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First Report on Argulus and *Aeromonas hydrophila* Infestations Affecting the Cultivation of the Endangered Fish Species, *Chitala lopis*

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ABSTRACT

This study aims to examine the environmental factors that promote the proliferation of the ectoparasites Argulus and Aeromonas, as well as the impact of infestation on *Chitala lopis*. Fish rearing was conducted in the ponds that are 20×30×2.5 m^3. Feeding with cyprinidae seeds and fresh vanamei prawns occurs *ad libitum* daily and was sustained for 60 days. DNA samples were obtained from the liver, gills, spleen, and kidneys of fish that died by Argulus disease. The study's test parameters included weight, survival rate, and fish health indicators (erythrocyte, hemoglobin, hematocrit, and glucose levels). The assessed water quality parameters were temperature, pH, dissolved oxygen (DO), conductivity, and total dissolved solids (TDS). A decrease in relative growth rate (-0.8±12.3%) and survival rate (84.3±11%) were observed. Positive DNA electrophoresis bands were observed in the liver and spleen. Erythrocytes, hemoglobin, hematocrit, and glucose were all within the normal range and did not decrease at all. However, the leucocyte value decreased to 125,291±77,929 $\times 10^3/mm^3$ at the end of the study. The water quality was within a suitable range for cultivation, except for low DO values (3.5±1.5mg/L), which might facilitate the development of pathogens. To avoid and safeguard *Chitala lopis* from attacks by Argulus and Aeromonas, it is imperative to implement periodic water quality optimization and quarantine of fry fishes outside the nursery.

Key words: Giant featherback, Pathogen, Conservation, Ectoparasite.

INTRODUCTION

One of the freshwater fisheries commodities that is in high demand in the domestic market and has a high economic value in Indonesia is the belida fish, also known as *Chitala lopis*. This fish is estimated to sell for $6.5 to $12.9/kg in the domestic market, according to the results of a local market survey. However, since 1980, the population has declined, and according to the IUCN Red List, it is classified as an extinct species on the island of Java, Indonesia (https://www.iucnredlist.org/species/157719927/89815479). In contrast, Wibowo et al. (2023) rediscovered that *Chitala lopis* had a distribution extending beyond the island of Java to include Kalimantan and Sumatra. On the other hand, the Indonesian government has implemented restrictions on the rearing and capturing of this species. Ex-situ conservation efforts, such as captive reproduction or cultivation, are one approach to preserving this species. The protection status results in negligible data and publications concerning the maintenance process of

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Chitala lopis. Furthermore, expertise in rearing fish within rearing ponds is a crucial component of the ex-situ conservation process.

Fish farming for ex-situ conservation encounters numerous problems, particularly with fish health due to parasite and bacterial illnesses. In the maintenance process there is a risk of death, both infectious and non-infectious (Cain 2022). Inadequate environmental conditions lead to the development of pathogens that infect fish kept in captivity as well as non-infectious diseases.

Aeromonas hydrophila and Argulus are typical flora in freshwater environments. Nonetheless, simultaneous detection of both will result in complications for the farmed fish. Argulus, more popularly known as fish lice, is a type of ectoparasite that is frequently found in freshwater environments, particularly in ponds used for agriculture and the natural habitats of freshwater fish. This parasite is capable of growing in a variety of aquatic environments, including artificial reservoirs utilized for fish cultivation, lakes, and rivers. They can swim freely in the water before adhering to their host's body, where they will consume the fish's blood and bodily fluids. Argulus habitat mainly includes fresh waters with environmental conditions that support their reproduction, such as warm water temperatures ranging from 20-30°C, low dissolved oxygen levels (<5.6mg/L) and anthropogenic pressure (Alsarakibi et al. 2012). Elevated concentrations of organic matter in aquatic environments, stemming from fish food waste and waste, frequently intensify Argulus infestations by creating optimal circumstances for the growth of this parasite.

Argulus is a fish pathogen that can cause mortality rates of up to 52% (Steckler and Yanong 2013; Vega-Villasante et al. 2017). *Aeromonas hydrophila* is the bacterium responsible for Motile Aeromonas Septicemia (MAS), which results in a mortality rate of up to 73% (Zhang et al. 2020; Semwal et al. 2023). The risk of mortality in fish can be elevated by the presence of both in a single host (Kotob et al. 2016). Freshwater fish cultivated in aquaculture that are susceptible to Argulus infestations include species from the Cyprinidae and Channidae families (Amriana et al. 2022; Kismiyati et al. 2022). The Notopteridae family has not been documented as hosts for Argulus; therefore, it is essential to investigate the extent of Argulus infestation in the endangered species *Chitala lopis* to prevent and address previously unreported cultivation issues.

MATERIALS AND METHODS

Ethical approval

The test fish surgery was conducted with the approval of the National and Innovation Research Agency (BRIN) Ethics Council under the number 069/KE.02/SK/04/2023.

Fish rearing

The fish rearing container uses a net located in a cement tank. The test fish were placed in a net measuring $3 \times 3 \times 1.5 m^3$ in a concrete pond measuring $20 \times 30 \times 2.5 m^3$. The fish farming location was at Animalium, KST Soekarno BRIN Cibinong, Bogor Regency, Indonesia. The research implementation (preparation and data collection) lasted for 6 months (May-October 2024).

The research utilised *Chitala lopis* as test fish, maintained at a density of three individuals per net. The initial average total length and weight of the test fish were 59cm and 1,200g, respectively. *Chitala lopis* were fed natural food consisting of cyprinidae seeds (5-7cm) and frozen vannamei prawns. Feeding occurred *ad libitum* during the morning and evening. The test fish were maintained for 60 days, during which their health status was visually monitored. The total length and weight were measured, and parasites and clinical symptoms were examined every 20 days.

Died fish showing signs of illness underwent organ sampling. We carried out the sampling by isolating target parasites and bacteria from organs and dissecting the liver, spleen, and kidney. We then extracted DNA from the obtained organs using the DNAZol kit, following the established protocol. According to the publication Nam and Joh (2007), we amplified the resulting extract using a set of primers targeting the Aerolysin gene. We conducted erythrocyte and leucocyte counts to determine the health condition using a hemocytometer. Blood profile measurements in the form of hemoglobin, hematocrit and glucose were analyzed using the Fora 6 Plus digital tool.

Water quality

The study measured water quality parameters including temperature, total dissolved solids (TDS), conductivity, dissolved oxygen (DO), and pH. The instrument employed was the Hanna HI2550 Multiparameter Water Quality Meter. Water quality assessments were conducted monthly.

Data and statistics analysis

The weight increase of the parent fish was quantified using the relative growth rate (%) formula during cultivation and the survival rate (%) was assessed at the conclusion of the study. The growth and survival data were analyzed using the methodologies of Lugert et al. (2016) and Wang et al. (2006). A statistical examination of average weight growth, survival rates, blood profiles, and water quality was conducted utilizing Analysis of Variance (ANOVA), with the smallest difference test performed by Tukey's test using statistical analysis of IBM SPSS version 23.

RESULTS

Growth and blood profile

Table 1 presents the average weight and the blood profile (erythrocyte values, hemoglobin, hematocrit and glucose) of *Chitala lopis*. The final weight (1,082±503.7g) is significantly lower than the initial (1,093±606.3g). The one-sample t-test in Table 2 indicated a decrease in the relative growth rate (-0.8±12.3%) and a survival rate of 84.3±11%.

The findings of the research that was carried out at the initial and the end of the study are shown in Table 1. These findings are based on the blood characteristics that were used to evaluate the health conditions of fish. The final erythrocyte, hemoglobin and hematocrit values ($2,191 \pm 932.9 \times 10^3/mm^3$, 8.3±1.8g/dL, 24.7±5.4%, respectively) were insignificantly higher than the initial ($1,908 \pm 684.4 \times 10^3/mm^3$, 7.6±1.9g/dL, 22.5±5.6%, respectively). Furthermore, the final glucose values (44.5±8.8mg/dL) revealed a significant increase compared to the initial values (34.5±6.8mg/dL), while the final erythrocyte count (125,291±77,929×10^3/mm³) showed a significant decrease relative to the initial count (216,979±114,075×10^3/mm³).

Table 1: The average of weight and the blood profile (erythrocyte values, hemoglobin, hematocrit and glucose) at the beginning and end of the study

Parameter	Initial±SD	Final±SD
Weight (g)	1,093±606.3	1,082±503.7*
Erythrocytes (×10^3/mm^3)	1,908±684.4	2,191±932.9
Leucocytes (×10^3/mm^3)	216,979±114,075	125,291±77,929*
Hemoglobin (g/dL)	7.6±1.9	8.3±1.8
Hematocrit (%)	22.5±5.6	24.7±5.4
Glucose (mg/dL)	34.5±6.8	44.5±8.8*

Note: *Superscript (row 2) indicates significant differences between populations.

Clinical symptoms

The sampled *Chitala lopis* were deceased, thus the observable clinical signs were restricted to outward manifestations. The symptoms exhibited by the internal organs are insufficient to support a precise diagnosis, as they already exhibit signs of decay (Fig. 1c and d). The clinical symptoms observed during the sampling procedure indicated that the fins of the belida fish had been wounded, indicating the presence of an infectious attack (Fig. 1a and b). The tip of the gill lamella exhibits a yellowish-white tone (Fig. 1e).

Dead fish organs were gathered in order to use DNA analysis to detect the presence of *Aeromonas hydrophila* infection. These organs include the kidneys, lymph nodes, gills, and liver. The gel electrophoresis reveals nucleotide band size of almost 400bp in the liver and spleen (Fig. 2). This indicates that the liver and spleen are positively infected.

Table 2: The average of relative growth rate, survival rate and water quality (temperature, total dissolved solids (TDS), conductivity, dissolved oxygen (DO), and pH levels)

Parameter	Average±SD
Relative Growth Rate (%)	-0.8±12.3
Survival Rate (%)	84.3±11
Temperature (°C)	28.6±0.5
TDS (mg/L)	78.1±7.9
Conductivity (µs)	165.6±16.5
DO (mg/L)	3.5±1.5
pH	8.3±0.5

Fig. 1: The clinical condition of *Chitala lopis*. A) The fish experiencing symptoms due to pathogenic bacteria in the form of wounds on the skin and fins, B) enlargement of images of clinical symptoms on damaged *Chitala lopis* fins, C) The fish organs experiencing decay, D) Enlargement of organs that are visible and undergoing decomposition in fish., E) The gill organs show a pale color and there is a yellowish color at the tip of the gill lamella.

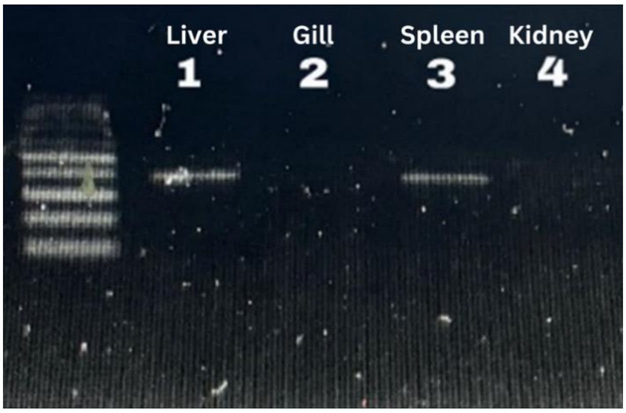

Fig. 2: Electrophoresis reveals DNA bands from *Aeromonas hydrophila* in the liver, gill, spleen, and kidney tissues of *Chitala lopis*.

The argulus affixed to the epidermis of *Chitala lopis* were gathered and examined using a binocular microscope at a magnification of 4×. The morphology of the argulus is illustrated in Fig. 3, depicting both the posterior and anterior positions (Fig. 3a and 3b).

Water quality

The water quality measurements data are presented in Table 2. The average temperature, Total Dissolved Solids (TDS), conductivity, Dissolved Oxygen (DO) and pH are 28.6±0.5°C, 78.1±7.9mg/L, 165.6±16.5µs, 3.5±1.5mg/L, and 8.3±0.5, respectively.

DISCUSSION

Fish farming practices of endangered species *Chitala lopis* represent a conservation initiative aimed at preventing the extinction of this species. Fish farming inherently involves risks of mortality, attributable to either disease or environmental factors. Argulus is a pathogen that impacts freshwater fish farming, influencing the health and growth of freshwater fish. It induces stress, diminishes feeding, and heightens vulnerability to other diseases. *Aeromonas hydrophila* can significantly worsen this issue, resulting in infections that undermine the fish's immune system and overall health. The interplay of these two factors may lead to a significant decline in fish farming activities. Infestation triggers immunomodulatory effects, inflammation and tissue damage, potentially resulting in elevated mortality rates within fish populations (Haridevamuthu et al. 2024). The mortality rate for *Channa striata* is reported to be between 73.3 and 96.7% (Amriana et al. 2022), while for Cyprinidae, it is 90% (Nurani et al. 2020).

The percentage of deaths observed in this study was comparable to that of previous research. Another disadvantage is the decrease in the weight of the fish. Argulus parasites adhere to fish and extract blood, resulting in anaemia and diminished growth rates. The blood-sucking behaviour significantly impacts the fish's weight gain (Ode 2012).

Argulus wounds may result in secondary bacterial, fungal, or viral infections that further impede growth and result in severe health issues (Ode 2012). *Aeromonas hydrophila*, a common flora in fresh to brackish waters and an opportunistic bacterium that infects aquatic animals and people, was the one that attacked in this investigation following an Argulus infestation (Matyar et al. 2007). Fish infections with Aeromonas can result in serious illnesses such as hemorrhagic septicaemia, which is marked by severe bleeding and a high death rate (Claudiano et al. 2020). Depigmentation, irregular swimming, and neurological abnormalities are common symptoms of systemic infection in infected fish. Aeromonas frequently infects the liver, as in gupy fish (Lazado and Zilberg 2018). The lymph, a component of the lymphatic system with blood vessel damage, is another organ that Aeromonas has been known to assault (Marinho-Neto et al. 2019).

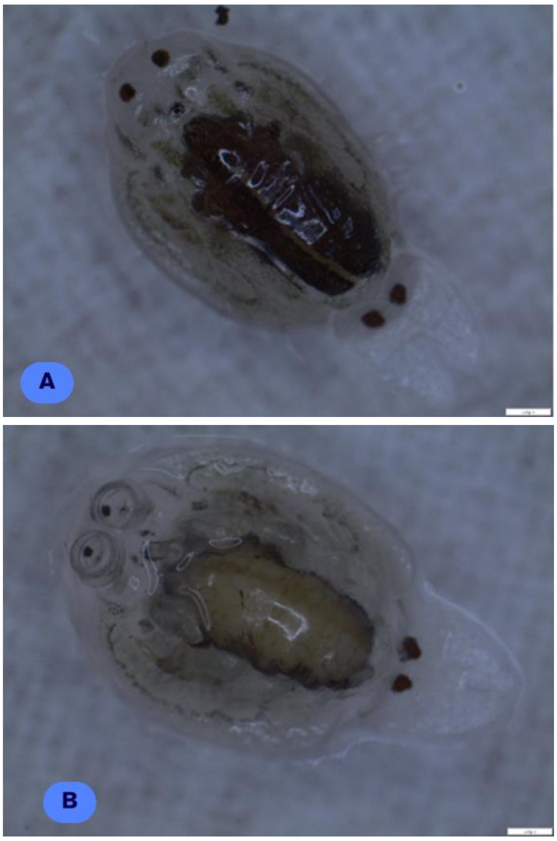

Fig. 3: Morphology of Argulus. A) Posterior and B) Anterior.

The health status of the fish that is being monitored can be described by measuring blood parameters. The cells known as erythrocytes are responsible for transporting oxygen and food throughout the body of the fish. Generally, the erythrocyte counts in this study fell within the normal range for fish, estimated at 1.41-4.55×10^6/mm^3 (Goel et al. 1981) and carnivorous fish, *Channa striata*, estimated at 1.45-3.22×10^6/mm^3 (Muslimin et al. 2023). The erythrocyte value aligns with the findings of this study, indicating that the condition of the erythrocytes is within the normal range. Nevertheless, the erythrocyte value at the end of this investigation increased. The increase in erythrocyte levels in the final study may result from a compensatory erythropoiesis process that recovers lost erythrocytes (Ode 2012). However, the number of leukocyte cells was reduced by the end of the trial. This could be due to Argulus infection influencing immune gene regulation, followed by a decrease in leukocyte cells, indicating that fish have a decreased ability to respond effectively to infections (Kar et al. 2015). There are no reported normal hemoglobin values for the Chitala. Normal hemoglobin values in freshwater fish range from 8 to 16.1g/dL (Goel et al. 1981). The hemoglobin is in a threshold condition, except for the initial. The hematocrit values among freshwater fish species exhibit considerable diversity. The normal level confirmed for hematocrit in this research is estimated to range from 18.6 to 38.9% (Goel et al. 1981; Prasad et al. 1978). The glucose levels in freshwater fish, particularly within the Genus Chitala, remain unreported. The general glucose range for freshwater fish is documented to be between 2.5 - 97.5mg/dL (Manera and Britti 2006; Jung et al. 2011), the glucose levels in this study is within the usual range. However, an elevation in glucose levels was observed at the final study. The elevation in glucose levels was observed to parallel the infestation of the Argulus ectoparasite in cantang grouper (Izzati et al. 2022). The increase in cortisol and epinephrine hormones triggers glycogenolysis, the process of breaking down glycogen into glucose, to enhance energy supply in response to parasite attacks (Datta et al. 2022).

The temperature range examined in this study aligns with the living and growth conditions of Argulus (20-28°C) and Aeromonas (25-30°C) (Austin and Austin 2016; Thune et al. 1993). The primary element affecting the presence of Argulus in aquaculture is the aquatic environment, specifically temperature fluctuations. An increase in temperature values demonstrates a positive correlation with the prevalence of the argulus parasite (Yunikasari et al. 2020). The ideal temperature for argulus hatching during its life cycle is 28°C, and it can hatch up to 75% of the eggs in 15 days (Sahoo et al. 2013). While various water quality parameters are recognized to affect one another (Olusegun and Oluwatoyin 2012), especially regarding temperature, pH, and ammonia levels (Levit 2010). The total dissolved solids (TDS) in aquatic environments for *Aeromonas hydrophila* range from 199-261mg/L (Ortega et al. 1996). The TDS value in this study was below the specified threshold, indicating normal conditions. The conductivity of the water in this study fell within the normal range of 100-500 µS/cm and did not promote the growth of pathogenic bacteria (Le Chevallier et al. 1996). In this study, low oxygen levels create a conducive water environment for the growth of *Aeromonas hydrophila*, which are facultative anaerobic bacteria. An ideal dissolved oxygen level to inhibit the development of both Argulus and *Aeromonas hydrophila* is greater than 5mg/L (Austin and Austin 2016). This aids in reducing stress and vulnerability to infection. The pH in this study was within the acceptable range of 6.5-8 (Thune et al. 1993). Water conditions significantly influence the proliferation of Argulus and *Aeromonas hydrophila*. Therefore, it is essential to anticipate drastic fluctuations in water quality during maintenance activities. Due to the ability of both pathogens to transmit and alter hosts, it is essential to enhance biosecurity measures and implement quarantine protocols for seeds used as feed for *Chitala lopis* to mitigate the transmission of Argulus and *Aeromonas hydrophila*.

Conclusion

Infections caused by Argulus and *Aeromonas hydrophila* lead to weight reduction and mortality in *Chitala lopis*. Those infections may wound the liver and spleen. A rise in glucose, erythrocytes, hemoglobin and hematocrit levels, and a conversely decrease on leucocytes was observed in the final study. Temperature and dissolved oxygen conditions also facilitate the proliferation of Argulus and *Aeromonas hydrophila*; therefore, environmental prevention and quarantine measures are essential, including those for fish seeds intended as sustenance for *Chitala lopis*.

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Author's contribution: The research management and draft paper writing were performed by Arif Wibowo, Boby Muslimin and Nunak Nafiqoh. Laboratory analyses and experimental research were carried out by Nunak Nafiqoh, Mochammad Zamroni and Boby Muslimin. Project administration was performed by Hermawan Budiantoro, Siti Rachmi Indahsari, Adi Firmansyah, and Indah Lestari. Data curation and and investigation were perfomed by Agus Priyadi, Andi Chadijah, Bastiar Nur, Mirna Dwirastina, Deni Irawan, Hessy Novita, Dewi Apriyanti, Indah Anggraini Yustani and Sujaka Nugraha. Data analyses and finalized the original paper conducted by Nunak Nafiqoh, Danang Yonarta and Boby Muslimin.

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