

Threat of extinction of *Macrobrachium esculentum* in the Serayu River (Central Java, Indonesia) confirmed by DNA barcoding

BARUNA KUSUMA^{1,4,*}, SLAMET BUDI PRAYITNO², ANINDITIA SABDANINGSIH³,
PETRUS HARY TJAHJA SOEDIBYA⁴, SURADI W. SAPUTRA³

¹Doctoral Program of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Jacub Rais, Semarang 50275, Central Java, Indonesia. Tel.: +62-24-76404447, *email: barunakusuma@unsoed.ac.id

²Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Soedarto, Semarang 50275, Central Java, Indonesia

³Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Soedarto, Semarang 50275, Central Java, Indonesia

⁴Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Jenderal Soedirman. Jl. Dr. Soeparno, Banyumas 53122, Central Java, Indonesia

Manuscript received: 24 July 2024. Revision accepted: 10 October 2024.

Abstract. Kusuma B, Prayitno SB, Sabdaningsih A, Soedibya PHT, Saputra SW. 2024. Threat of extinction of *Macrobrachium esculentum* in the Serayu River (Central Java, Indonesia) confirmed by DNA barcoding. *Biodiversitas* 25: 3531-3539. Sweet river prawn (*Macrobrachium esculentum* Thallwitz 1891) is one of the amphidromous shrimp. Serayu River is one of the habitats of this shrimp. Information about the *M. esculentum* in the Serayu River, is still minimal, so this research is essential. The Serayu Weir affects the population of *Macrobrachium esculentum*, which impacts the balance of the Serayu River ecosystem. This study aims to determine the condition of the *M. esculentum* population in the lower reaches of the Serayu River in order to maintain its sustainability and avoid the threat of extinction. This research was conducted for one year (January-December 2023). The method used was the descriptive method. We collected *M. esculentum* catches both upstream and downstream of the weir for one year to determine the presence of shrimp as a result of the Serayu Weir. The catch data was compared with the catch data from previous research. *M. esculentum* has morphological characteristics with a blackish-gray pattern with stripes along its abdomen and has an upper rostrum of 11-14 teeth and 2-4 on the lower rostrum. *M. esculentum* in the Serayu River has an mtDNA fragment of 686 bp and shows the same species as *M. esculentum* in GenBank. A total of 65 *M. esculentum* were collected. This shrimp was not found above the weir (Station 1) but only at station 2 (below the weir) due to the presence of weir. The results showed the importance of constructing migration routes for aquatic biota in the Serayu Weir. Further research is also needed, especially related to the reproductive system of *M. esculentum*.

Keywords: Habitat narrowing, migration breaker, weir

INTRODUCTION

Freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Infraorder Caridea; Family Palaemonidae), comprise a diverse group of over 240 species that inhabit a variety of aquatic environments, including coastal lagoons, lakes, tropical rivers, ponds, and streams (Wowor et al. 2009; Jose et al. 2016). Species of this genus are characterized by an elongated second pair of locomotor appendages, usually equal to or larger than body size, with prominent chelae (Short 2004; Molina et al. 2020). Despite having different habitat preferences, these shrimp are believed to have evolved from a marine ancestor (Jose and Harikrishnan 2019). Indonesia is the country with the highest biodiversity of *Macrobrachium* species in the world. *Macrobrachium* is found in 27 countries around the world: 113 species in Indonesia, 67 in India, 41 in the United States, 29 in Malaysia, 24 in Brazil, 20 in Bangladesh, 14 in Kenya, 13 in Colombia, 10 in Panama, 7 in Australia, and several other countries (BOLD system).

Tropical region is considered the biogeographic origin of this genus. *Macrobrachium* species can be categorized into two types based on their life cycle: shrimps that

complete their life cycle entirely in freshwater and *Macrobrachium* that require salinity levels brackishwater environments for larval development (Wowor et al. 2009). The majority of naturally occurring *Macrobrachium* are amphidromous (Pescinelli et al. 2016). Amphidromous shrimp exhibit complex migratory behavior where they can move between freshwater and estuarine environments to complete their life cycle (Beesley et al. 2023). These migrations can span hundreds of kilometers upstream and face numerous challenges (Novak et al. 2017; Jarvis and Closs 2019). Factors such as habitat destruction, river siltation, overfishing, and the presence of dams that alter migration pathways can all contribute to stress during migration (Silva-Junior et al. 2017; Heim-Ballew et al. 2020; Ribeiro et al. 2020). Research conducted by De Grave et al. (2015) showed that the shrimp genus *Macrobrachium* is increasingly under threat of extinction.

Previous studies on the genus *Macrobrachium* have historically relied heavily on morphological characteristics for species identification, which often yielded inconclusive results (Jose et al. 2016; Jurniati et al. 2021). Molecular identification is essential in shrimp conservation efforts to mitigate extinction threats. Through DNA barcoding and

Polymerase chain reaction PCR-based methods, researchers can accurately identify shrimp species, detect cryptic species, and distinguish closely related species (Khan et al. 2014; Jose and Harikrishnan 2019). This precision in species identification is important for understanding biodiversity, population structure and genetic diversity, which are essential for effective conservation strategies (González-Castellano et al. 2020).

One of the shrimp genus *Macrobrachium* that is amphidromous and plays an important role is *M. esculentum* (Wowor et al. 2009). This shrimp is one of the freshwater shrimp found in various regions and is characterized by its relatively large size. This shrimp is consumed locally without international commercialization. *M. esculentum* is usually found in the upper reaches of rivers with sand or mud substrates, but can also be found in the lower reaches of rivers with brackish water (Jurniati et al. 2021). One river that serves as a habitat for *M. esculentum* is the lower Serayu River. This river is one of the longest rivers in Central Java, Indonesia, with a length of 153 km (Sinaga et al. 2024). It crosses five districts and empties into Cilacap District (Pranoto et al. 2019).

Research conducted on the shrimp genus *Macrobrachium* in the Serayu River is minimal. Siregar et al. (2001) found six species of shrimp genus *Macrobrachium* from three tributaries of the Serayu River located in Banyumas District (Banjaran, Pelus, and Logawa Rivers) namely: *Macrobrachium cowlesi* Holthuis 1950, *Macrobrachium idae* Heller 1862, *Macrobrachium oenone* De Man 1902, *Macrobrachium lanchesteri* De Man 1911, *Macrobrachium esculentum* Thallwitz 1891, and *Macrobrachium* sp.. Recent research by Kusbiyanto (2009) showed that only four shrimp species were found, namely *M. cowlesi*, *M. idea*, *M. esculentum* and *M. oenone*. This resulted in serious concerns about the sustainability of shrimp in the Serayu River, especially *M. esculentum*. This study aimed to determine the condition of the *M. esculentum* population in

the Serayu River due to the impacts caused by the construction of the weir.

MATERIALS AND METHODS

Research location and sampling method

This research was conducted in the lower reaches of the Serayu River that crosses Banyumas and Cilacap Districts, Indonesia (Pranoto et al. 2019), with coordinates ranging from 77°29'35.5 "S 109°20'13.1 "E to 7°41'06.5 "S 109°06'29.1 "E (Figure 1). Sampling was divided into two stations, station 1 above the weir (isolated) and station 2 below the weir to the mouth of the Serayu River. Station 1 was chosen at a location right above the Serayu Weir, extending to the mouth of the lowermost tributary. This was done to represent previous research, where the study was conducted in the tributaries of the Banyumas area. Sampling was divided into two stations, namely station 1 above the weir (isolated) and station 2 below the weir to the mouth of the Serayu River.

Data collection was conducted every two weeks for a year, from January to December 2023. *M. esculentum* was caught using nets (mesh size 1.5 cm, length 3 m, and height 1.5 m), fishing rods, and shrimp traps. The shrimp trap is shaped like an umbrella net with 8 entry holes. Trash fish bait is placed right in the center of the trap's spokes and submerged with a stone weight at a depth of 1-2 meters. A marker line is left floating so we can pull it after 12 hours (Silva et al. 2020). Shrimp were caught at night (18:00-24:00) and in the morning (06:00-10:00). At night, we fishing and set shrimp traps, doing this 1-2 times a week. Capturing shrimp with regular nets is done during the day 1-2 times a week, though sometimes it is also done at night. The captures are assisted by local community members experienced in catching shrimp.

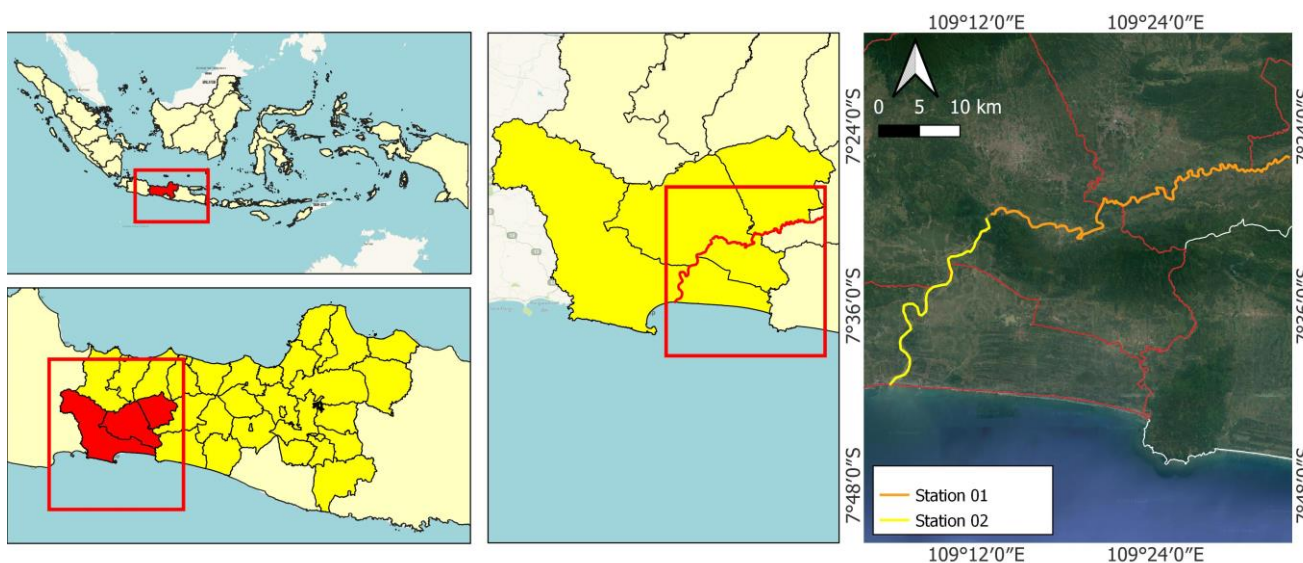


Figure 1. Research location in the lower reaches of the Serayu River, Central Java, Indonesia. Station 01 with coordinates: 77°29'35.5 "S 109°20'13.1 "E to 7°31'31.5 "S 109°12'05.1 "E), Station 02 with coordinates: 7°31'33.0 "S 109°12'03.4 "E to 7°41'06.5 "S 109°06'29.1 "E

This serayu motion weir was built in 1993 and was inaugurated in 1996, and until now, this weir is still active. The weir is equipped with eight large radial sluices (10.70×9.00 m) that regulate the Serayu River's flow. In addition, the weir has a 273 m long sluice gate consisting of 2 lanes and entrance and exit gates measuring 2.50×2.00 m each. The weir operating water level is between ±12.20 to 12.90 meters above sea level, with the peak reaching ±15.50 meters above sea level. The weir structure is quite large, reaching 121.20 m in length and 109.60 m in width (Sinaga et al. 2024).

M. esculentum collected were 43 males and 22 females. The captured shrimp was stored in a cool box and brought to the Faculty of Fisheries and Marine Science laboratory, Universitas Jenderal Soedirman, to be preserved and identified. Morphological identification was carried out based on previous research by Jurniati et al. (2021). Observations were made based on color patterns, the number of upper and lower rostrums, and other identifications sex of this species. A literature study was conducted on the distribution of *M. esculentum* in the Serayu River, based on previous research, as a comparison of the presence of *M. esculentum* from 1998 to 2003 (at the time of the study). The sex ratio (male: female) was analyzed by dividing the number of male and female specimens by the total number of specimens examined (Ahmed et al. 2021).

Genetic analysis

Meat tissue samples (approximately 10 g) were stored in separate 50 mL vials filled with 96% ethanol, labeled accordingly, and sent to BIONESIA Laboratory for mtDNA isolation. Qiagen protocol was used for extraction. The extracted material was then analyzed via PCR (Polymerase Chain Reaction) using procedures established by the BIONESIA laboratory. For amplification of *M. esculentum* samples, primers jgLCO (5'-TIT CIA CIA AYC AYA ARG AYA TTG G-3') and jgHCO (5'-TAI ACY TCI GGR TGI CCR AAR AAY CA-3') were used (Geller et al. 2013). The total volume for the PCR reaction was 26 µL, which included 2 µL of extracted DNA template, 1.25 µL of each primer with a concentration of 10 mM, 9 µL of ddH₂O, and 12.5 µL of Ready-mix. Amplification was performed using an Applied Biosystems™ 2720 Thermal Cycler. The PCR protocol involved initial denaturation at 94°C for 3 minutes, followed by 38 cycles of denaturation at 94°C for 30 seconds, annealing at 50-55°C for 30 seconds, and extension at 72°C for 60 seconds, ending with a final extension at 72°C for 2 minutes. PCR products were visualized on a 1% agarose gel stained with GelRed®. Samples showing DNA bands were sequenced using the Sanger deoxyribonucleotide method at PT Genetika Science Jakarta.

Data analysis

The morphology, distribution, and population of *M. esculentum* were analyzed descriptively during the study. Computer software was used for molecular analysis. Sequence data were edited and aligned using the ClustalW method in the MEGA XI program. Each base sequence was carefully checked to ensure its quality, and sequences with

substandard results were reprocessed through PCR and re-sequencing. The data were then compared with the GenBank database (NCBI) using the Basic Local Alignment Search Tool (BLAST) on the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Each sequence was recorded for similarity and accuracy. In addition to BLAST analysis, the data were evaluated using a phylogenetic tree to determine relationships between samples and verify species-level identification. This kinship tree was constructed using the Neighbor-Joining (NJ) method with 1000 bootstrap replications on MEGA XI software.

RESULTS AND DISCUSSION

Morphology of *M. esculentum*

Samples of freshwater prawns recovered from the lower reaches of the Serayu River show a grey-black color with striated stripes along the abdomen. It has a short, downwardly curved rostrum that does not reach the antennae, with 11-14 teeth on the upper and 2-4 teeth on the lower rostrum. Its overall length varies between 3.46 and 8.77 cm (Figure 2).



Figure 2. *Macrobrachium esculentum* found in the lower reaches of the Serayu River. A. Female; B. Male; C. Rostrum; D. Uropod; E. Sex (sex is in the way of being at the foot of the third path (left female; right male)); F. *M. esculentum* side view

Figure 3. Phylogenetic tree of *Macrobrachium esculentum* in the lower reaches of Sugai Serayu (Red color is the species found in this study)

Distribution of *M. esculentum*

The distribution of *M. esculentum* is still limited to date. Based on its life cycle, this shrimp is euryhaline (Wowor et al. 2009). Euryhaline shrimp have an unlimited natural distribution compared to terrestrial and marine species. The natural distribution of euryhaline shrimp is also well recorded in *M. rosenbergii*, which can adapt to freshwater environments and has a wide natural distribution in tropical and subtropical areas in South and Southeast Asia, reaching up to the Western Pacific (Hurwood et al. 2014). This study still cannot answer the puzzle of the natural distribution of *M. esculentum*. Based on available data, *M. esculentum* is distributed from Indonesia-China (Figure 4). The amphidromous distribution of *Macrobrachium* species depends on brackish or seawater for its larvae (Han et al. 2022). The ability of larvae to adapt to varying levels of salinity is critical to their dispersal and developmental success (Hiraga et al. 2021).

The population of *M. esculentum* in the Lower Serayu River

Research conducted in the lower reaches of the Serayu River did not find *M. esculentum* above the weir (station 01). This shrimp was only found below the weir (station 02). The dominant catch was found in November (Figure 5). The most common *M. esculentum* was male, with a sex ratio of male: female of 1.95. Although *M. esculentum* is one of the freshwater shrimp species that is not included in the endangered species according to the International Union for Conservation of Nature IUCN Red List, the absence of *M. esculentum* above the dam emphasizes the importance of protecting the native habitat of this shrimp. This further supports the notion that conservation efforts are essential to maintain populations of *M. esculentum* species.

The severe threat to *M. esculentum* is also shown based on previous research (Table 2). Since 1989, *M. esculentum* has still been found in Banyumas District. This shrimp's distribution was known deep in the weir basin. However, due to the nature and characteristics of the weir, the distribution space is narrowed. Weir can disrupt the natural movement of migratory *Macrobrachium* shrimp, thus affecting their reproductive behavior and life cycle (Sokolow et al. 2017). Dam construction has also been reported in *Macrobrachium vollohovenii* Herklots 1857 to disrupt shrimp access to breeding sites, affecting these shrimp (Savaya et al. 2014). This constriction also results in competition with predators.

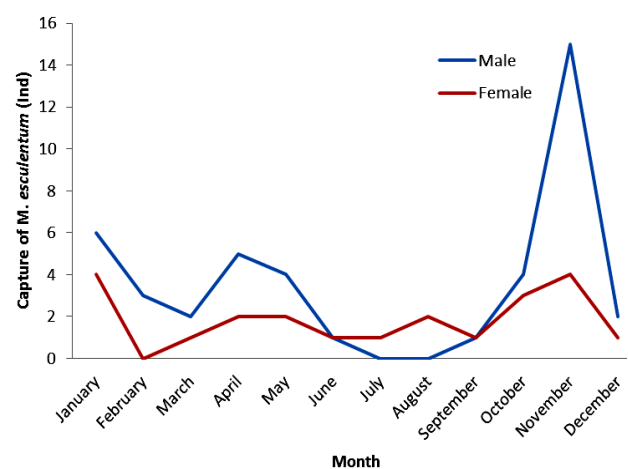


Figure 5. *Macrobrachium esculentum* catch at station 02 (Below the weir)

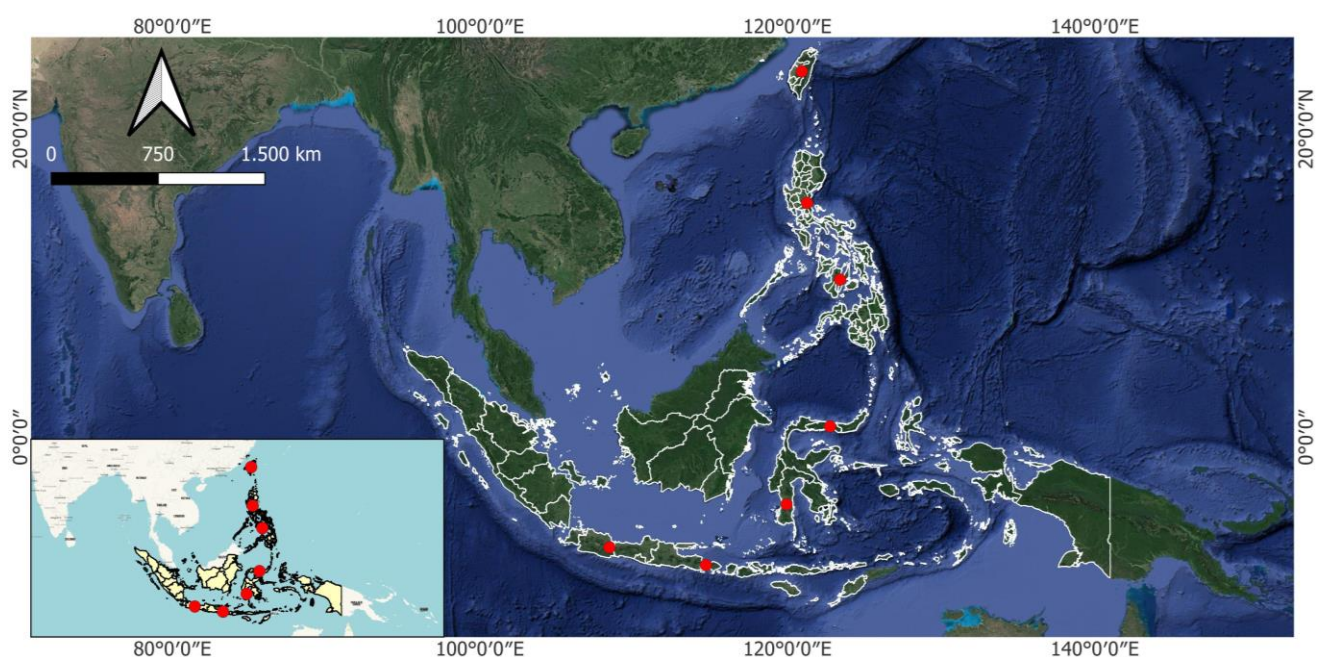


Figure 4. Distribution of *Macrobrachium esculentum*

Discussion

Male *M. esculentum* had larger chelipeds than females (Jurniati et al. 2021). This gives males an advantage in terms of dominance, ability to obtain resources, and increasing reproductive opportunities. However, in shrimp populations that do not have a dominant morphotype, male reproductive success is not determined by large cheliped size but rather by the ability to search for females actively (Silva et al. 2019; Nascimento et al. 2020). Other social and reproductive behaviors, such as 'pure search', played an essential role in male sexual selection during the evolution of the species. According to Nogueira et al. (2023), in 'pure search', there are no aggressive encounters between males, so mating success depends more on the ability to find as many mating-ready females as possible.

M. esculentum has only been documented in Indonesia, the Philippines, and Chinese Taipei (Figure 4). According to the Global Biodiversity Information Facility (GBIF), the species has been recorded from Mimanga River, Minahasa, North Sulawesi, Indonesia (Senckenberg 2004); Pongkeru River, South Sulawesi, Indonesia (The International Barcode of Life Consortium 2016); Rangkong River basin, South Sulawesi, Indonesia (Jurniati et al. 2021) Tempe Lake, South Sulawesi, Indonesia (Goud et al. 2020); Java and Bali, Indonesia (Hernawati et al. 2020); Philippines (Akiba and Sasaki 2020); and Chinese Taipei (De Grave 2017; Santos et al. 2024). *M. esculentum* has a habitat with sand/mud substrate near the sea where fresh and brackish water meet (De Grave et al. 2013).

The genetic distance of *M. esculentum* found in the lower reaches of the Serayu River has a genetic distance ranging from 0-0.277, with the closest genetic similarity with *M. esculentum* (GenBank code: MN526199.1) and the farthest distance with *Macrobrachium forcipatum* Ng 1995 (GenBank code: MW845472.1) (Figure 3). GenBank data shows that *M. esculentum* found in the same region has a bootstrap value of 54-100%. Compared to other species, *M. esculentum* showed a 16-88% bootstrap value in this study. The phylogenetic tree showed strong terminal phylogenetic specificity with deeper relationships, indicating the

effectiveness of DNA barcodes in identifying closely related species.

The results of research conducted by Jose and Harikrishnan (2019) show that, *M. esculentum* is close to *Macrobrachium lanatum* Cai and Ng 2002 and *Macrobrachium scabriculum* Heller 1862 and forms one clade with *Macrobrachium striatum* N.N.Pillai 1991, *Macrobrachium equidens* Dana 1852, *Macrobrachium idella* Hilgendorf 1898, *Macrobrachium idae* Heller 1862 and *Macrobrachium mammillodactylus* Thallwitz 1892. While research conducted by Chen et al. (2009) and Wowor et al. (2009) using 16S and 28S showed that *M. esculentum* is closely related to *M. latidactylus*. Jurniati et al. (2021) showed that *M. esculentum* is closely related to *Macrobrachium nipponense* De Haan 1849. To comprehensively understand the evolutionary rate and timing of branch emergence on the phylogenetic tree, expanding and completing the inventory of *M. esculentum* in Indonesia is essential.

During the research period conducted in the lower reaches of the Serayu River, males were dominant in the *M. esculentum* population. An unbalanced sex ratio in a population can significantly impact mating, reproduction and population dynamics. The sex ratio observed in the study showed instability compared to the expected 1.95:1 ratio, with a higher proportion of males each month. This phenomenon is common in crustaceans and can be influenced by various factors such as seasonal migration, birth rate, mortality, longevity, and predation (Nogueira et al. 2019).

Favorable sex ratios were observed in certain *Macrobrachium* species, such as *Macrobrachium olfersii* Wiegmann 1836 (Pescinelli et al. 2016), *Macrobrachium jelskii* Miers 1877 (Rocha and Barbosa 2017), *M. vollenhovenii* (Ukagwu and Deekae 2016), and *Macrobrachium rosenbergii* De Man 1879 (Indarjo et al. 2021) *Macrobrachium brasiliense* Heller 1862 (de Oliveira et al. 2019), with a higher proportion of females. Understanding the drivers of sex ratio imbalances is critical for predicting population growth, survival, and species vulnerability to extinction (Silva et al. 2019).

Table 2. Development of *Macrobrachium esculentum* research in the lower Serayu River from 1989-2023

Research year	Research location	Catch results	Sex ratio (Male:Female)	Reference
1989	Banyumas District	There were 8 species of freshwater shrimp and <i>M. esculentum</i> was found.	Unknown	Darbohoesodo (1987)
2001	Banjaran River, Pelus River and Logawa River (Upper Serayu River Tributary of Serayu Motion Weir)	There were 6 species of freshwater shrimp and <i>M. esculentum</i> was found.	Unknown	Siregar et al. (2001)
2005	Banjaran River (Upper Serayu River Tributary of Serayu Motion Weir)	<i>M. esculentum</i> found	1.22:1 (multiple males)	Kusbiyanto (2009)
2023	Downstream of Serayu River (Above Serayu weir)	Not found <i>M. esculentum</i>	-	this research
2023	Downstream of Serayu River (Under Serayu weir)	<i>M. esculentum</i> found	1.95:1 (multiple males)	this research

This study showed male dominance at station 02 (below the weir) (Figure 5). Sex ratio imbalances at the population level can have far-reaching evolutionary and ecological consequences, impacting population dynamics and mating behavior (Sethi et al. 2014). According to Taddei et al. (2017), an increase in the number of females in a population leads to a higher fertilization index, mainly due to the continuous reproductive nature of the species (Taddei et al. 2017). The more significant number of males in the *M. esculentum* population in the lower Serayu River indicates a threat to the sustainability of the species.

Nonetheless, males are generally larger than females, possibly due to dominance and territoriality behaviors favoring more prominent individuals. Reproductive effectiveness depends on the ability of males to find and fertilize as many receptive females as possible (Nogueira et al. 2023). The larger size of males compared to females likely increases reproductive success and provides an advantage during intraspecific competition, as in *Macrobrachium lar* J.C.Fabricius 1798 (Rismawati et al. 2024); *Macrobrachium potiuna* Müller 1880 (De Melo and Masunari 2017), *Macrobrachium amazonicum* Heller 1862 (Augusto and Masui 2014). However, in some shrimps of the genus *Macrobrachium* there are also females larger than males such as *M. jelskii* (Nascimento et al. 2020), *Macrobrachium pantanalense* Dos Santos, Hayd and Anger 2013 (Nogueira et al. 2023).

Built-in 1996, the Serayu River Weir has eight radial gates that control the flow of river water. With a height of 15 meters, a length of 121.20 meters, and a width of 109.60 meters, the weir was built without fish passage. Its presence in the lower reaches of the Serayu River changes its hydrographic characteristics by increasing surface runoff and sedimentation rates. This is consistent with research conducted by Pescinelli et al. (2016) in an urban river in southeastern Brazil. In addition, dams increase pollutant concentrations and decrease species diversity (Reid et al. 2019). Increased sedimentation, riverbank alteration, and changes in rivers' physical and chemical composition can lead to reduced habitat diversity and loss of sensitive species (Pérez-Reyes et al. 2016).

The absence of *M. esculentum* at station 01 (above the weir) indicates that these shrimp did not successfully return or breed above the weir. This finding supports the notion that the height of the dam and high river flow may inhibit migration, causing shrimp to remain downstream in search of better habitat. Extreme environmental conditions, such as storms or droughts, can affect shrimp densities, either increasing or decreasing (Pérez-Reyes et al. 2015).

Several factors may contribute to the absence of *M. esculentum* in the lower Serayu River, namely the impact of human activities cannot be ignored, changes in water chemistry (Arinda et al. 2023), population isolation due to habitat fragmentation, and larval and juvenile mortality caused by dams. Sudden changes in shrimp populations can disrupt other organisms in the ecosystem. Similar observations were noted in the study by Pescinelli et al. (2016) on other shrimp species found in urban rivers in southeastern Brazil, such as *Atya scabra* Leach 1816 and *Potimirim brasiliiana*

F.Villalobos 1960. These changes may impact the abundance of shrimp, algae and insects in these environments.

Crustaceans are often used as bioindicators in aquatic environments due to their presence in various ecosystems and their essential role in the food chain (Bertrand et al. 2018). The results of this study, where *M. esculentum* was found from 1989-2005, and recorded its absence in 2023, suggest environmental stresses that disrupt species migration. A similar study in Costa Rica (Devi et al. 2013) found that *M. olfersii* populations in degraded rivers showed an 87% decrease in relative abundance compared to populations in protected rivers. Similarly, *M. olfersii* populations in the Taquaral River may be affected by human activities, potentially disrupting migration patterns and thus threatening the survival of the species in dense urban ecosystems.

Previous studies have also noted extinction threats due to dams (Table 3). For example, the Dama Dam has been associated with a decline in *M. vollenhovenii* populations, which impacts their ability to prey on snails and indirectly contributes to the spread of schistosomiasis (Secor 2014). Furthermore, proposed dam projects such as the Inter-Oceanic Canal in Nicaragua are expected to have a significant impact on migratory species with complex life cycles, such as *Macrobrachium carcinus* Linnaeus 1758, further highlighting the vulnerability of these species to dam construction (Huete-Perez et al. 2016). Research shows that the presence of dams, often built for energy generation purposes, agricultural drainage causes environmental degradation, thus impacting the reproductive performance and behavior of shrimp such as *M. amazonicum* (Pantaleão et al. 2018; Silva et al. 2020).

Macrobrachium esculentum has a grey-black color with stripes along its abdomen and has 11-14 teeth on the upper rostrum and 2-4 teeth on the lower rostrum. The mtDNA fragment of this shrimp reaches 686 bp. This shrimp forms a clade with *M. esculentum* with GenBank data indicating this species's correctness. *M. esculentum* in the lower reaches of the Serayu River is under serious threat. The weir existence limits the distribution of this shrimp so that at station 01 (above the weir) *M. esculentum* is no longer found. The condition is exacerbated by the proportion of this shrimp sex ratio between males and females is 1.95: 1. The results showed the importance of constructing migration routes for aquatic biota in the Serayu Weir. The results of this study can be a reference in conservation management efforts of *M. esculentum*, which is threatened with extinction in the lower reaches of the Serayu River.

ACKNOWLEDGEMENTS

We want to thank PUSLAPDIK (Education Financing Service Center), the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia, BPI (Indonesian Education Scholarship) and LPDP (Education Fund Management Institute), the Ministry of Finance of the Republic of Indonesia, for financial assistance. We also thank the entire academic community at the Faculty of Fisheries and Marine Sciences, Universitas Diponegoro, Semarang, Indonesia, for all the support provided. We also

thank Prof. Abdul Ghofar was the initial supervisor of this research.

REFERENCES

- Ahmed ZF, Ahamed F, Rahman MM, Fatema MK. 2021. Spawning season, recruitment, and growth of the freshwater prawn *Macrobrachium lamarrei* (H. Milne-Edwards, 1837) in a perennial wetland, northeastern Bangladesh. *Nauplius* 29: 1-10. DOI: 10.1590/2358-2936e2021021.
- Akiba M, Sasaki T. 2020. Crustacea specimens of Ryukyu University Museum (Fujikan). Version 1.2. National Museum of Nature and Science, Japan. Occurrence Dataset 1. DOI: 10.15468/vdwqzo accessed via GBIF.org on 2020-11-19.
- Arinda ES, Wahyono HD, Santoso A. 2023. Penentuan status mutu air Sungai Serayu menggunakan teknologi online monitoring (Onlimo) dengan metode analisa storet. *Jurnal Manajemen Sumberdaya Perairan* 19: 102-113. DOI: 10.30598/TRITONvol19issue2page102-113. [Indonesian]
- Atminarso D, Lee JB, Robyn JW, Meaghan LR, Jennifer B, Arif W. 2023. Evidence of fish community fragmentation in a tropical river upstream and downstream of a dam, despite the presence of a fishway. *Pac Conserv Biol* 30 (1): 1-11. DOI: 10.1071/PC22035.
- Augusto A, Masui DC. 2014. Sex and reproductive stage differences in the growth, metabolism, feed, fecal production, excretion and energy budget of the Amazon River prawn (*Macrobrachium amazonicum*). *Mar Freshw Behav Physiol* 47 (6): 373-388. DOI: 10.1080/10236244.2014.942547.
- Beesley LS, Savannah K, Daniel CG, Bradley JP, Michael MD, Peter AN, Thiago CT, Chris SK, Mark JK, Caroline AC, Samantha AS. 2023. Modelling the longitudinal distribution, abundance, and habitat use of the giant freshwater shrimp (*Macrobrachium spinipes*) in a large intermittent, tropical Australian River to inform water resource policy. *Freshw Biol* 68 (1): 61-76. DOI: 10.1111/fwb.14009.
- Bertrand L, Monferrán MV, Mouneyrac C, Amé MV. 2018. Native crustacean species as a bioindicator of freshwater ecosystem pollution: A multivariate and integrative study of multi-biomarker response in active river monitoring. *Chemosphere* 206: 265-277. DOI: 10.1016/j.chemosphere.2018.05.002.
- Bhaumik U, Mukhopadhyay MK, Shrivastava NP, Sharma AP, Singh SN. 2017. A case study of the Narmada River system in India with particular reference to the impact of dams on its ecology and fisheries. *Aquat Ecosyst Health Manag* 20 (1-2): 151-59. DOI: 10.1080/14634988.2017.1288529.
- Burnett MJ, Bradley VZ, Colleen TD. 2023. The migration of aquatic macrocrustaceans over an artificial barrier in the Uthukela River, South Africa. *Afr J Ecol* 62 (1): 1-7. DOI: 10.1111/aje.13234.
- Chappell J, Kyle SM, Mary CF, Catherine MP. 2019. Long-term (37 Years) impacts of low-head dams on freshwater shrimp habitat connectivity in Northeastern Puerto Rico. *River Res Appl* 35 (7): 1034-43. DOI: 10.1002/rra.3499.
- Chen RT, Tsai CF, Tzeng WN. 2009a. 16S and 28S rDNA sequences in phylogenetic analyses of freshwater prawns (*Macrobrachium* Bate, 1868) from Taiwan. *J Crustac Biol* 29 (3): 400-412. DOI: 10.1651/08-3069.1.
- CBOL [Consortium for the Barcode of Life]. 2016. International Barcode of Life project (iBOL). Occurrence Dataset. DOI: 10.15468/inygc6 accessed via GBIF.org on 2020-11-19
- Darbohoesodo. 1987. Potensi Udang Air Tawar di Daerah Banyumas. Makalah Workshop tentang Potensi *Macrobrachium* spp. Pusat Antar Universitas Ilmu Hayati Institut Teknologi Bandung, Bandung. [Indonesian]
- De Grave S, Wowor D, Shy J. 2013. *Macrobrachium esculentum*. The IUCN Red List of Threatened Species 2013: e.T198185A2515154. DOI: 10.2305/IUCN.UK.2013-1.RLTS.T198185A2515154.en. [16 Oktober 2024]
- De Grave S. 2017. Global caridean shrimp fauna. Version 2.3. BioFresh. Occurrence dataset DOI: 10.13148/bfcf7 accessed via GBIF.org on 2024-10-16. <https://www.gbif.org/occurrence/1262422670>
- De Grave S, Kevin GS, Nils AA, Dave JA, Fernando A, Arthur A, Yixiong C, Savrina FC, Werner K, Fernando LM, Timothy JP, Jhy YS, José LV, Daisy W. 2015. Dead shrimp blues: A global assessment of extinction risk in freshwater shrimps (Crustacea: Decapoda: Caridea). *PLoS One* 10 (3): e0120198. DOI: 10.1371/journal.pone.0120198.
- De Melo MS, Masunari S. 2017. Sexual dimorphism in the carapace shape and length of the freshwater palaemonid shrimp *Macrobrachium potiana* (Müller, 1880) (Decapoda: Caridea: Palaemonidae): Geometric and traditional morphometric approaches. *Anim Biol* 67 (2): 93-103. DOI: 10.1163/15707563-00002522.
- de Oliveira LJF, Sant'Anna BS, Hattori GY. 2019. Population biology of the freshwater prawn *Macrobrachium brasiliense* (Heller, 1862) in the Middle Amazon Region, Brazil. *Trop Zool* 32 (1): 19-36. DOI: 10.1080/03946975.2018.1542195.
- Devi TSR, Shah DN. 2013. Evaluation of benthic macroinvertebrate assemblage for disturbance zonation in urban rivers using multivariate analysis: Implications for river management. *J Earth Syst Sci* 122 (4): 1125-1139. DOI: 10.1007/s12040-013-0317-8.
- Geller J, Meyer C, Parker M, Hawk H. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Mol Ecol Resour* 13 (5): 851-861. DOI: 10.1111/1755-0998.12138.
- González-Castellano I, González-López J, González-Tizón AM, Martínez-Lage A. 2020. Genetic diversity and population structure of the rockpool shrimp *Palaemon elegans* based on microsatellites: Evidence for a cryptic species and differentiation across the Atlantic-Mediterranean transition. *Sci Rep* 10: 10784. DOI: 10.1038/s41598-020-67824-7.
- Goud J, Van der Bijl B, Creuwels J. 2020. Naturalis Biodiversity Center (NL)-Crustacea. Occurrence Dataset. DOI: 10.15468/vjoltu accessed via GBIF.org on 2020-11-19
- Han CC, Lai CH, Huang CC, Wang IC, Lin H, Du, Wang WK. 2022. Phylogeographic structuring of the kuroshio-type prawn *Macrobrachium japonicum* (Decapoda: Palaemonidae) in Taiwan and Ryukyu Islands. *Diversity* 14 (8): 617. DOI: 10.3390/d14080617.
- Heim-Ballew H, Moody KN, Blum MJ, McIntyre PB, Hogan JD. 2020. Migratory flexibility in native Hawaiian amphidromous fishes. *J Fish Biol* 96: 456-468. DOI: 10.1111/jfb.14224.
- Hernawati R, Nurhaman U, Busson F, Suryobroto B, Hanner R, Keith P, Wowor D, Hubert N. 2020. Exploring community assembly among Javanese and Balinese freshwater shrimps (Atyidae, Palaemonidae) through DNA barcodes. *Hydrobiologia* 847 (2): 647-663. DOI: 10.1007/s10750-019-04127-7.
- Hiraga H, Azuma K, Kusaka T, Kinoshita I, Fujita S. 2021. Downstream drifting of *Macrobrachium* (Decapoda: Palaemonidae) larvae in the Shimanto river, Japan. *Plankton Benthos Res* 16 (4): 301-307. DOI: 10.3800/pbr.16.301.
- Huete-Perez JA, Ortega-Hegg M, Urquhart GR et al. 2016. Critical uncertainties and gaps in the environmental-and social-impact assessment of the proposed interoceanic canal through Nicaragua. *BioScience* 66 (8): 632-645. DOI: 10.1093/biosci/biw064.
- Hurwood DA, Dammannagoda S, Krosch MN, Jung H, Salin KR, Youssef MABH, Bruyn MDe, Mather PB. 2014. Impacts of climatic factors on evolution of molecular diversity and the natural distribution of wild stocks of the giant freshwater prawn (*Macrobrachium rosenbergii*). *Freshw Sci* 33 (1): 217-231. DOI: 10.1086/675243.
- Indarjo A, Salim G, Nugraeni CD, Zein M, Ransangan J, Prakoso LY, Suhiawan, Anggoro S. 2021. Length-weight relationship, sex ratio, mortality and growth condition of natural stock of *Macrobrachium rosenbergii* from the estuarine systems of North Kalimantan, Indonesia. *Biodiversitas* 22: 846-857. DOI: 10.13057/biodiv/d220239.
- Jarvis MG, Closs GP. 2019. Water infrastructure and the migrations of amphidromous species: Impacts and research requirements. *J Ecohydraulics* 4 (1): 4-13. DOI: 10.1080/24705357.2019.1611390.
- Jose D, Hari Krishnan M. 2019. Evolutionary history of genus *Macrobrachium* inferred from mitochondrial markers: A molecular clock approach. *Mitochondrial DNA Part A: DNA Map Seq Anal* 30 (1): 92-100. DOI: 10.1080/24701394.2018.1462347.
- Jose D, Nidhin B, Anil Kumar KP, Pradeep PJ, Hari Krishnan M. 2016. A molecular approach towards the taxonomy of fresh water prawns *Macrobrachium striatum* and *M. equidens* (Decapoda, palaemonidae) using mitochondrial markers. *Mitochondrial DNA* 27 (4): 2585-2593. DOI: 10.3109/19401736.2015.1041114.
- Jurniati, Arfiati D, Andriyono S, Hertika AMS, Kurniawan A, Tanod WA. 2021. The morphological characters and dna barcoding identification of sweet river prawn *Macrobrachium esculentum* (Thallwitz, 1891) from Rongkong watershed of south Sulawesi, Indonesia. *Biodiversitas* 22: 113-121. DOI: 10.13057/biodiv/d220116.

- Khan SR, Akter H, Sultana N, Khan MGQ, Wahab MA, Alam MS. 2014. Genetic diversity in three river populations of the giant freshwater prawn (*Macrobrachium rosenbergii*) in Bangladesh assessed by microsatellite DNA markers. *Intl J Agric Biol* 16 (1): 195-200.
- Kusbiyanto K. 2009. Bioekologi udang *Macrobrachium* spp. di Sungai Banjara Kabupaten Banyumas. *Biosfera* 26 (1): 23-29. [Indonesian]
- Lagarde R, Teichert N, Boussarie G, Grondin H, & Valade P. 2015. Upstream migration of amphidromous gobies of La Réunion Island: Implication for management. *Fish Manag Ecol* 22 (6): 437-449. DOI: 10.1111/fme.12142.
- Lázaro-Vázquez A, Castillo MM, Jarquín-Sánchez A, Carrillo L, Capps KA. 2018. Temporal changes in the hydrology and nutrient concentrations of a large tropical river: Anthropogenic influence in the lower Grijalva River, Mexico. *River Res Appl* 34 (7): 649-660. DOI: 10.1002/rra.3301.
- Molina WF, Costa GWWF, Cunha IMC, Bertollo LAC, Ezaz T, Liehr T, Cioffi MB. 2020. Molecular cytogenetic analysis in freshwater prawns of the genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae). *Intl J Mol Sci* 21: 2599. DOI: 10.3390/ijms21072599.
- Nascimento WM, Isis CD, Rayury SM, Pinheiro P. 2020. Sexual size dimorphism of the freshwater shrimp *Macrobrachium jelskii* (Miers, 1877) (Decapoda : Palaemonidae) and its relationship to rensch's rule. *Invertebr Reprod Dev* 64 (2): 106-114. DOI: 10.1080/07924259.2020.1726513.
- Nogueira CS, Perroca JF, Piantkoski EL, Costa RC, Taddei FG, Fransozo A. 2019. Relative growth and population dynamics of *Macrobrachium iheringi* (Decapoda, Palaemonidae). *Papéis Avulsos de Zoologia (São Paulo)* 59: e20195908. DOI: 10.11606/1807-0205/2019.59.08.
- Nogueira CS, Gois GVMR, Pescinelli RA, Costa RC. 2023. Different strategies and shapes: The relationship between mating system and sexual dimorphism in two freshwater prawn species. *NZ J Zool* 50 (2): 329-340. DOI: 10.1080/03014223.2022.2043394.
- Novak PA, Bayliss P, Crook DA, Garcia EA, Pusey BJ, Douglas MM. 2017. Do upstream migrating, juvenile amphidromous shrimps, provide a marine subsidy to river ecosystems ?. *Freshw Biol* 12 (January): 1-14. DOI: 10.1111/fwb.12907.
- Pantaleão JAF, Carvalho-batista A. 2018. The influence of environmental variables in the reproductive performance of *Macrobrachium amazonicum* (Heller, 1862) (Caridea: Palaemonidae) females in a continental population. *Anais Da Academia Brasileira de Ciências* 90 (2): 1445-1458. DOI: 10.1590/0001-3765201820170275.
- Pérez-Reyes O, Crowl TA, Covich AP. 2015. Effects of food supplies and water temperature on growth rates of two species of freshwater tropical shrimps. *Freshw Biol* 60: 1514-1524. DOI: 10.1111/fwb.12584.
- Pérez-Reyes O, Crowl TA, Covich AP. 2016. Comparison of decapod communities across an urban-forest land use gradient in Puerto Rican streams. *Urban Ecosyst* 19 (1): 181-203. DOI: 10.1007/s11252-015-0490-4.
- Pescinelli RA, Carosia MF, Pantaleão JAF, Simões SM, Costa RC. 2016. Population biology and size at the onset of sexual maturity of the amphidromous prawn *Macrobrachium olfersii* (Decapoda, Palaemonidae) in an urban river in southeastern Brazil. *Invertebr Reprod Dev* 60 (4): 254-262. DOI: 10.1080/07924259.2016.1202338.
- Pranoto WA, Johan J, Reynaldo. 2019. Study of suspended sediment transport discharge of Serayu River, Central Java, Indonesia in laboratory. *IOP Conf Ser: Mat Sci Eng* 650 (1): 012058. DOI: 10.1088/1757-899X/650/1/012058.
- Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PTJ, Kidd KA, MacCormack TJ, Olden JD, Ormerod, SJ, Smol JP, Taylor WW, Tockner K, Vermaire JC, Dudgeon D, Cooke SJ. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol Rev* 94 (3): 849-873. DOI: 10.1111/brv.12480.
- Ribeiro CC, Lopes VHP, Bertini G. 2020. Abundance and spatio-temporal distribution of the amphidromous shrimp *Macrobrachium olfersii* (Caridea: Palaemonidae) along the Ribeira de Iguape River (São Paulo, Brazil). *Nauplius* 28: e2020017. DOI: 10.1590/2358-2936e2020017.
- Rismawati R, Krisanti M, Farajallah A. 2024. Sexual dimorphism phenomenon of first record *Macrobrachium lar* (Fabricius, 1798) from the southern Sukabumi, West Java, Indonesia. *Biodiversitas* 25 (5): 1929-1937. DOI: 10.13057/biodiv/d250509.
- Rocha SSda, Barbosa RdeJ. 2017. Population biology of *Macrobrachium jelskii* (Miers, 1877) (Decapoda, Palaemonidae) from an artificial pond in Bahia, Brazil. *Nauplius* 25 (1): e2017023. DOI: 10.1590/2358-2936e2017023.
- Santos MNM, Wowor D, Ikeda M, Padilla PI, Romana-Eguia MR. 2024. Morphological and genetic diversity assessment of freshwater prawns (*Macrobrachium* spp.) in the Cairawan River, Antique Province, Panay Island, Philippines. *Phillipp J Fish* 31 (1): 1-14. DOI: 10.31398/tjpf/31.1.2023-0009.
- Savaya AAT, Ohad R, Susanne HS, Yacinthe PWF, Djibril SF, Eliahu DA, Nicolas J, Dina Z, Elizabeth H, Amir S. 2014. The prawn *Macrobrachium vollenhovenii* in the Senegal River Basin: Towards sustainable restocking of all-male populations for biological control of schistosomiasis. *PLoS Negl Trop Dis* 8 (8): e3060. DOI: 10.1371/journal.pntd.0003060.
- Senckenberg. 2004. *Collection Crustacea-ZMB*. Occurrence Dataset. DOI: 10.15468/fwghff accessed via GBIF.org on 2020-11-19.
- Secor WE. 2014. Water-based interventions for schistosomiasis control. *Pathogens Glob Health* 108 (5): 246-254. DOI: 10.1179/2047773214Y.0000000149.
- Senckenberg. 2004. *Collection Crustacea-ZMB*. Occurrence Dataset DOI: 10.15468/fwghff. <https://www.gbif.org/occurrence/251627986>. [19 November 2020]
- Sethi SN, Ram N, Venkatesan V. 2014. Reproductive biology of *Macrobrachium lar* (Fabricius, 1798) in Andaman Islands. *Indian J Geo-Mar Sci* 43 (12): 2269-2276.
- Short JW. 2004. A revision of Australian river prawns, *Macrobrachium* (Crustacea: Decapoda: Palaemonidae). *Hydrobiologia* 525 (1-3): 1-100. DOI: 10.1023/B:HYDR.0000038871.50730.95.
- Silva GMF, Liziane BG, Marcelo CA, Breno RMS, Ingrid SP, Rossineide MR, Maria APF. 2020. Has a river dam affected the life-history traits of a freshwater prawn. *Ecol Evol* 10 (13): 6536-6548. DOI: 10.6536-6548.
- Silva RCE, Marina CC, Emerson CM, Giuliano BJ. 2019. Population structure of *Macrobrachium amazonicum* (Heller, 1862) (Decapoda: Palaemonidae) in Miranda hydroelectric plant reservoir, Araguari River, Minas Gerais, Brazil. *Acta Limnol Brasiliensia* 31: 1-12. DOI: 10.1590/s2179-975x4318.
- Silva-Junior EF, Silva-Araújo M, Moulton TP. 2017. Distribution and abundance of freshwater decapods in an Atlantic rainforest catchment with a dammed future. *Braz J Biol* 77 (4): 820-829. DOI: 10.1590/1519-6984.01916.
- Sinaga S, Yunita M, Arning WE, Maheno SW. 2024. The impact of artificial barriers on the *Varuna litterata* migration route in the lower Serayu River, Central Java and its molecular identification. *Omni-Akuatika* 20 (1): 50-60. DOI: 10.20884/1.0a.2024.20.1.1140.
- Siregar AS, Sinaga TP, Setjanto. 2001. Studi ekologi fauna benthik (*Macrobrachium* spp) pada Sungai Banjara, Pelus dan Logawa di Kabupaten Banyumas. *Biosfera* 3 (1): 1-6. [Indonesian]
- Sokolow SH, Jones IJ, Jocque M, La D, Cords O, Knight A, Lund A, Wood CL, Lafferty KD, Hoover CM, Collender PA, Remais JV, Lopez-Carr D, Fisk J, Kuris AM, de Leo GA. 2017. Nearly 400 million people are at higher risk of schistosomiasis because dams block the migration of snail-eating river prawns. *Philos Trans R Soc B: Biol Sci* 372 (1722): 20160127. DOI: 10.1098/rstb.2016.0127.
- Taddei FG, Reis SDS, David FS, Da Silva TE, Fransozo V, Fransozo A. 2017. Population structure, mortality, and recruitment of *Macrobrachium amazonicum* (Heller, 1862) (Caridea: Palaemonidae) in the eastern Amazon region, Brazil. *J Crustac Biol* 37 (2): 131-141. DOI: 10.1093/jcbiol/rux006.
- Ukagwu JI, Deekae S. 2016. Sex population structure of *Macrobrachium felicinum* and *Macrobrachium vollenhovenii* in the Akor river, Ibere Ikwuano, Abia State. *Intl J Fish Aquat Stud* 4 (4): 19-23.
- Wowor D, Muthu V, Meier R, Balke M, Cai Y, Peter KLN. 2009. Evolution of life history traits in asian freshwater prawns of the genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae) based on multilocus molecular phylogenetic analysis. *Mol Phylogenet Evol* 52 (2): 340-350. DOI: 10.1016/j.ympev.2009.01.002.