freshwater *L. calcarifer* is higher than that of seawater-grown *L. calcarifer*. This could be brought on by different living conditions or habitats, as well as the food that is ingested. The higher protein content in dried freshwater *L. calcarifer* compared to seawater-reared fish may be associated with differences in energy allocation related to osmoregulation. Fish cultured in marine environments require greater energy expenditure to maintain ionic and osmotic balance, which can reduce the energy available for somatic growth and protein deposition. This interpretation is in line with the research of Glencross (2009), who highlighted that environmental factors and energy partitioning, especially under different salinity regimes, have a significant impact on fish nutrient utilization efficiency. Similar findings were documented by Orban et al (2008), who discovered that freshwater fish like striped catfish, *Pangasius hypophthalmus* (Sauvage, 1878) had a notable protein content (80-85%), emphasizing the influence of habitat on proximate composition.

Freshwater *L. calcarifer* greater protein content is consistent with recent research highlighting the significant influence of ecological circumstances, feeding practices, and water quality on nutritional profiles in farmed species (Boyd 2015; Saskia & Kusuma 2025). Since lipid deposition is impacted by both energy intake and environmental stresses, variations in lipid content may potentially indicate metabolic responses to salinity gradients and dietary fluctuation (Glencross 2009).

Table 3

Dry meat proximate composition of juvenile *Lates calcarifer*

Parameter (% DM)	*(A) Seawater*	*(B) Freshwater*
Ash content	5.67	5.52
Fat	9.11	10.55
Protein	82.14	84.62

Note: DM = dry matter.

Water quality. Both freshwater and seawater treatments largely stayed within acceptable parameters for *L. calcarifer* aquaculture, according to the experimental water quality data (Table 4). While freshwater showed larger variations that might be due to air anomalies and climate-driven variability, seawater's temperature values (27.5-30.2°C) and freshwater's (23.1-30.8°C) were mostly within the ideal thermal range of 26-32°C, supporting metabolic activity and growth (Katersky & Carter 2007; Yadav et al 2024). However, the amounts of dissolved oxygen (DO), which ranged from 5.2-6.1 mg L^{-1} in saltwater and 4.8-5.7 mg L^{-1} in freshwater, were marginally below the optimal threshold of 6.5-12.5 mg L^{-1} (Jobling 1981). Although *L. calcarifer* can withstand moderate hypoxia, extended exposure below 5 mg L^{-1} might hinder growth, lower feed conversion efficiency, and raise the risk of mortality, highlighting the significance of aeration and ongoing observation (Jerry et al 2013; Vo et al 2020).

The appropriate range of 7.0-8.5 for *L. calcarifer* cultivation, which promotes physiological homeostasis and lessens stress, was maintained by stable pH values (7.0-8.0 in saltwater and 7.3-7.8 in freshwater) (Boyd 2015). A more serious issue was nitrite concentrations: freshwater stayed within safer ranges (0.02-0.4 mg L^{-1}), whereas seawater levels reached 1.0 mg L^{-1}, beyond the recommended limit of < 0.5 mg L^{-1}. Methemoglobinemia, which impairs oxygen delivery and raises stress and mortality risk, is known to be caused by elevated nitrite. To reduce hypoxia and nitrogenous waste buildup, aeration, biofiltration, and ongoing monitoring are advised (Yadav et al 2024; Sakia & Kusuma 2025).

Table 4

Range of water quality during the experiment

Variable	*(A) Seawater*		*(B) Freshwater*	
	Min	*Max*	*Min*	*Max*
Temperature (°C)	27.5	30.2	23.1	30.8
Dissolved oxygen (mg L^{-1})	5.2	6.1	4.8	5.7
pH	7	8	7.3	7.8
Nitrite (mg L^{-1})	0.2	1	0.02	0.4

Conclusions. This study provides a comprehensive comparative biological parameters, including growth performance, osmoregulation, and metabolic profiles, in *L. calcarifer* cultured under both freshwater and seawater conditions. Based on changes in the fish's blood profile, osmoregulation, and other biological parameters, we concluded that *L. calcarifer* culture must adapt gradually when it transfers from high to low salinity. The impact of gradual salinity increases on physiological osmoregulatory processes generally requires more investigation.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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