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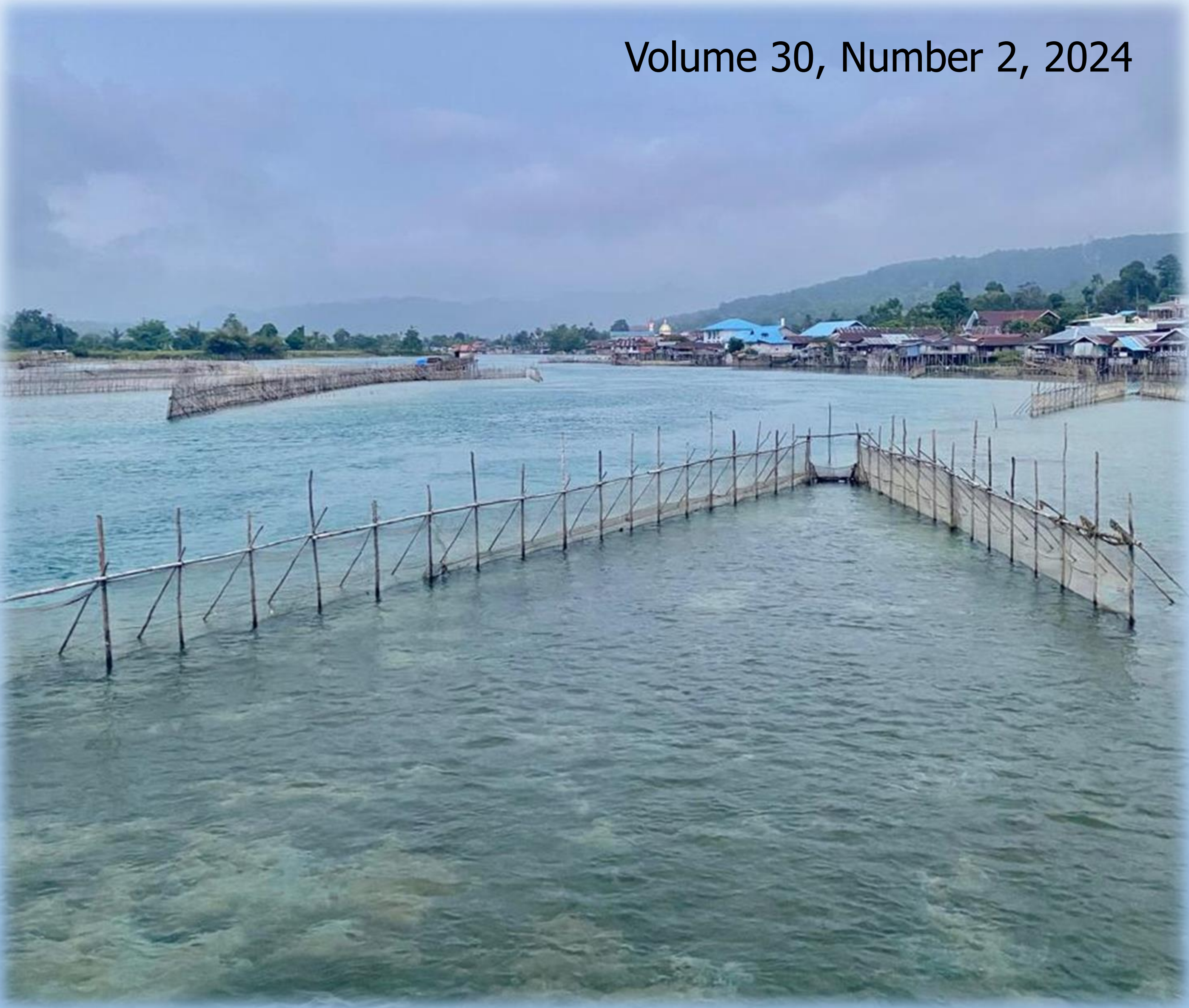


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**Cover Image:** "*Waya Masapi*," the traditional fishing gear used for catching eels in Lake Poso, courtesy of Prof. Dr. Gadis Sri Haryani (Research Centre for Limnology and Water Resources, National Research and Innovation Agency - BRIN, Indonesia).

# SERTIFIKAT

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# Morphometric and Genetic Variations of Freshwater Eels (*Anguilla* spp.) in Poso River, Central Sulawesi: Implications for Conservation Strategies

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**Abstract:** The freshwater eel, Anguillids, is a valuable nutrition and commodity fish found in various freshwater environments. However, the world's population of Anguillids is declining because of habitat degradation, pollution, and barriers to migration, all of which are prevalent threats in freshwater ecosystems such as the Poso River in Central Sulawesi. Establishing conservation areas is one of the efforts to protect eels and their habitats, which requires information on the anguillid's morphometrics and genetics, where high morphometric and genetic variations are indicators of adaptation or evolution of the species to survive environmental changes. Therefore, the study aims to assess the morphometric and genetic variations s in the Poso River, Central Sulawesi. Samples were collected in May 2021 and August 2023 along the Poso River. Different fishing gears were used depending on the location and the eel's phase of life. 150 eel samples were used for morphometric analysis, of which 38 were selected randomly for the genetic one. Genetic diversity analysis was performed using Cytochrome c Oxidase I (COI). The study identified three species: *A. bicolor*, *A. celebesensis*, and *A. marmorata*. The key characteristic distinguishing the three species was ADL/TL ratio. Most coefficients of variation of morphometric characters of each species were above 10%, indicating medium to high variation. A total of 11 haplotypes were identified, of which six belong to *A. marmorata* and five to *A. celebesensis*. Generally, haplotype diversity was low, ranging from 0.2923 to 0.9333, and nucleotide diversity ranged from 0.0005 to 0.0046. The low genetic diversity observed in this study is likely a result of the migratory nature of Anguillid eels. Morphometric and genetic variations can support restocking as a conservation strategy to bolster wild populations. However, comprehensive studies must be conducted to understand all aspects impacting Anguillid resources and establish conservation areas to protect their populations and habitats.

**Keywords:** Freshwater eel conservation, Anguillids, taxonomy validation, DNA barcoding, COI gene, genetic diversity

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## 1. Introduction

The freshwater eel, Anguillids, is a migratory fish species inhabiting various habitats from the ocean to the river ecosystem (Tsukamoto and Arai, 2001). Anguillids migrate and metamorphose as catadromous fish, beginning by spawning in the deep sea, where eggs hatch into larvae called leptocephalus. The larva floats towards the coast and estuary then transforms into an eel-like phase called glass



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eel. The river serves as a habitat for elvers and yellow eels for physical growth and gonad maturation, leading to the silver eel phase. Once the Anguillids eel reaches maturity, they migrate back to the ocean to spawn once in their lifetime (Kurogi *et al*., 2011).

Anguillids have high nutritional values, especially protein, fat, and vitamins A and E. Wijayanti *et al*. (2018) highlighted that the protein content of *A. bicolor* reached 17.51%, while Bote *et al*. (2024) mentioned that *A. anguilla* contains about 271.6 grams of protein per kilogram. As a high-value food commodity, the global demand for Anguillids continues to rise. The primary consumers are Japan, South Korea, China, parts of Southeast Asia and Europe, and the United States and Canada, with Japan leading the import market by bringing in 60,000 tons in 2002 (FAO, 2009). In 2012 – 2013, Japan's consumption was still the highest, estimated at 30-45% of global eel production (Shiraishi and Crook, 2015).

Despite their high economic value, the population of Anguillids is decreasing worldwide, particularly in subtropical regions. The juvenile abundance dropped by 99% for European and 80% for Japanese eels (Dekker, 2003). According to IUCN (Pike *et al*., 2020), 10 out of 20 species worldwide are endangered (EN) or critically endangered (CR). Indonesia is a tropical country and has nine different species/sub-species of freshwater eels, four of which are found in the Poso waters: *A. marmorata*, *A. celebesensis*, *A. interioris*, and *A. bicolor pacifica* (Sugeha *et al*., 2008; Fahmi *et al*., 2012). Among these species, *A. bicolor* is categorized as near threatened (NT), while the other three species are least concerned (LC) and data deficient (DD). Therefore, it is crucial to carry out further research to ensure their conservation status. Unfortunately, freshwater eel stocks in Poso waters have declined due to overfishing of the broodstock in Tentena (outlet of Lake Poso), not eco-friendly glass eel fishing at the estuary of the Poso River, and the construction of a dam for the Hydroelectric Power Plant in Sulewana, which has cut off the freshwater eels' migration path (Krismono and Kartamihardja, 2012).

Numerous studies have been conducted on freshwater eels in Poso waters, focusing on conservation, recruitment, and capture fisheries. Additionally, genetic studies have targeted genes such as D-loop, Cyt b, and 16S rRNA (Triyanto *et al*., 2008; Sugeha *et al*., 2008; Fahmi, 2015;). However, most of these studies have only taken samples from Lake Poso or the Poso River (estuary). More information on morphometric and genetic variation is needed, using mtDNA markers with COI target genes and wider sampling locations within the Poso River. DNA barcoding is the most commonly used and effective method for identifying fish species and validating taxonomy (Bhattacharya *et al*., 2015). The benefits include its ability to identify species when traditional morphological approaches fail, such as during the larvae phase, from partial specimens, or when dealing with damaged samples (Ward *et al*., 2009).

Morphometric and genetic information are crucial for fisheries management when creating conservation strategies. The information derived from genetics confirms taxonomy, which is a critical first step in species conservation (Fahmi, 2015). Moreover, morphometric and genetic analysis can help evaluate population structure and identify stocks for restocking and determining conservation zones to prevent genetic homogenization (Mojekwu and Anumudu, 2015 ; Pimentel *et al*., 2020;). Therefore, the study aims to assess the morphometric and genetic variations and their implications for eel conservation strategies in the Poso River, Central Sulawesi.

## 2. Materials and Methods

### 2.1 Location and Sampling

Samples were collected from three locations along the Poso River, Central Sulawesi (Figure 1), and conducted from May 2021 to August 2023 with varying times for each station. The sampling of eels in Poso 1 was carried out in May–June 2021, January–February and July–December 2022, and January–July 2023, while in Poso 2 and 3, it was only done in 2023, with June–July and May–August, respectively.

Different fishing gears were used to catch Anguillids depending on the sampling location and their phase of life. *Waya Masapi* was used to catch yellow eel in Poso 1 (outlets of Lake Poso), longline and folding traps were used for Poso 2 (middle part of the Poso River), and ATG

(*Gorong-gorong* fishing gear), a local fish trap, was used to catch for glass eel in the estuary, Poso 3 sampling site (Figure 2).

The Anguillids caught at each location were then randomly subsampled, resulting in 150 for morphometric analysis. The yellow eel was directly measured at the research site, while the glass eel was measured at the BRIN Laboratory in Cibinong West Java. Additionally, 38 samples, excluding *A. bicolor*, were randomly chosen and underwent a comprehensive genetic analysis at the BRIN Laboratory in Cibinong.

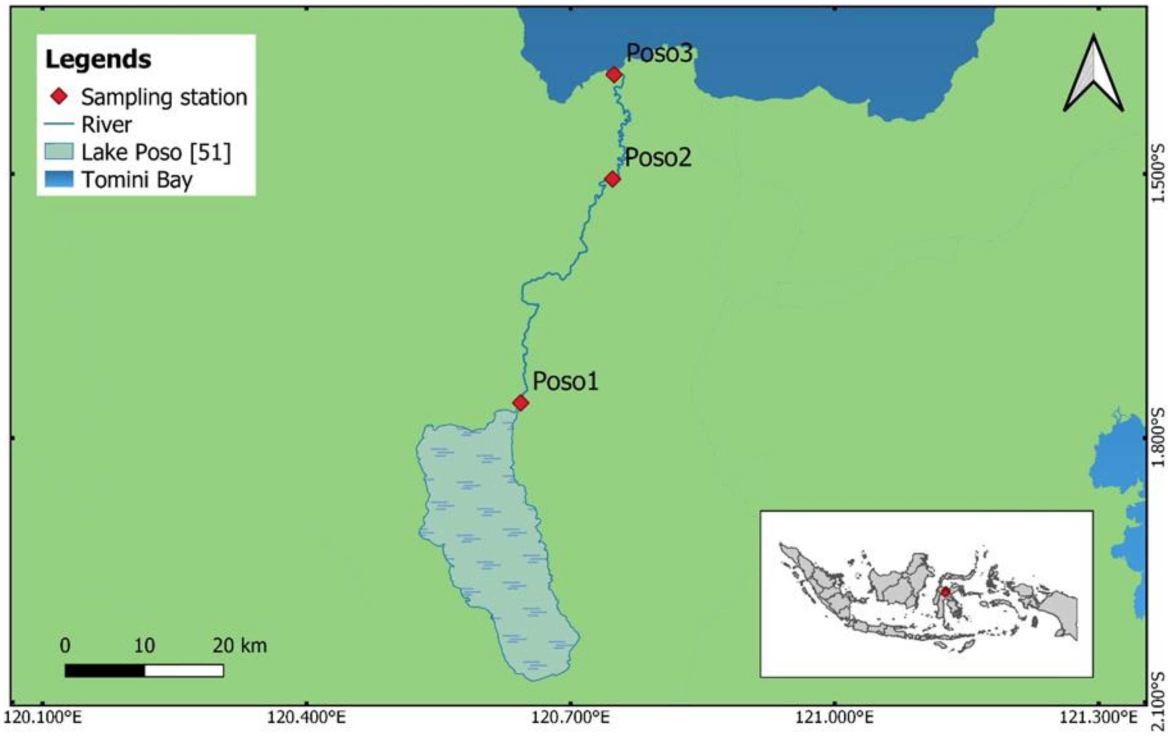



Figure 1. Map of Sampling locations in Poso River, Sulawesi Island, Indonesia

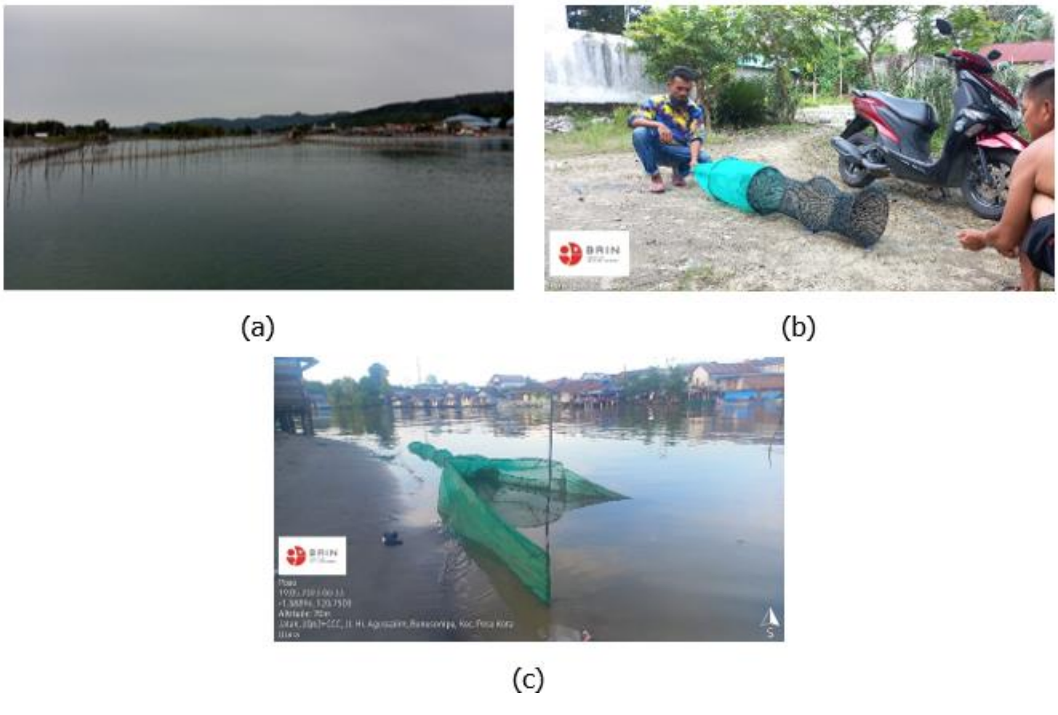


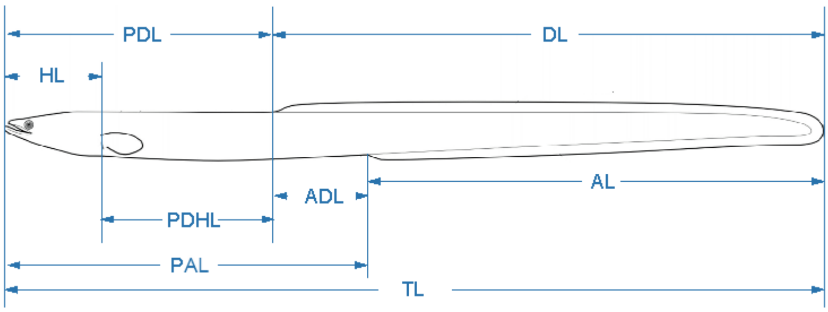

Figure 2. Fishing gears used for catching the Anguillids: (a) *Waya Masapi*/fence trap, (b) folding trap (modified fish trap), and (c) ATG (*Alat Tangkap Gorong-Gorong*)

The Anguillids caught at each location were then randomly subsampled, resulting in 150 for morphometric analysis. The yellow eel was directly measured at the research site, while the glass eel was measured at the BRIN Laboratory in Cibinong West Java. Additionally, 38 samples, excluding *A. bicolor*, were randomly chosen and underwent a comprehensive genetic analysis at the BRIN Laboratory in Cibinong.

## 2.2. Morphometric Analysis

Seven morphometric characteristics (Figure 2) were measured for the yellow eel using a ruler with 1 mm precision, while a macro microscope was operated for the glass eel. Based on Schindler and Schmidt (2006), measurement data was transformed using the following formula: Mtrans is the transformation result data, M is the measurement data, and TL is the Total Length).

$$Mtrans = \frac{(M \times 100)}{TL}$$




The freshwater eel morphometric characteristics (Silvergrip, 2009, modified). HL: Head Length, PDHL: Pre-Dorsal Head Length, PDL: Pre-Dorsal Length, DL: Dorsal Length, PAL: Pre-Anal Length, AL: Anal Length, and ADL: Ano-Dorsal Length.

The transformation data was then analyzed using the Kruskal-Wallis test to determine the effect of species differences on morphometrics. Further, Mann-Whitney U tests were conducted to identify the key characteristics distinguishing different species. Finally, discriminant analysis was applied to analyze the relationships between the different species based on morphometric characteristics. The entire morphometric analysis was conducted using SPSS 2016 (Shin *et al.*, 2022) for all the statistics tests and PAST 4.03 (Hammer *et al.*, 2001) software for running the discriminant analysis.

## 2.3. Genetic Analysis

### 2.3.1. Tissue Sampling

A total of 38 samples were collected for genetic analysis. Tissue samples were taken from either a yellow eel's pectoral fin or a glass eel by cutting approximately 1 cm with a sterile scissor, then preserved in a pro-analytic ethanol solution. Subsequently, all samples were continued for the DNA extraction.

### 2.3.2. DNA Extraction

The DNA extraction was performed using the gSYNC$^{TM}$ DNA Extraction Kit (Geneaid, Taiwan), following the manufacturer’s protocol ver. 09.14.23. DNA concentration and purity were measured using Thermo Scientific NanoDrop Spectrophotometers based on Desjardins and Conklin (2010). The DNA was stored at -20°C for subsequent use.

### 2.3.3. PCR Amplification, Sequencing, and Analysis

The entire phase, including the primers selection, amplification, and visualization of PCR results, was carried out based on Ward *et al.* (2005) with modifications and optimization following the protocol of the product provider. Amplification of the COI gene on mtDNA using primary Fish F1 (5'TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGG-AC3') and Fish R1 (5'TAG-ACT-TCT-GG G-TGG-CCA-AAG-AAT-CA3'). A total of 25 µl of PCR reaction volumes were prepared by considering the volume ratio of each reagent according to the Thermo Scientific DreamTaq DNA Polymerase User Guide 2022. This consisted of 19.9 µl of Nuclease-free water, 2.5

µl of 10X PCR buffer, 0.5 µl of 10 mM dNTP, 0.5 µl of 10 pM each primer, 0.1 µl of 5 U/µ Taq DNA polymerase, and 1 µl DNA sample. The temperature was adjusted according to the following steps: initial denaturation of 2 minutes at 95°C, continued with 35 cycles denaturation of 30 seconds at 94°C, annealing of 30 seconds at 52°C, extension of 1 minute at 72°C, and the final extension of 10 minutes at 72°C was executed after those all cycles. The temperature was then held at 12°C. PCR products were visualized on 1.5% agarose gel by electrophoresis at 100 volts for approximately 30 minutes. The PCR product was sent for Sanger sequencing, with one part sent to 1st BASE Laboratories in Malaysia and another to the Center Laboratory of Sequencing BRIN using "E-Layanan Sains".

The DNA sequencing results were analyzed and modified using MEGA XI software version 11.0.13 (Kumar *et al*., 2008). In this analysis, additional sequences from GenBank were also used to confirm and compare intra-species and inter-species within the family and inter-family within the order. The accession numbers of these sequences include MW275927 and OR674041 for *A. marmorata*, OQ137029 for *A. celebesensis*, NC006536 for *A. borneensis*, and GU674219 for *Uroconger lepturus* (Family: Congridae).

The sequences were then compared with those in the NCBI (https://www.ncbi.nlm.nih.gov/) and BOLD (https/www.boldsystems.org/) databases by aligning them. The Kimura-2-parameter (K2P) model in MEGA XI software was used to estimate intra and interspecific genetic distances. The COI gene phylogenetic tree was constructed using the Neighbour Joining (NJ) method with 1000 bootstrap replications set on the Kimura-2-parameter model (K2P). In addition, DNASP 5.10 software was used to examine haplotype distribution and other genetic diversity analyses (Librado and Rozas, 2009).

## 3. Results and discussion

### 3.1. Morphometric

Morphometric analysis was conducted on three species of Anguillids, with sample sizes of 5 (*A. bicolor*), 34 (*A. celebesensis*), and 111 (*A. marmorata*). *Anguilla bicolor*, the least common among the three identified eel species, was found in limited numbers, with only five individuals discovered. This scarcity may be attributed to the brief sampling period at the mouth of the Poso River, which spanned only four months. Arai *et al*. (2001) revealed that *A. bicolor* in the Poigar River of North Sulawesi was only present during specific months due to variations in the duration of the leptocephalus metamorphosis phase and the age at recruitment of each species. The low number of *A. bicolor* individuals caught in Sulawesi waters, including in this study, suggests that the natural population of this species is limited compared to *A. marmorata* and *A. celebesensis* (Arai *et al*., 2001 and Sugeha *et al*., 2001).

The Total Length (TL) measured ranged from 36.22 to 1,315.00 mm. The Kruskal-Wallis test indicated a significant influence of species differences on morphometric characteristic variations ($p < 0.05$). Subsequently, the Mann-Whitney U test revealed that only one morphometric characteristic (ADL) differs significantly between the three species, as indicated in Table 1. *Anguilla bicolor* shares six morphometric characteristic similarities (HL, PDHL, PDL, DL, PAL, AL) with *A. celebesensis* and two similarities (PDL and DL) with *A. marmorata*. However, all seven morphometric characteristics in *A. celebesensis* differ relatively from *A. marmorata*.

It has been observed that there are many similarities between Anguillid species, which makes it difficult to distinguish them based on morphometric characteristics alone. Commonly, several Anguillid species have similar or overlapping morphometric measures. Sugeha and Suharti (2008) confirmed that distinguishing *A. celebesensis* and *A. interioris* can be challenging. Morphological analysis showed that all Anguillids were classified as *A. celebesensis*. However, the genetic analysis revealed that one sample was A. *interioris*.

Morphological similarities frequently appear in two or more species in the same habitats. The shape and size of a fish's body parts are closely linked to their environment. Environmental factors such as food can influence the Anguillid's size of fin and head in a habitat (Watanabe *et al*., 2009). By comparing the size morphometric characteristics of these three species, it can be concluded that *A. bicolor* and *A. celebesensis* share similar habitats, while *A. marmorata* occupies a distinct habitat.

Seven morphometric characteristics describe the size of the dorsal, anal, and caudal fins. Fish fins generally play a vital role in regulating their stability while swimming. According to Chalchisa (2023), the shape and size of fins are related to the fish's behavior, especially movement. Additionally, the habitat or physical condition of the water, such as the boundary in the water, is also related to the fish's fin appearance. *Anguilla bicolor* and *A. celebesensis* have greater DL and AL to TL ratios than *A. marmorata*, indicating more active movements due to survival in challenging physical habitats.

Table 1. The Average (± SD) of transformed morphometric characteristic data for three Anguillid species in Poso River. Averages ± SD on the same line with different superscripts indicate significant differences ($p < 0.05$). All morphometric values are in per cent (%), except TL in millimetres (mm)

| **Characteristic Code** | **Species** | | |
|---|---|---|---|
| | ***A. bicolor*** | ***A. celebesensis*** | ***A. marmorata*** |
| HL | $11.04 \pm 1.97^{a}$ | $11.86 \pm 1.10^{a}$ | $13.40 \pm 1.71^{b}$ |
| PDHL | $22.59 \pm 11.42^{a}$ | $15.99 \pm 1.74^{a}$ | $6.09 \pm 6.50^{b}$ |
| PDL | $41.56 \pm 8.67^{ab}$ | $43.30 \pm 4.04^{a}$ | $57.60 \pm 5.50^{b}$ |
| DL | $58.44 \pm 8.67^{ab}$ | $56.70 \pm 5.04^{a}$ | $42.40 \pm 4.50^{b}$ |
| PAL | $46.49 \pm 12.34^{a}$ | $52.14 \pm 5.28^{a}$ | $72.89 \pm 7.65^{b}$ |
| AL | $53.51 \pm 12.34^{a}$ | $47.86 \pm 4.28^{a}$ | $27.11 \pm 2.65^{b}$ |
| ADL | $1.86 \pm 0.65^{a}$ | $9.53 \pm 9.29^{b}$ | $17.84 \pm 1.54^{c}$ |
| N | 5 | 34 | 111 |
| TL (min-max) * | 48.37 - 910.00 | 37.52 - 1,110.00 | 36.22 - 1,315.00 |

According to the research conducted by Itakura and Wakiya (2020), *A. marmorata* tends to prefer riverbank habitats with vegetation and avoids waters with concrete substrates and sand. The study also found that the river's depth and velocity influence the Anguillid's size. Small-sized Anguillids (less than 24 cm) prefer riverine habitats with fast currents, while larger ones can be found in any depth and current. On the other hand, *A. bicolor* prefers marshy habitats and is commonly found in narrow and short rivers, as creeks with deeper rock-bottom waters and pools, but rarely in large rivers (Menon, 1999; Pethiyagoda, 1991; Arai and Kadir, 2017).

The morphological characteristic also includes the Anguillid's head because it is related to the size of some organs, such as the mouth. The size of the mouth consequently affects their feeding behavior and environment. Lammens and Visser (1989) reported that the breadth of the mouth in *A. anguilla* is adaptable to their environmental conditions, such as the size and availability of prey. They prefer an appropriate habitat based on their physical condition and function. Upon comparing the two groups of Anguillids, it is evident that the head size of *A. marmorata* is greater than that of *A. bicolor* and *A. celebesensis*. However, this does not necessarily imply that *A. marmorata* prey on larger animals than the other two species. *A. bicolor* and *A. marmorata* prey on relatively similar animals, with crabs and shrimps being their dominant prey (Sidqi *et al.*, 2018; Romanda *et al.*, 2019). Hence, further studies are required to confirm this, specifically regarding the size of the mouth breadth of each species.

The Discriminant Function Analysis (DFA) has identified two functions: Function 1 has an eigenvalue of 3.331 and explains 99.48% of all variances, while Function 2 has a 0.017 and 0.52%. Function 1 has two high-loading values, ADL and PDHL (0.967 and -0.316, respectively), while Function 2 has three high-loading values, AL, DL, and HL, as shown in Table 2. Function 1 significantly impacts the differences between the three species. It has an eigenvalue (EV) of 3.331, 99.5% of the variance, and a correlation coefficient 0.877. Among the morphometric characteristics within Function 1, ADL has the highest loading value of 0.967, significantly different from other characteristics. Therefore, ADL could be a key identification feature that distinguishes the three species.

Table 2. The eigenvalue, % variance, and DFA loading of morphometric characteristics in the Poso River. Characteristics with high loading are marked with an asterisk.

| **Function** | **1** | **2** |
|---|---|---|
| Eigenvalue | 3.331 | 0.017 |
| Percentage Variance (%) | 99.48 | 0.52 |
| ADL | 0.967* | -0.075 |
| PDHL | -0.316* | -0.307 |
| AL | -0.275 | -0.782* |
| PAL | 0.275 | 0.782* |
| DL[a] | -0.165 | -0.601 |
| PDL[a] | 0.165 | 0.601 |
| HL | 0.253 | 0.400* |

[a]) This variable was not used in the analysis.

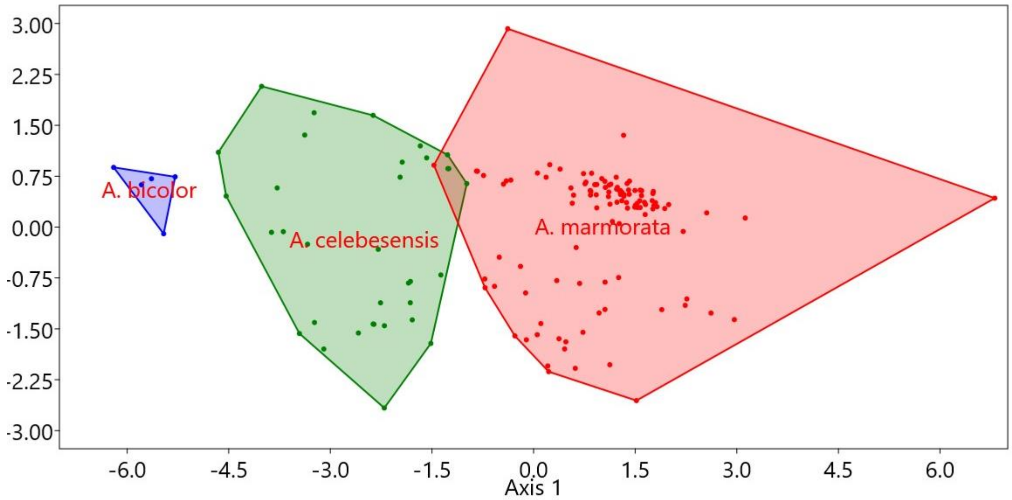



Figure 4. The Scatter Plot Function 1 and Function 2 of the three Anguillids morphometric characteristics. Different colors of the convex hulls represent each species: blue (*A. bicolor*), green (*A. celebesensis*), and red (*A. marmorata*).

According to Ege (1939), the range of AD/TL ratio in some Anguillid species is as follows: *A. bicolor* pacifica -6 – 3 %, *A. bicolor* bicolor: -3 – 4%, *A. celebesensis*: 6 – 12%, and *A. marmorata*: 12 – 20%. Some Anguillid samples analyzed in this study overlapped between species or were outliers. This variation can be caused by an individual's adaptation to their habitat, commonly called phenotypic plasticity. West-Ebenhard (2003) defines phenotypic plasticity as the ability of a genotype to produce more than one morphology, physiology, or behavior in response to environmental conditions. Different habitats will cause individual morphological differences, even within one species.

The DFA scatter plot shows that the three Anguillids species are separated into distinct groups, slightly overlapping *A. celebesensis* and *A. marmorata* (Figure 4). *Anguilla bicolor* is a distinct group, with its unique AD/TL ratio not overlapping with other species. This ratio is the most significant contributor to the composition of Function 1, as displayed by axis 1 on the graph. In contrast, *A. celebesensis* and *A. marmorata* share an AD/TL ratio of 0.12, with *A. celebesensis* at the upper limit and *A. marmorata* at the lower limit. Watanabe (2003) notes that *A. celebesensis* has an AD/TL ratio of 0.06–0.12, while *A. marmorata* has a ratio of 0.12–0.20. Generally, the ADL can separate these three groups. However, the grouping or species identification will be more precise when considering other morphological organs, such as tooth bands and vertebrae (Silvergrip, 2009).

Besides their measurable morphometric characteristics, skin appearance can be used to differentiate between Anguillid species. By direct observation, Anguillids can be divided into plain and patterned groups. Among the three species, *A. bicolor* can be distinguished from *A. marmorata* and *A. celebesensis* by their skin. *Anguilla bicolor* has plain skin with darker or black on the dorsal side, while the ventral side is lighter or white. On the other hand, *A. celebesensis* and *A. marmorata* have the patterned skin. Although the pattern is almost the same, it is still relatively easy to differentiate them morphologically by the ratio of ADL and TL. Nonetheless, validation with genetic analysis is necessary due to the high similarity of morphometric and overlapping key characteristics in some species, as conducted in this study.

Table 3 The coefficient of variation for three eel species in the Poso River.

| **Species** | **Coefficient of Variance (%)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **HL/TL** | **DHL/TL** | **PDL/TL** | **DL/TL** | **PAL/TL** | **AL/TL** | **ADL/TL** |
| *A. bicolor* | 17.87 | 50.57 | 20.87 | 14.84 | 26.54 | 23.06 | 35.03 |
| *A. celebesensis* | 9.28 | 67.14 | 43.98 | 33.59 | 36.97 | 40.28 | 24.01 |
| *A. marmorata* | 12.75 | 123.18 | 37.32 | 50.70 | 25.59 | 68.81 | 14.24 |

Morphometric variation in each eel species can be seen from the coefficient of variance (Table 3); < 10% means low variation, 10-30% medium, and > 30% means high variation (Sokal and Rohlf, 2012). Almost all morphometric parameters in the three species showed moderate to high variation; only the HL/TL ratio of *A. celebesensis* showed low variation. As explained in the discussion earlier, head size is related to the size of other organs in the head, such as the mouth. In this case, it is presumed that the prey size of *A. celebesensis* in all sampling locations is relatively the same despite the differences in habitat.

Anguillid are considered carnivorous. *Anguilla bicolor* feeds on fish, worms, crabs, and shrimp (Sidqi *et al.*, 2018); the same was found in *A. marmorata* (Hartanto *et al.*, 2015). The feeding habits of fish may change, influenced by age, availability, and density of food sources in the water. Eels feed on invertebrates when small and become fish eaters when more significant (Rupasinghe and Attygalle, 2006). In this study, the identified samples of *A. celebesensis* were dominated by glass eel, which influenced the calculation results of the relatively small variation in the HL/TL ratio compared to *A. bicolor* and *A. marmorata*. Although there was high variation within each species, it did not lead to species differences that have been confirmed in subsequent molecular discussions.

### 3.1. Genetic

A genetic analysis was conducted on 38 samples presumed to be species of *A. celebesensis* and *A. marmorata* based on their morphometric characteristics. Samples of *A. bicolor* were excluded from the analysis because this species can be easily identified based on its skin pattern.

Table 4. Species validation using BLAST and BOLD

| **No.** | **N** | **Species** | **Similarity (%)** | |
|---|---|---|---|---|
| | | | **BLAST** | **BOLD** |
| 1 | 32 | *A. marmorata* | 98.28–100 | 99.52–100 |
| 2 | 6 | *A. celebesensis* | 98.93–99.85 | 94.18–100 |

Validation of species through BLAST and BOLD databases revealed similarity values ranging from 94.18% to 100%, confirming the validity of both species (Table 3). Bhattacharjee *et al.* (2012) classified the similarity range between the query and the database sequence into three groups: 97%–100% (significant), 92%–96% (moderate), and ≤91% (insignificant).

According to the BLAST and BOLD databases, *A. marmorata* had the lowest similarity percentages of 98.28% and 99.52%, respectively, indicating significant similarity. *Anguilla celebesensis* also showed a significant similarity of 98.93% in the BLAST database. However, in the BOLD database, *A.*

*celebesensis* had the lowest similarity of 94.18%, indicating moderate similarity (Table 4). Overall, these species were validated as such. The lower similarity percentages in BOLD were due to the need for more sufficient data compared to BLAST. In some cases, such as *A. celebesensis*, BOLD did not have more sequence variations than BLAST. The BOLD database was pre-curated, and then sequences were uploaded. On BLAST, anyone could upload the result of a species sequence without curation (Meiklejohn *et al*., 2019).

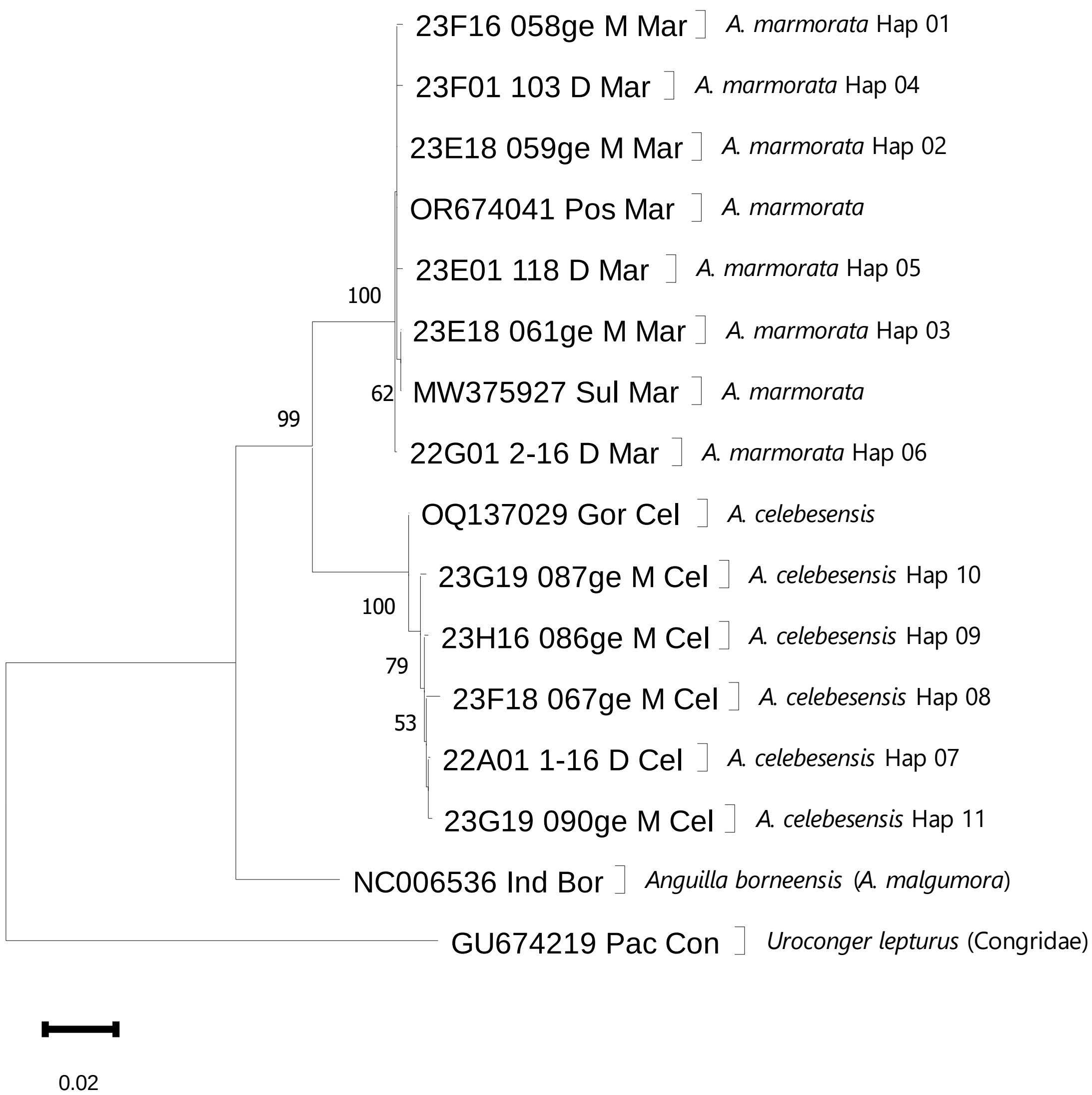



Figure 5. The phylogenetic tree grouped by haplotype in the Poso River. The branch number shows the NJ bootstrap's confidence level (1000 replications).

The reconstruction of the phylogenetic trees illustrated that the Anguillids samples were divided into two clades or species: *A. marmorata* and *A. celebesensis*, which were further grouped into six and five clusters, respectively (Figure 5). Sequences from the BLAST-NCBI database were used to confirm both species. Accession numbers MW275927 and OR674041 correspond to *A. marmorata*, and accession number OQ137029 corresponds to *A. celebesensis*. In addition, the accession number NC006536 for *A. borneensis* is used as a comparison of another species in the same family (Anguillidae), and the accession number GU674219 for the *Uroconger lepturus* of another family (Congridae) in the same order Anguilliformes.

The phylogenetic tree is constructed using the haploid of each species to simplify the appearance. The 0.01 scale represents a genetic change of 1 per 100 nucleotide sites in Figure 5. The length of the horizontal line on the branch indicates the degree of change, with longer lines representing greater changes and shorter lines indicating less change. The percentage value of each node reflects the large-scale support of the node. This trust value depends on the ratio of samples in each clade; the larger the sample size, the greater the trust value in the nodes. *Anguilla celebesensis* showed a lower percentage than *A. marmorata* because the sample size analyzed was smaller, with 6 and 32 in total samples, respectively.

Table 5 presents the results of a detailed genetic distance analysis on 11 haplotypes and several sequences from GenBank as comparators. The genetic distance between *A. marmorata* from GenBank and haplotypes 01–06 (*A. marmorata*) ranges from 0 to 0.0032, while the genetic distance between *A. celebesensis* from GenBank and haplotypes 07–11 (*A. celebesensis*) ranges from 0.0048 to 0.0081. These ranges, with less than 3% genetic distances, provide clear evidence that each haplotype group represents the same species as the sequences obtained from GenBank (Aoyama *et al*., 2000).

Conversely, the genetic distance between *A. marmorata* and *A. celebesensis* haplotypes is more distinct, ranging from 0.0555 to 0.0628. The analysis results between the two species' haplotypes and *A. borneensis* show a minimum genetic distance ranging from 0.0730 to 0.0840. These genetic distances, exceeding the 3% threshold, are crucial in establishing them as distinct species. As Watanabe *et al*. (2009) demonstrated, the genetic distance threshold between 2–3% in the COI gene is a significant marker for differentiating *A. rostrata* and *A. anguilla* species.

The highest genetic distance is shown between *Uroconger lepturus* (Family: Congridae) and haplotypes of *A. marmorata* and *A. celebesensis*, with minimum values of 0.2334 and 0.2360, respectively. The minimum ranges effectively prove that these two haplotypes belong to different families than *Uroconger lepturus*. For mtDNA markers, such as the COI gene, with an average genetic distance of 15.46%, species from different families can already be distinguished (Ward *et al*., 2005).

When comparing intraspecies, the *A. celebesensis* haplotype shows a higher genetic distance, ranging from 0.0016 to 0.0064, compared to *A. marmorata* haplotype, which ranges from 0.0016 to 0.0032 (Table 5). The genetic distance measures genetic differences between species or populations within a species (Nei, 1987). The value of genetic distance is represented by an index ranging from 0 to 1. A value closer to 0 means that the genetic distance between two populations is smaller, indicating that both populations have similar genetic diversity. On the other hand, a value closer to 1 signifies that the genetic distance between the two populations is greater. In this study, the genetic difference between *A. celebesensis* and *A. marmorata* populations is low (the maximum is 0.0628), indicating that the two populations have relatively low genetic differences. Watanabe *et al*. (2008) found that the genetic distance between *A. celebesensis* and *A. marmorata*, collected from different geographical locations, was 0.042, which is remarkably low, indicating almost no genetic difference despite the distant locations of collection sites such as Madagascar, Japan, Sulawesi, and Tahiti.

Table 5 Genetic distances by haplotype species of Anguillid from Poso River

| Genetic Distance per Haplotype | Bor | Cel | Mar | Mar | Hap01 | Hap02 | Hap03 | Hap04 | Hap05 | Hap06 | Hap07 | Hap08 | Hap09 | Hap10 | Hap11 | Uro |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. borneensis* | | | | | | | | | | | | | | | | |
| *A. celebesensis* | 0.0823 | | | | | | | | | | | | | | | |
| MW375927_ *A. marmorata* | 0.0730 | 0.0538 | | | | | | | | | | | | | | |
| OR674041_ *A. marmorata* | 0.0749 | 0.0521 | 0.0016 | | | | | | | | | | | | | |
| Hap01_ *A. marmorata* | 0.0767 | 0.0538 | 0.0032 | 0.0016 | | | | | | | | | | | | |
| Hap02_ *A. marmorata* | 0.0749 | 0.0521 | 0.0016 | 0.0000 | 0.0016 | | | | | | | | | | | |
| Hap03_ *A. marmorata* | 0.0730 | 0.0538 | 0.0000 | 0.0016 | 0.0032 | 0.0016 | | | | | | | | | | |
| Hap04_ *A. marmorata* | 0.0767 | 0.0538 | 0.0032 | 0.0016 | 0.0032 | 0.0016 | 0.0032 | | | | | | | | | |
| Hap05_ *A. marmorata* | 0.0767 | 0.0503 | 0.0032 | 0.0016 | 0.0032 | 0.0016 | 0.0032 | 0.0032 | | | | | | | | |
| Hap06_ *A. marmorata* | 0.0767 | 0.0503 | 0.0032 | 0.0016 | 0.0032 | 0.0016 | 0.0032 | 0.0032 | 0.0032 | | | | | | | |
| Hap07_ *A. celebesensis* | 0.0841 | 0.0081 | 0.0592 | 0.0574 | 0.0592 | 0.0574 | 0.0592 | 0.0592 | 0.0556 | 0.0556 | | | | | | |
| Hap08_ *A. celebesensis* | 0.0879 | 0.0081 | 0.0628 | 0.0610 | 0.0628 | 0.0610 | 0.0628 | 0.0628 | 0.0592 | 0.0592 | 0.0064 | | | | | |
| Hap09_ *A. celebesensis* | 0.0841 | 0.0048 | 0.0592 | 0.0574 | 0.0592 | 0.0574 | 0.0592 | 0.0592 | 0.0556 | 0.0556 | 0.0032 | 0.0064 | | | | |
| Hap10_ *A. celebesensis* | 0.0840 | 0.0048 | 0.0591 | 0.0573 | 0.0591 | 0.0573 | 0.0591 | 0.0591 | 0.0555 | 0.0555 | 0.0064 | 0.0064 | 0.0032 | | | |
| Hap11_ *A. celebesensis* | 0.0860 | 0.0064 | 0.0610 | 0.0592 | 0.0610 | 0.0592 | 0.0610 | 0.0610 | 0.0574 | 0.0574 | 0.0016 | 0.0048 | 0.0016 | 0.0048 | | |
| *Uroconger lepturus* | 0.2173 | 0.2358 | 0.2334 | 0.2358 | 0.2381 | 0.2358 | 0.2334 | 0.2381 | 0.2381 | 0.2334 | 0.2452 | 0.2452 | 0.2405 | 0.2360 | 0.2428 | |

More similar species have a lower genetic distance, which is indicated by a value close to 0. Compared to *A. celebesensis*, the intraspecies of *A. marmorata* are lower, with a maximum genetic distance of 0.0032 compared to 0.0064 for *A. celebesensis*. Starting with the same minimum genetic distance of 0.0016, the genetic diversity of *A. celebesensis* is greater than that of *A. marmorata*. The low genetic distance within each population in our research underscores the significant genetic similarities individuals share within each species. In an ecological context, this low genetic distance is a crucial indicator of the well-connected nature of each species' population despite the diverse and complex habitats from which individuals originate (Sadler *et al.*, 2023). This connectivity results from the unique catadromous behavior of Anguillids, which migrate along the Poso River from the sea in Tomini Bay to Lake Poso upstream.

All sample sequences of the COI gene have been amplified with a base length of 625 bp. This amplification has identified 11 haplotypes, with six belonging to *A. marmorata* and five to *A. celebesensis* (Table 6).

Table 6. The Anguillids haplotype from the Poso River.

| **Species** | **Haplo-type** | **N** | **Sample Code** |
|---|---|---|---|
| *A. marmorata* | 1 | 1 | 23F16_058ge_M_Mar |
| *A. marmorata* | 2 | 27 | 23E18_059ge; 23G19_071ge; 23G20_076; 23G20_071; 23G20_070; 23G20_069; 23G20_068; 23G20_067; 23G20_066; 23G20_065; 23G20_060; 23G20_059; 23F26_032; 23F16_004; 23G01_106; 21F01_PS7G; 21F01_PS1; 21E01_PS2; 21E01_PS3G; 21E01_PS4G; 21F01_PS5; 21F01_PS6; 22G01_7-16; 22H01_8-18; 22J01_11-20; 23A01_A0123; 23A01_B0123 |
| *A. marmorata* | 3 | 1 | 23E18_061ge_M_Mar |
| *A. marmorata* | 4 | 1 | 23F01_103_D_Mar |
| *A. marmorata* | 5 | 1 | 23E01_118_D_Mar |
| *A. marmorata* | 6 | 1 | 22G01_2-16_D_Mar |
| *A. celebesensis* | 7 | 1 | 22A01_1-16_D_Cel |
| *A. celebesensis* | 8 | 2 | 23F18_067ge; 23G19_089ge |
| *A. celebesensis* | 9 | 1 | 23H16_086ge_M_Cel |
| *A. celebesensis* | 10 | 1 | 23G19_087ge_M_Cel |
| *A. celebesensis* | 11 | 1 | 23G19_090ge_M_Cel |

Table 7. Genetic Diversity Analysis

| **Population** | **n** | **Hn** | **Hd** | $\pi$ |
|---|---|---|---|---|
| *A. celebesensis* | 6 | 5 | 0.93333 | 0.00459 |
| *A. marmorata* | 32 | 6 | 0.29234 | 0.0005 |
| Total | 38 | 11 | 0.49929 | 0.01569 |

n: Sample; Hn: Haplotype; Hd: Haplotype diversity; π (phi): nucleotide diversity

Further genetic diversity analysis showed that *A. celebesensis* and *A. marmorata* populations have haplotype diversities of 0.933 and 0.29234, respectively, with a total of 0.499 (Table 7). Additionally, the nucleotide diversity (π) in *A. celebesensis* and *A. marmorata* is 0.0046 and 0.0005, respectively, with a maximum of 0.01569.

The result above reveals that the populations of *A. celebesensis* and *A. marmorata* have different levels of haplotype diversity. *Anguilla celebesensis* has a haplotype diversity (Hd) of 0.933, which is high according to Nei's (1987) classification of 0.8 – 1.0. On the other hand, *A. marmorata* has a lower haplotype diversity of 0.29234 and is classified as the lowest haplotype diversity category (0.1 – 0.4). The sample size analyzed impacts the level of haplotype diversity. *Anguilla marmorata* shows lower haplotype diversity because it has a larger sample size with a relatively similar number of haplotypes compared to *A. celebesensis*, which has a smaller sample size.

Haplotype diversity is important for the population's survival and adaptation to environmental changes. The number of haplotypes is one of the factors influencing genetic diversity. Low genetic diversity raises the risk of extinction because it restricts the potential of species to adapt to environmental changes. Organisms that tend to settle have a lower genetic structure than active or migratory organisms (Hellmair and Kinziger, 2014).

### 3.2. Morphometric and genetic analyses as fundamental conservation principles

Accurate identification of fish species is a fundamental step in fisheries conservation. Both morphometric and genetic analyses are essential for this initial task, completing each other with their respective advantages and limitations. Genetic methods can validate morphometric analyses, especially for species that exhibit morphometric similarities. Morphometric methods, on the other hand, play a critical role in identifying new species, especially for species that still need to be available in gene banks.

The regulation of the Government of The Republic of Indonesia (Peraturan Pemerintah) No. 6/2007 provides a comprehensive guide to fish resource conservation, encompassing ecosystem, species, and genetic levels. As the smallest unit, genetic conservation is a key principle in fish conservation. Heyden *et al.* (2015) emphasize the importance of conservation efforts for populations with unique genetic ancestry or low genetic diversity. The present study on *A. marmorata* and *A. celebesensis*, which reveals a low genetic diversity, raises concerns about the *potential* impact on these species. This underscores the urgent need for conservation management in the Poso River for Anguillid species.

Furthermore, environmental conditions have been observed to reduce current genetic diversity levels. Dam-building and habitat changes can disrupt the connection between the upstream (Lake Poso) and downstream (Poso River estuary), forming new species. Some species may evolve due to this disconnection, which can lead to adaptations (such as physiological, morphological, or other changes) to inhabit specific environments better (Heyden *et al.*, 2015).

Two species of Anguillids in the Poso River, *A. marmorata* and *A. celebesensis*, have been verified morphometrically and genetically. *Anguilla marmorata* was more commonly sampled in this study than *A. celebesensis*, suggesting that the latter has a relatively smaller population. Fahmi *et al.* (2012) noted that *A. celebesensis* has a limited distribution, found only from the Northern to the central parts of Sulawesi waters, thus classifying it as an endemic species. Small biota populations tend to have low genetic variation due to inbreeding, which can reduce population fitness (Meffe, 1986; Frankham *et al.*, 2002). Consequently, it is recommended that these species be protected or caught in limited numbers to ensure their sustainability. Conversely, *A. marmorata* has a broader distribution and a larger population than *A. celebesensis*. However, catching *A. marmorata* must be cautiously approached as it shares the same habitat and appears similar to the other species, *A. celebesensis* and *A. interioris,* which have similar skin patterns (Fahmi, 2015).

Population enhancement and habitat protection, including the population itself, can be viable approaches to addressing the challenges faced by Anguillids in the Poso River. Population enhancement through restocking requires the species to have genetic traits similar to those found in nature to avoid introducing genetic characteristics (Laikre *et al.*, 2010).

On the other hand, the morphometric variation in the Poso River underscores the need for Anguillid conservation. The high morphometric variation indicates the diverse habitat in the Poso River and the need for habitat protection through the identification of conservation areas. Both the morphometric and genetic variation studies can contribute to this. However, it is crucial to understand that fisheries management is not a standalone task but a complex, interdisciplinary field that requires further consideration before determining the conservation area (Abell *et al.*, 2007).

## 4. Conclusion

This study has successfully identified three species of freshwater Anguillid in the Poso River using morphological analysis for *Anguilla bicolor* and a combination of morphological and genetic analysis for *A. celebesensis* and A. marmorata. While the three species exhibit similar morphometric characteristics, the Ano-Dorsal (AD) length emerged as a key differentiating feature. Additionally, the genetic analysis revealed low genetic variation within *A. celebesensis* and *A. marmorata* population in the Poso River.

Identifying these species and determining key morphometric differences are significant for enhancing our understanding of species diversity and aiding in the accurate classification of Anguillid eels. This is particularly critical for conservation strategies, as accurate species identification can inform targeted conservation efforts and policies to preserve genetic diversity. Some conservation efforts that can be applied based on morpho-genetic aspects include restocking and identifying conservation areas. By advancing our knowledge of species differentiation and genetic diversity, this study lays the groundwork for more effective conservation strategies. It contributes to the broader scientific understanding of freshwater Anguillid eel and their ecological significance.

Future research should aim to include comprehensive genetic analyses for all identified species, including *A. bicolor*, with consistent sample size ratios to ensure fair comparison. Expanding the geographical scope of studies to include regions with significant geographical boundaries could provide deeper insights into morphometric and genetic variations. Employing other targeted genes in genetic analyses could further refine species differentiation and contribute to our broader understanding of Anguillid eel populations globally.

### Data availability statement

The authors confirm that all necessary data have been included and accurately stated in this manuscript.

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### Conflict of interests

The authors declare no conflict of interest in conducting and publishing this research.

### Authors contributions

All authors in this paper are major contributors, with the authors' contributions following their respective competencies and roles. This research is part of a primary research (LPDP Rispro Invitasi) led by **T**. **OS, who** conceived and designed the study under the supervision of **MMK**, **RK**, and **SL**. **OS** and **T** conducted on-site sample collection and measurement. **MRW** conducted genetic analysis in the laboratory, including DNA extraction and PCR amplification. **OS** conducted data analysis and discussed to answer the research questions and obtain the conclusions with **RK** for statistical analysis, **SL** for genetic analysis, and **MMK** and **T** for conservation strategies in eel management. **OS** led the drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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# Effect of Light Intensity on Ammonium Removal and Biomass Growth in Different Levels of Aquaculture Effluent Using Duckweed (*Lemna perpusilla*)

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**Abstract:** Cultivating duckweed in aquaculture effluent offers a viable approach to eliminating contaminants. The duckweed biomass obtained can be utilized for the generation of bioenergy. However, elevated ammonium (NH4+) levels in aquaculture effluent, combined with variations in light intensity, can hinder biomass formation. The precise mechanisms underlying this inhibition remain incompletely elucidated. The study assessed the efficacy of duckweed (*Lemna perpusilla)* as a treatment agent for wastewater from catfish farms. The objective was to evaluate the growth response of duckweed and its efficacy in reducing ammonium levels. The research demonstrated that daily light intensity fluctuated using shade nets and that the ammonium concentration of aquaculture wastewater varied according to the age of the fish. The shade nets, which blocked 25% of the sunlight and had an average daily light intensity of 3433.34–15199.56 lux, demonstrated a slightly elevated $NH_4^+$ removal efficiency and duckweed productivity of 69.34% and 0.050 kg/m²/day, respectively. However, these values were not statistically significant compared to conditions without shade nets, with a removal efficiency of 63.97% and duckweed productivity of 0.042kg/m²/day ($P<0.05$). Implementing shade structures that effectively decrease solar exposure by 25% shows promise for enhancing duckweed productivity and optimizing nutrient reduction in wastewater from fish cultivation systems. This approach contributes to the promotion of sustainable integrated aquaculture.

**Keywords:** duckweed, ammonium removal, aquaculture wastewater, light intensity, shade net

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## 1. Introduction

The global demand for fishery products, including aquaculture, continues to increase. It is estimated that world per capita fish consumption will reach 21.2 kg in 2030, representing an average increase of 20.5 kg since 2018–2020 (FAO, 2021). In Indonesia, fish consumption per capita was 54.5 kg in 2019 and increased by 3.47% to 56.39 kg per capita in 2020 (Harianto *et al.*, 2021), with the current level of fisheries cultivation production reaching 15.5 million tons in 2023 (KKP, 2024). This growth will continue and accelerate over the next decade (Obiero *et al.,* 2019). The government and industry are pursuing further intensification of aquaculture to meet the growing demand for fishery products. The intensification of aquaculture activities will have an impact on the increased use of inputs, primarily feed and water per unit area of land, as well as implications for the increased concentration of wastewater produced from the production system (Henriksson *et al*., 2018; Dauda *et al*., 2019). The nutrient content of aquaculture waste presents a potential increase in the pollution burden if discharged directly into the aquatic environment. The contribution of aquaculture activities to ocean and coastal waste nutrient inputs has increased sixfold worldwide, from 0.43 x $10^9$ kg N/year in 1985 to 2.60 x $10^9$ kg N/year in 2005 (Malone & Newton, 2020). The absence of waste management strategies can potentially negatively impact water quality and the equilibrium of aquatic ecosystems. This can result in the onset of eutrophication, a reduction in oxygen levels, and a decline in biodiversity.

In aquaculture waste management, the nutrient content of waste can be utilized and reused through the bioconversion process, whereby plants are employed to transform the waste into a form that can be used again. The utilization of duckweed in wastewater treatment techniques has been observed to reduce nutrient levels effectively. The protein content of duckweed biomass makes it a significant source of bioenergy and organic feed for fish farming operations (de Matos *et al*., 2014; Popa *et al*., 2017). In integrated aquaculture, using duckweed as feed can reduce cultivation costs and provide a remediation impact that reduces pollutant levels in wastewater, thereby reducing water pollution. Studies indicate that duckweed can save up to 85% on water conservation and up to 40% on feed expenditures (Chrismadha *et al*., 2019; Chrismadha, 2021; Paolacci *et al*., 2022).

Nevertheless, the cultivation of duckweed frequently encounters obstacles that impede its optimal growth, diminish production, and lead to inadequate nutrient absorption. Consequently, agricultural wastewater continues to exhibit elevated levels of nutrient concentrations. The substantial release of this wastewater into water bodies has the potential to induce pollution and eutrophication. Examining the environmental consequences associated with intensifying fishing output is important since it can result in heightened inputs, such as feed, and higher concentrations of wastewater.

The efficacy of duckweed in wastewater treatment and its integration into integrated aquaculture depends on the scale of growth or productivity exhibited by the duckweed. Two primary factors that impact productivity are nutrition and light intensity. $NH_4^+$ is a readily absorbable nutrient in cultivation wastewater, essential for duckweed growth. However, specific amounts of $NH_4^+$ have been found to exhibit toxicity (Tian *et al*., 2021; O'Mahoney *et al*., 2022). Furthermore, duckweed productivity is also influenced by light intensity. Both excessive and insufficient sunlight can negatively impact duckweed growth (Walsh *et al*., 2021; Megahud and Dalumpines, 2021). Furthermore, the rate of waste nutrient removal is contingent upon the productivity value of duckweed.

The concentration of $NH_4^+$ in aquaculture effluent strongly correlates with several factors of the cultivation cycle, such as fish age, feed quantity, and density. Currently, there is a lack of accessible data on duckweed's growth response and phytoremediation capacity when utilizing wastewater generated during a fish cultivation production cycle. This lack of information is particularly relevant to the impact of variations in ammonium concentration and fluctuations in natural sunlight intensity. The main objective of this study was to assess and improve the response and phytoremediation capacity of duckweed using aquaculture wastewater. By effectively incorporating duckweed into wastewater management and integrated aquaculture, the aim is to achieve sustainable implementation and generate additional value. This approach aligns with the government's promotion of the blue economy concept (Yadav *et al*., 2023; Bappenas, 2023).

## 2. Materials and Methods

The experiment was conducted in a greenhouse at the Limnology and Water Resources Research Center, National Research and Innovation Agency (RCLWR-BRIN), and observed for 18 days. The use of a greenhouse is the initial stage of this research to minimize limiting factors such as rainfall and pests. *Lemna perpusilla* species of duckweed, collected from the culture pond at RCLWR-BRIN, was employed in this study. Shade nets with 25 percent and 50 percent sunlight blocking were used as shade to ascertain the extent of reduction. A total of 36 plastic containers with dimensions of 0.61 m x 0.43 m x 0.38 m were utilized to cultivate duckweed. The experimental setup comprised a recirculation system with tanks measuring 2.0 m x 1.0 m x 0.5 m, a digital scale, and a rake for collecting duckweed. A randomized block design with three replications was employed to assess the influence of variations in catfish wastewater concentration on duckweed. A randomized block design was selected to regulate environmental variability within the greenhouse, thereby ensuring the accuracy of the assessment of the impact of light intensity on duckweed growth. Furthermore, the objective was to ascertain the response results obtained from the two sides of the factorial treatment and its interaction. The wastewater was classified into three categories based on the age of the fish (L1: 2 months, L2: 3 months, and L3: 4 months) and subjected to shade net treatment at 25% shade (N25), 50% shade (N50), and no shade (N0). The ammonium concentration test is carried out based on the APHA AWWA 4500-NH3-F-phenate method (2017) with a UV-Vis Spectrophotometer. Water quality in the cultivation media (pH and temperature) was measured using the HORIBA U-50 series multi-parameter water quality checker and a lux meter logger (Lutron LX-1128SD) installed in the greenhouse for 24 hours.

## 2.1 Configuration Design of Experiment

Duckweed cultivation containers are positioned within the recirculating system. The recirculation system is arranged using fiber tubs measuring 2x1x0.5 m3, arranged in tiers, and forms a recirculating water flow assisted by pumps and water towers (Figure 1a). The recirculation system is designed to regulate environmental conditions to ensure minimal variation, particularly in temperature, across different treatments. According to the treatment, each cultivation container is filled with media such as catfish cultivation waste (Figure 1b). Each container was filled with 50 liters of wastewater generated from catfish farming. This wastewater, categorized according to the age of the fish, contained different levels of ammonium, as shown in Table 1. Subsequently, 50 grams of duckweed were planted in each container. An 18-day observation period was conducted, during which samples were collected every three days to analyze the quality of duckweed and water.

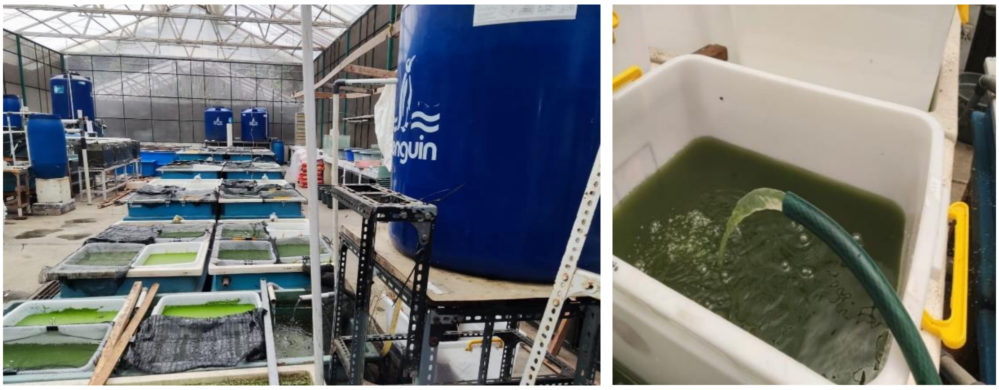

Figure 1. The installation of the recirculation system in experiment (a) and the filling of media/wastewater in (b).

Table 1. The ammonium concentration of wastewater sources

| Wastewater Code | Fish Age in catfish farming | Ammonium (mg/L) |
|---|---|---|
| L1 | ± 2 months | 3.57 |
| L2 | ± 3 months | 19.27 |
| L3 | ± 4 months | 35.64 |

During cultivation, a shade net was used to differentiate variations in sunlight intensity. The shading net used is a commercially available shading net with a 25% and 50% reduction range. The shade net is first calibrated to ensure the value of the percentage reduction range. Calibration was performed indoors by placing the lux meter under a commercial LED lamp at several measurement distances. Based on the measurement results, the light reduction value at N25 is 25.3±1.0%, and the 50% (N50) average reduction range is 49.8±2.2%. In this research treatment, there were two control groups (N0) without shade and (N0TL) without shade and duckweed. A lux meter logger was installed in the greenhouse for 24 hours to measure sunlight intensity at the research site.

### 2.2 Data analysis

Duckweed productivity is calculated referring to the following formula (Chrismadha *et al*. 2016):

$$P = \frac{W_t - W_0}{t.\ A} \quad ...(1)$$

where: P = productivity, $W_t$ = biomass at the time t, $W_0$ = initial of biomass, t = time dan A = surface area of the pond or container.

The removal efficiency of ammonium was calculated following the formula of Wang dan Sample (2013):

$$RE = \frac{C_0 - C}{C_0} \times 100 \quad ...(2)$$

where: RE = removal efficiency (%), C = final concentration of pollutant (mg/L), dan $C_0$ = initial concentration the pollutant (mg/L).

## 3. Result and Discussion

### 3.1. The Light Intensity

According to the measurement results, the light intensity values in the greenhouse range from 0 to 50,100 lux. The light intensity value in the greenhouse is the same as the light intensity in the N0 treatment, with a daily average ranging from 3,433 to 15,199 lux (Table 2). Figure 3 shows the fluctuation of daily average light intensity values in each shade net treatment. These values are calculated as the average of the logging data per minute each day and will be used as data related to the correlation between parameters.

Table 2. Light Intensity in different shade nets during observation

| Shade Net | Light intensity (lux) | Daily Average (lux) |
|---|---|---|
| N0 | 0- 50,100 | 3,433.34 – 15,199.56 |
| N25 | 0- 37,575 | 2,575.01 – 11,399.67 |
| N50 | 0- 25,050 | 1,716.67 – 7,599.78 |

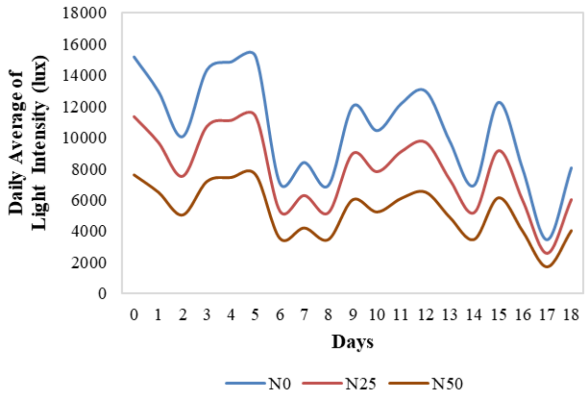


Figure 3. Light intensity fluctuation on shade nets

The intensity of outdoor sunlight varies depending on the weather, geographic location, and elevation, with illumination levels ranging from 130,000 lux on a sunny day to 15,000 lux in shady or cloudy conditions (Lanca *et al*. 2019). The fluctuation value outdoors is still higher than in the greenhouse, which served as the research location.

### 3.2 Duckweed productivity

The productivity of duckweed is inextricably linked to its growth rate, which is contingent upon the efficacy of photosynthesis. Peeters *et al.* (2013) stated that there is a linear correlation between photosynthesis and light intensity. In photosynthesis, plants need nutrients for optimal growth; plants' leaves and roots are required to capture light, water, and nutrients (Evans, 2013; Romand, 2024). Nutrient concentration and light intensity can have an interactive effect on duckweed growth. The application of shade nets to modify light intensity exposure, when integrated with disparate nutrient concentrations, elicits a dynamic response in duckweed productivity, as evidenced in Figure 4. The use of N0 and N25 when given low and medium nutrient concentrations (L1 and L2), productivity still gave a good response, compared to when at the highest nutrient concentration (L3), optimal growth only occurred on the third and sixth days then significantly decreased until the end of the study. In contrast, when employing the N50 method, the combination with L1 and L2 still exhibited productivity values, albeit lower than those observed with N0 and N25. Furthermore, when combined with L3, duckweed productivity was notably the lowest since the third day. In general, the N0 and N25 values, when combined with the three media (L1, L2, and L3), have higher average productivity values than the use of N50. Furthermore, there is no significant difference between the two. Nevertheless, there is a discernible tendency for N25 to yield slightly higher values than N0. The final results demonstrated that the productivity at N25 was 0.050 kg/m$^2$/day, which was observed to be greater than the productivity at N50, which was recorded at 0.016 kg/m$^2$/day. However, the productivity at N25 was similar to that without shade (N0), which was 0.042 kg/m$^2$/day.

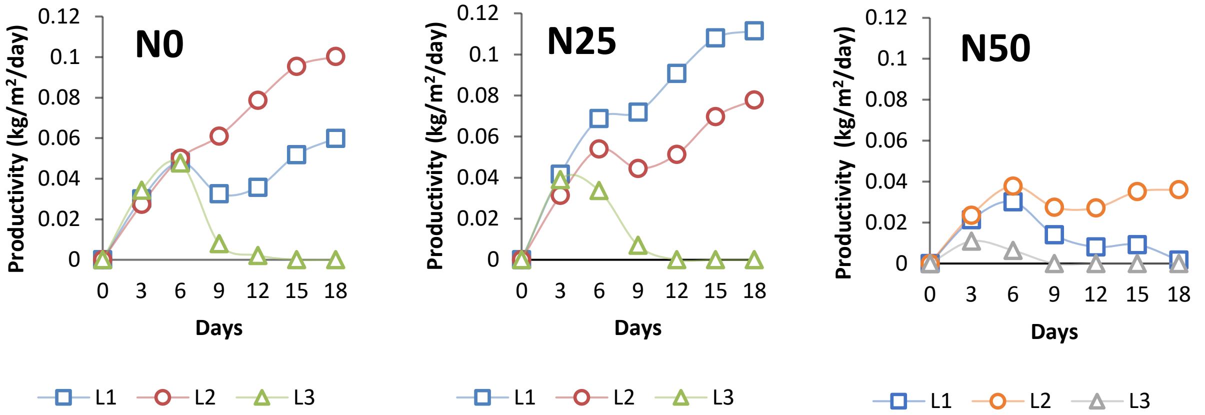


Figure 4. The duckweed productivity based on different shade nets on type media treatment

The productivity values declined when subjected to daily light intensity values of N50 compared to N25 and N0, even though duckweed was given media with small and medium concentrations (L1 and L2). The maximum light intensity value obtained by N50 was 25,050, with a daily average of 1,716.67–7,999.78 lux. This value remains within the range of optimal photosynthesis values. However, it is postulated that using N50 results in a lower frequency of optimal light exposure when compared to N0 and N50. The light intensity range encompasses the minimum threshold necessary to initiate photosynthesis, with the saturation point ranging from 300 μmol/ m$^2$.s (16,216 lux) to 600 μmol/m$^2$s (32,432 lux) at a temperature of 30°C. Oxygen evolution takes place within the light intensity range. Under photoinhibition conditions, duckweed's photosynthesis commences at a light intensity of 1,200 $^1$mol/m$^2$s (64,864.86 lux). According to Landolt *et al.* (1987) and Wedge and Burris (1982), the ideal temperature for oxygen production in duckweed photosynthesis is 30°C, but the temperature range for $CO_2$ fixation is between 20 – 30°C.

The greenhouse, designated a research location of equivalent value to N0, exhibited a maximum light intensity of 50,100 lux (with a daily average of 3,433.34–15,199.56 lux). This value exceeded the optimal threshold but remained below the duckweed photoinhibition value. Conversely, N25 has the highest value, 37,575 lux (daily average 2,575.01 - 11,399.67 lux), within the optimal range. Therefore, N25 treatment can enhance duckweed photosynthesis, increasing growth and phytoremediation capacity. Good nutrient absorption, assimilation, transportation, photosynthesis, respiration, and enzymatic activity all play roles in the growth rate of duckweed (Landolt *et al.*, 1987). Photoinhibition occurs at a light intensity of

64,864 lux (Petersen *et al.* 2022), but light-induced stress in duckweed begins to appear at a light intensity of 54054.05 lux (Adams *et al.* 2020; Stewart *et al.* 2020). Despite being conducted in a partially enclosed environment (greenhouse) with a shade net as a treatment, the productivity value remained consistent with the previously reported findings. In a previous study, duckweed was cultivated in an integrated common carp (Cyprinus carpio) system with close recirculation aquaculture in an open area, resulting in a productivity range of 0.028 - 0.053 kg/m$^2$/day (Chrismadha *et al.*, 2016).

Based on the relationship between daily light intensity and productivity shown in Figure 5, the $R^2$ value of N0 and N25, when given media L1 and L2, duckweed productivity is stronger than when given media L3. While the R2 value at N50 when given low media concentration (L1) and high (L3), the value is smaller when compared to when given medium media L2; this indicates that when the light intensity is not optimal for growth, the presence of nutrients at the maximum growth points up to 18 days of maintenance, duckweed can still grow and survive. Megahud and Dalumpines (2021) reported that the highest growth or maximum growth point was achieved at a particular nutrient concentration and light intensity. Growth can continue to decline when nutrient concentrations decrease or successively increase beyond the maximum growth point, and growth will decline when light intensity is decreased or increased from the maximum growth point. Statistically, it was shown that the highest productivity value was shown in the use of N0 and N25 combined with L1 and L2 media, compared to when cultured in L3 media. Meanwhile, using L3 media in almost all combinations obtained the lowest value, especially when combined with N50 (Table 3.).

Table 3. The final productivity of duckweed

| **Treatment** | **N0** | **N25** | **N50** |
|---|---|---|---|
| L1 | 0,043$^{abcd}$ | 0.083$^{d}$ | 0.013$^{ab}$ |
| L2 | 0,070$^{cd}$ | 0,053$^{bcd}$ | 0,033$^{abc}$ |
| L3 | 0,013$^{ab}$ | 0,013$^{ab}$ | 0,003$^{a}$ |
| Average | 0.042 | 0.050 | 0.016 |

Note: Different letters in the final productivity data indicate differences using the Duncan multiple range test (P<0.05)

The low productivity value is shown when using L3 media in all combinations with shade net. Specifically, L3 is a 4-month-old catfish wastewater with the highest initial $NH_4^+$ concentration of 35.64 mg/L. Duckweed productivity does not necessarily increase with high $NH_4^+$ concentrations, even though $NH_4^+$ is the compound most easily absorbed by plants. *Lemna minor* is known to grow well at $NH_4^+$ concentrations ranging from 7 to 138 mg/L, indicating that duckweed can tolerate high levels of $NH_4^+$. However, optimal growth occurs at a concentration of 28 mg/L (Huang *et al.*, 2013; Wang *et al.*, 2014). Although the $NH_4^+$ concentration in L3 is within the tolerance range, it exceeds the optimal value. As a result, duckweed survived until the end of the study but exhibited lower productivity (Figure 6). Optimal light intensity positively impacts productivity only if the media conditions support optimal growth. Which, in this case, is influenced by the ammonium concentration.

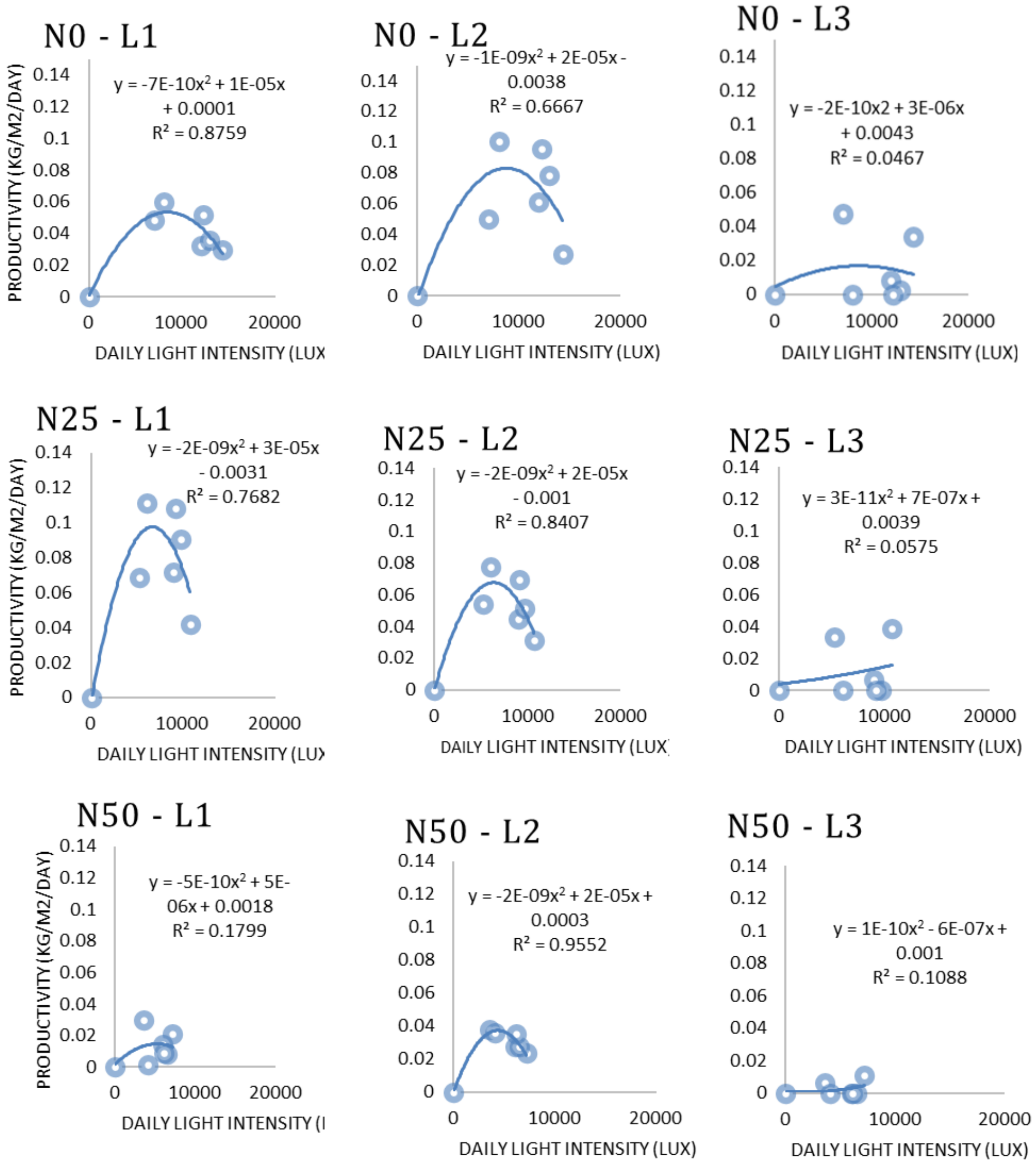


Figure 5. The daily light intensity-productivity relationship

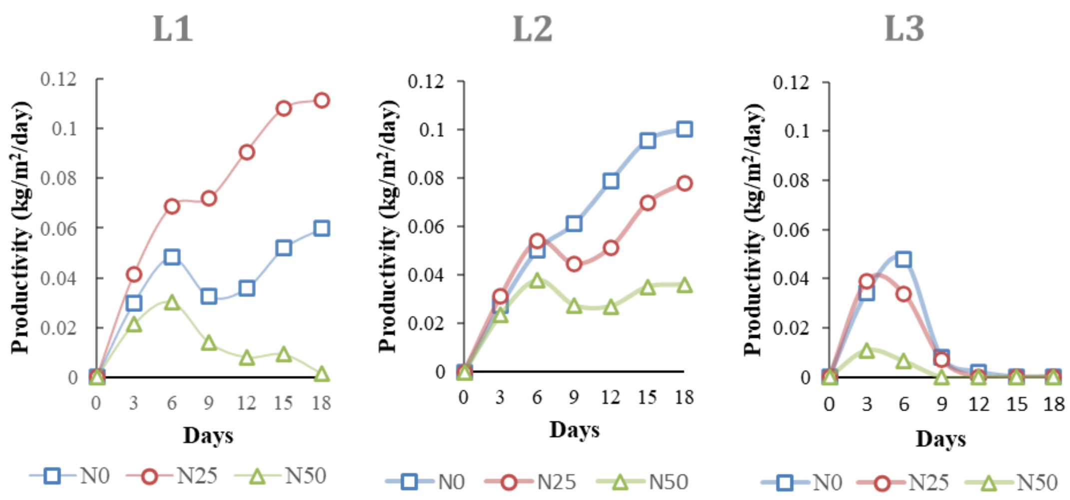


Figure 6. The duckweed productivity based on type media with shade net treatment

Environmental factors such as temperature and pH also influence duckweed productivity. Temperature can be influenced by light intensity; the higher the light intensity, the more the media temperature will increase. In this study, the media temperature was controlled by utilizing a recirculating system in duckweed culture, which can maintain the temperature within a range that did not vary significantly. Similarly, the pH levels in this study were within a range of values that were not significantly different except for the control, as seen in Table 4. The average media temperature and pH values remained within optimal ranges, namely 25 – 30 $^0$C for temperature (Vymazal, 2008) and pH 5.0 – 7.5 (Mkandawire and Dudel, 2005; Vymazal, 2008). In the control treatment, the absence of duckweed caused higher pH and temperature values.

Table 4. Average temperature and pH of the media based on the shade net

| Shade net | Temperature ($^0$C) | pH |
|---|---|---|
| N0 | 27.38 ± 0.2$^a$ | 7.78 ± 0.18$^a$ |
| N25 | 27.41 ± 0.2$^a$ | 7.78 ± 0.15$^a$ |
| N50 | 27.24 ± 0.3$^a$ | 7.75 ± 0.19$^a$ |
| N0TL | 27.69 ± 0.3$^b$ | 8.18 ± 0.21$^b$ |

Note: Different letters in the same column indicate differences using the Duncan multiple range test (P<0.05)

Table 5. The average removal efficiency of $NH_4^+$ in the duckweed culture media with the provision of shade nets

| Treatment | N0 | N25 | N50 | N0TL |
|---|---|---|---|---|
| L1 | 67,25 | 62,15 | 26,65 | 7,10 |
| L2 | 74,35 | 83,90 | 72,35 | 36,80 |
| L3 | 50,30 | 61,90 | 34,25 | 57,85 |
| Average | 63,97$^b$ | 69,32$^b$ | 44,42$^a$ | 33,92$^a$ |

### 3.3. Removal Efficiency Ammonium ($NH_4^+$)

During the 18-day observation period, the concentration of ammonium ($NH_4^+$) decreased in shade and unshaded media, indicating increased $NH_4^+$ removal efficiency by duckweed. The removal efficiency value fluctuated until the end of the research (Figure 7). The results show that N25 had a higher average removal efficiency (69.32%) compared to N50 (44.42%) and N0TL (33.92%). However, there was no significant difference between N25 and N0 (63.97%) (Table 5).

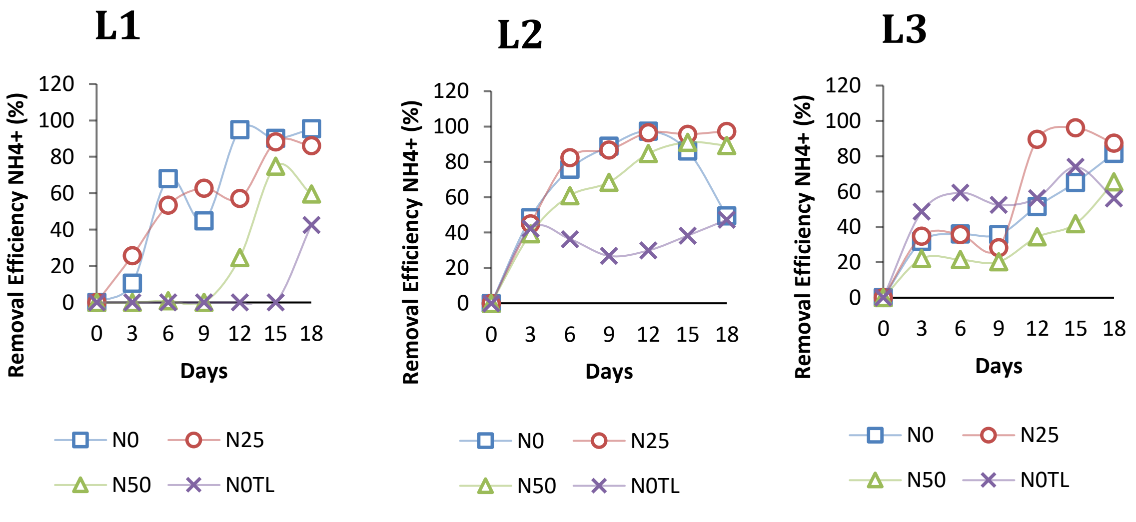


Figure 7. Fluctuation $NH_4^+$ removal efficiency on type media with shade net treatment

Daily light intensity is generally associated with duckweed growth and higher protein production (Femeena *et al*., 2023). Duckweed growth is also associated with the reduction or uptake of nutrients in the media. The results of the experiment demonstrate that the use of N0 and N25 shade net on low (L1) and medium (L2) concentration waste resulted in higher removal efficiency values than when using high-concentration waste (L3). This finding aligns with the duckweed productivity value observed in Figure 6. The low productivity observed in L3 also resulted in a reduction in efficiency value that was low, even several times lower than that observed for N0TL. The addition of nutrients to L3 from duckweed that died due to high ammonium concentrations caused the rate of reduction efficiency to be slower in all three shade treatments.

Based on experimental results, high ammonium reduction, such as N25 and N0, is associated with the highest duckweed productivity, while low productivity results in low ammonium reduction values, such as N50. In nature, the Lemnaceae group grows in sunny and shady habitats, but shady habitats are preferred because of the lower light intensity and less extreme temperatures (Landolt, 1986). Plant reactions to different light intensities are also influenced by other abiotic factors such as temperature and nutrition (Francis and Gilman, 2019) and are also influenced by species (Petersen *et al*., 2022). Several studies report that the light saturation of *Lemna minor* ranges from 342 to 400 µmol/m$^2$/s (18,486.49 – 21,621.62 lux) (Petersen *et al*., 2022). Lack or excess of light intensity will have an impact on biomass growth, frond size, leaf pigments, root length, protein and starch content, as well as plant hormonal production (Femeena *et al*., 2023; Strzalek and Kufel, 2021; Brini *et al*., 2022). According to studies on *Lemna gibba*, light intensity higher than 1,000 µmol/m$^2$/s (54,054.05 lux) causes increased levels of zeaxanthin, which is a chlorophyll /photoprotection hormone against damage caused by intense light, detoxification of oxidants (reactive oxygen species/ROS, and other free radicals), and overall structural and functional maintenance of plant biological membranes. Zeaxanthin is also an essential micronutrient for humans if duckweed is developed for food (Adams *et al*., 2020; Stewart et al., 2020; Petersen *et al*., 2022). Another impact of very high light intensity exceeding the maximum limit for plants is that the rate of photosynthesis will decrease and can result in plant damage due to oxygen stress (photoinhibition) (Petersen *et al*., 2022).

The effect of variations in daily light intensity through providing shade on $NH_4^+$ reduction efficiency can be seen directly in the correlation value. At N25, a firm determination coefficient ($R^2$) of 0.7598 and 0.8203 was obtained when combined with L1 and L2, compared to when combined with L3; this indicates that the optimal light intensity value will not produce good reduction efficiency if the optimal $NH_4^+$ concentration does not influence it. While the use of N0 indicates approaching the N25 value for the strength of the relationship, there is also an indication that it is equivalent to the use of N50 and NOTL. The light intensity produced by N0 indicates approaching a value close to the minimum limit of the stress level due to the influence of photoinhibition, which results in disrupted growth and reduced efficiency. Even though the values are not significantly different, this is a sign that increasing light intensity beyond N0 can cause suboptimal growth and improve the effectiveness of duckweed remediation in removing $NH_4^+$ (Figure 8).

Other factors besides light intensity that also influence $NH_4^+$ reduction efficiency in the use of aquatic macrophytes such as duckweed include the initial $NH_4^+$ concentration, macrophyte tolerance level, biomass, and media water quality such as temperature and pH (Kinidi and Salleh, 2017; Walsh *et al*. 2021; Fahmi *et al*., 2023).

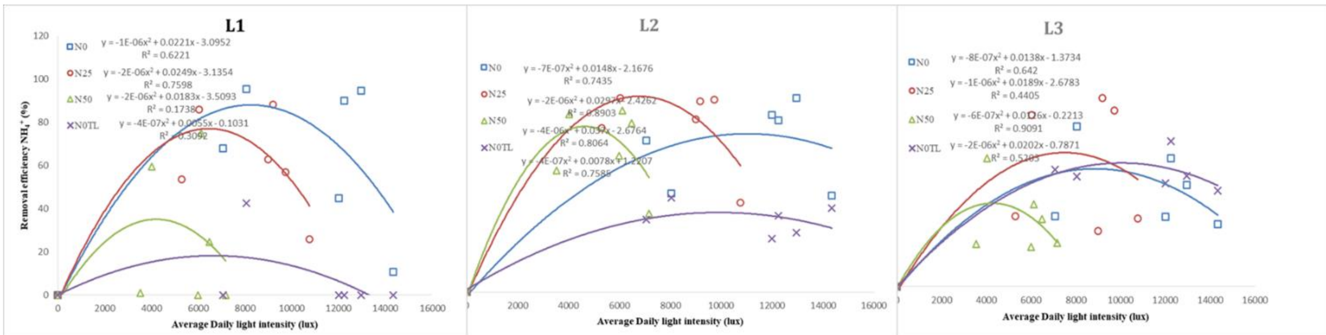


Figure 8. Relationship between daily average light intensity and $NH_4^+$ removal efficiency on different media with varying shade net

The initial concentration of $NH_4^+$ in the media is thought to determine the growth rate of duckweed biomass and its phytoremediation capacity. The initial concentrations of $NH_4^+$ in this study were L1 (3.57 mg/L), L2 (19.27 mg/L), and L3 (35.64 mg/L), which according to quality standards were in the high category (PP No. 22 of 2021). Despite being duckweed's most preferred compound, a high concentration of $NH_4^+$ does not guarantee good duckweed growth and good nutrient uptake. High levels of $NH_4^+$ can inhibit the absorption of cations such as calcium and magnesium from the substrate, causing a deficiency of these elements in plants. A decrease in the uptake of these essential cations can cause more problems for plant growth and metabolism (Zhao *et al*. 2016). As in the results of this study, in terms of media, the best average reduction efficiency value is owned by L2 with a value of 76.87% and is significantly different from L1 (52.02%) and L3 (48.82%) (Figure 7. Despite L3 having the highest $NH_4^+$ content, it exhibits lower duckweed uptake compared to L2 media, which is in line with the previous explanation that a high $NH_4^+$ concentration does not guarantee good duckweed growth and nutrient uptake, even though $NH_4^+$ is the compound most easily absorbed by plants. Meanwhile, in L1, as the medium with the lowest initial concentration of $NH_4^+$, a high $NH_4^+$ reduction efficiency value should be obtained, but the results show that the value tends to be low; it is suspected that there is an influence of shade in the process, where the shade of 50% (N50) when using L1 media has the lowest reduction efficiency level.

$NH_4^+$ is a nitrogen compound most easily absorbed by plants and nitrate ($NO_3^-$). However, ammonia can decrease duckweed productivity at high concentrations because it can be toxic, causing root and leaf rot and inhibiting nutrient uptake in the media. Symptoms of $NH_4^+$ poisoning in plants include reduced growth, leaf chlorosis, changes in root shape, decreased root/shoot ratio, decreased root gravitropism, and triggering oxidative stress (Caicedo *et al*., 2000; Liu and Wiren, 2017; Tian *et al*., 2021). Oxidative stress can be caused by oxygen deficiency, including direct photoreduction of $O_2$ to $O_2^-$ via reduced electron transport associated with the photo-respiration cycle (Wang *et al*., 2014). Excessive increases in reactive oxygen species (ROS) due to oxidative stress can also result in oxidative damage to proteins, DNA, and lipids. ROS production is one of the leading causes of reduced productivity, injury, and death accompanying these plant stresses (Mittler *et al*., 2004; Huang *et al*., 2013). Most plants will experience toxicity when $NH_4^+$ is in the millimolar (mM) concentration range or is the only nitrogen source, and in the micromolar range (lower than mM), most species roots prefer ammonium uptake over nitrate (Gazarrini *et al*., 1999; Britto and Kronzucker, 2002; Liu and Wiren, 2017).

According to Nasr *et al*. (2009), although duckweed is susceptible to $NH_4^+$ concentrations, it can still treat wastewater containing very high concentrations of total ammonia if a certain pH level is not exceeded. Through additional oxygen supply, degradation of organic matter can be increased by duckweed and additional area surface for the growth of bacteria and algae, which can contribute to the total loss of nutrients in shallow systems, regardless of loading rates (Korner *et al.*, 2003).

Ammonium ($NH_4^+$) and nitrate ($NO_3^-$) are the primary forms of inorganic nitrogen in wastewater that plants can absorb directly. The energy required for the assimilation of $NH_4^+$ is lower than that required for the assimilation of $NO_3^-$. $NH_4^+$ is the preferred nitrogen source for many plants in the micromolar (μM) concentration range (Tian *et al.* 2021). Unlike other plants, duckweed has better ammonium absorption than other nitrogen sources (Porath and Pollock, 1982). However, at high concentrations, ammonium ions inhibit duckweed growth. Growth inhibition by total ammonia ($NH_4^+$ + $NH_3$) is generally caused more by the $NH_3$ form than by the $NH_4^+$ form (Caicedo *et al.*, 2000).

Duckweed biomass also has a crucial role in the level of $NH_4^+$ reduction efficiency. The amount of biomass, density, and surface area of duckweed cover significantly influence the level of reduction efficiency. High duckweed density can negatively impact growth and has implications for developing duckweed-based remediation systems (Paolacci *et al.*, 2022). High density can result in some individuals lacking sufficient sunlight, not being adequately exposed to the media, or being submerged up to the leaves, hindering their photosynthesis ability. Duckweed density also affects oxygen levels in the medium (Walsh *et al.*, 2021). The influence of light intensity on duckweed productivity has been demonstrated to be significant. To understand how duckweed productivity influences $NH_4^+$ reduction efficiency, the correlation between productivity and $NH_4^+$ reduction efficiency is depicted in Figure 9. At N25, there is a slightly stronger correlation with no shade (N0) than with duckweed at N50, indicating that optimal light conditions (N25) enhance both productivity and $NH_4^+$ reduction efficiency.

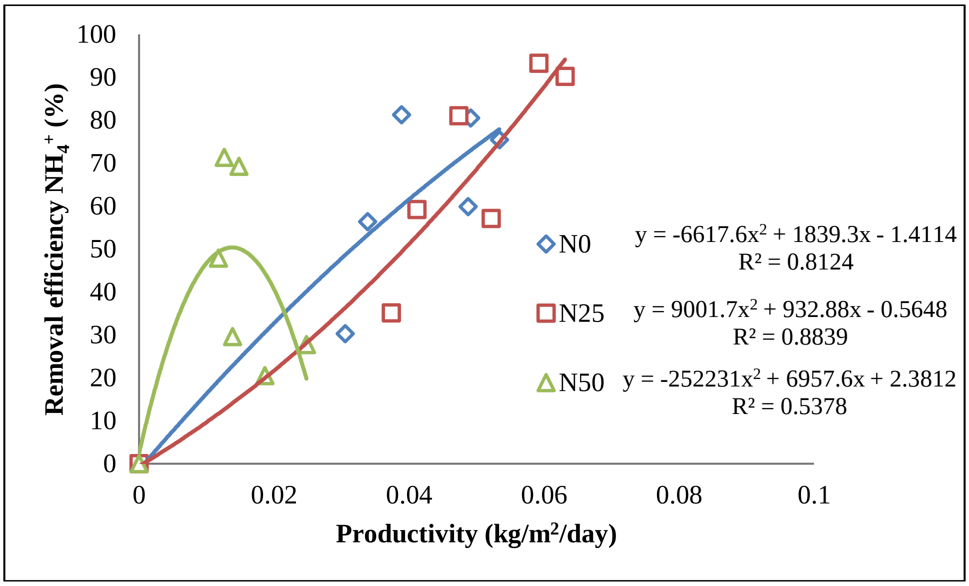


Figure 9. Duckweed productivity-removal efficiency $NH_4^+$ relationship based on shade net variations

The response of duckweed to ammonium ($NH_4^+$) and ammonia ($NH_3$) levels is widely reported in the literature, but the conclusions do not always agree. Shen *et al.* (2019) reported that the $NH_4^+$ removal efficiency from the combination of *Acinetobacter* sp. and duckweed strains in media and cultivation wastewater samples at a temperature of 15°C exceeded 99%. Ahmadi and Dursun (2024) reported that the removal efficiency of $NH_4^+$ from the secondary clarifier tank of a conventional biological treatment system after the settling process reached 72%. Sarkheil and Safari (2020) explained that the use of *Lemna minor* in phytoremediation in the cultivation of African cichlid fish (*Labidochromis lividus*) was able to reduce total ammonia nitrogen (TAN) by 43.7% after 48 hours and seven days. It isn't easy to compare the results of the studies mentioned above because they were obtained under different conditions of temperature, pH, wastewater type, and media composition.

The differences in $NH_4^+$ removal efficiency revealed in this study are consistent with previous studies, emphasizing the complex relationship between duckweed productivity, light intensity, and $NH_4^+$ concentration. The correlation between duckweed productivity and $NH_4^+$ removal efficiency, shown in Figure 9, emphasizes the significance of managing environmental conditions to improve phytoremediation outcomes. Specifically, delivering optimal light intensity (observed with N25) appears to support higher productivity and improved $NH_4^+$ reduction efficiency.

## 4. Conclusion

The media's light intensity and nutrient concentration are critical factors for duckweed growth. These factors can independently or collaboratively influence growth outcomes. The optimal conditions for light intensity and ammonium concentration yield better growth effects. In contrast, suboptimal conditions or conditions that exceed the optimal value limit can lead to reduced productivity, induce toxic effects, and impede the efficacy of duckweed-based remediation. The use of shade nets that reduce sunlight by 25% (N25), which produces an average daily intensity of 2575.01 - 11399.67 lux, encourages slightly higher growth than duckweed without shade (N0), with an ammonium concentration of 19.57 mg/L still obtained good duckweed productivity, while at a concentration of 35.64 mg/L obtained low productivity. It is anticipated that the findings of this study will serve as a point of reference for developing strategies for utilizing duckweed in waste management, water quality control, and biomass production in integrated aquaculture systems. The results of this research still need to be tested outdoors with environmental factors such as weather, land/pond conditions, pests, and other factors that may have different effects. Future research could focus on refining the balance between nutrient levels and light conditions to maximize duckweed's utility in various environmental and aquaculture settings. Overall, this study provides valuable insights into optimizing conditions for duckweed in aquaculture, offering a promising approach for enhancing wastewater treatment and supporting sustainable fish farming practices.

## Data availability statement

The data included and used in this study is not confidential and is available upon request.

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## Conflict of interests

All authors have stated that there is no conflict of interest in relation to the manuscript's writing or submission.

## Authors contribution

**AW**, **KN**, **AS**, **YPH**, **TC** and **ES** as the main contributors, conceptualized the study, data analysis, writing-review, editing and funding acquisition. **FSL**, **EN**, **SG**, and **NM** support laboratory analysis, data collection, and data analysis.

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# A Systematic Review of Research Trends in Methane Emissions from Rice Fields in Asia

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**Abstract:** Global greenhouse gas levels are significantly impacted by methane emissions from rice fields, especially in Asia, where most of the world's rice is produced. This review analyzes research trends on methane emissions from rice fields in East, Southeast, and South Asia, focusing on factors influencing emissions and the effectiveness of mitigation strategies. We synthesized data about 169 papers published between 2000 and 2023 from Web of Science and Google Scholar, which were merged in Mendeley. The results were visualized using VOSviewer. It covers key aspects such as water management, soil types, farming practices, and rice varieties. Our findings suggest that water management practices, including intermittent drainage and pulse irrigation, are critical in reducing methane emissions. Soil types, farming practices, and rice varieties also influence variations in emissions levels. The research highlights significant regional differences, with China and Indonesia major contributors to emissions, while countries such as Japan and South Korea have implemented effective mitigation measures. Emerging research topics include the impact of organic matter inputs and innovative rice cultivars on emission levels. This review underscores the need for region-specific strategies and research in less studied, such as rainfed and peatland rice fields, to enhance global understanding and control of methane emissions from rice cultivation. The boundary of this review is this manuscript only focuses on methane emissions in artificial wetlands, such as rice field areas, not other water bodies. Therefore, further research review in other freshwater ecosystems is encouraged.

**Keywords:** methane emissions, Asia, rice field, research trends

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## 1. Introduction

Anthropogenic factors are the main source of methane emissions, with about 70% of emissions coming from agriculture, mining, natural gas use, enteric fermentation in ruminants and insects, manure storage systems, wetland soils, wastewater treatment, landfill sites, wetland soils, forest fires, hydroelectric reservoirs, transportation, biogas production and industrial processes such as coal burning or cement production (Khalil *et al.*, 1993; Topp and Pattey, 1997; Mer *et al.*, 2001; Minamikawa *et al.*, 2006; Choi *et al.*, 2017). In Asia, where most of the world's rice is produced, and 90% of rice fields are flooded, most methane emissions come from the agricultural sector (Wassmann *et al.*, 2009). Since anaerobic conditions facilitate methane production by methanogens, inundated rice fields and domesticated ruminants are responsible for up to 40% of emissions and are considered the major anthropogenic sources (Mer *et al.*, 2001; Ariani *et al.*, 2021).

In Asia, numerous studies have focused on methane emissions from rice fields, with much of the research investigating the factors influencing these emissions. Factors such as irrigation management, cultivation techniques, rice varieties, soil types, soil amendments, and their interactions remain dominant research topics in several Asian countries. In addition, recent studies have focused on emission modeling such as process-based, empirical and statistical, remote sensing and geospatial, machine learning and data-driven models, and top-down inverse models (Schulz *et al*., 2006; Van Dingenen *et al*., 2018; Zhu *et al*., 2018; Conrad, 2020; Gwon *et al*., 2022; Mboyerwa *et al*., 2022; Ouyang *et al*., 2023). However, no comprehensive review synthesizes previous studies to identify recent knowledge gaps, particularly in Asia, the largest methane producer. This study addresses that gap, with the primary objective of synthesizing existing research on methane emissions from rice fields in Asia, focusing on identifying knowledge gaps and emerging trends.

To address this gap, we have prepared a follow-up manuscript that expands on the existing research and examines the status of methane research in East, Southeast, and South Asian countries, focusing on rice field ecosystems. A systematic review was conducted using keywords related to methane emissions and rice fields in Asia. Combining traditional review techniques and novel visualization methods allowed for a more comprehensive analysis of research trends across different Asian countries.

The review is limited by variability in the quality and availability of data across regions and challenges in merging bibliometric network outputs with empirical field data. Despite these limitations, this synthesis provides valuable insights into methane emission patterns across Asia, filling a critical gap in understanding global methane emissions and their environmental impacts.

## 2. Methods

This study was a comprehensive literature review to synthesize existing research on methane emissions from rice field ecosystems in Asia. A literature review was chosen over primary research to consolidate existing knowledge and identify trends and gaps in the literature across different regions. This approach allows for a more efficient approach to provide an overview of existing studies and inform future research directions, ensuring a broad understanding of methane emissions without primary data collection that needs intensive resources.

Keywords such as "methane and climate change issues," "methanogenesis," "factors influencing methane emissions in aquatic ecosystems," and " methane research methods in rice field ecosystems" were used to gather references for this review from search engines such as Google Scholar, Research Rabbit, and Web of Science (Clarivate). Numerous sources were identified, focusing on specific regions of Asia and rice field ecosystems from 2000 to 2023. The scope was limited to this time frame to capture recent developments in methane research while ensuring enough data to analyze trends over time. Exclusion criteria included studies that addressed methane emissions unrelated to rice fields or focused outside the region of Asia. A total of 169 articles met these criteria, which provided a robust yet manageable sample size for analysis.

Search results from Web of Science and Google Scholar were merged and organized in Mendeley, a reference manager software, to track the sources and remove the duplicates systematically. Articles were further screened based on titles and abstracts to ensure relevance to the focus on methane emission in Asian rice field ecosystems.

In addition, to enhance the literature analysis, the search results were visualized using the VOSviewer, a software tool for constructing and visualizing bibliometric networks (van Eck and Waltman, 2010; Kirby, 2023). The VOSviewer was employed to map key research trends, identify collaborations among institutions, and detect emerging themes in methane emissions research across Asia. This visualization revealed underexplored areas and provided a clearer picture of the evolving research landscape. For a brief step on the methodology, please refer to Figure 1.

As with any literature review, this study is subject to limitations, including the potential for publication bias, where unpublished studies or those not indexed in the selected databases may have been missed. The review focused on English-language and some Korean articles with English abstracts, potentially excluding research in other languages. Diverse methodologies and measurement techniques also challenged consistent conclusions.

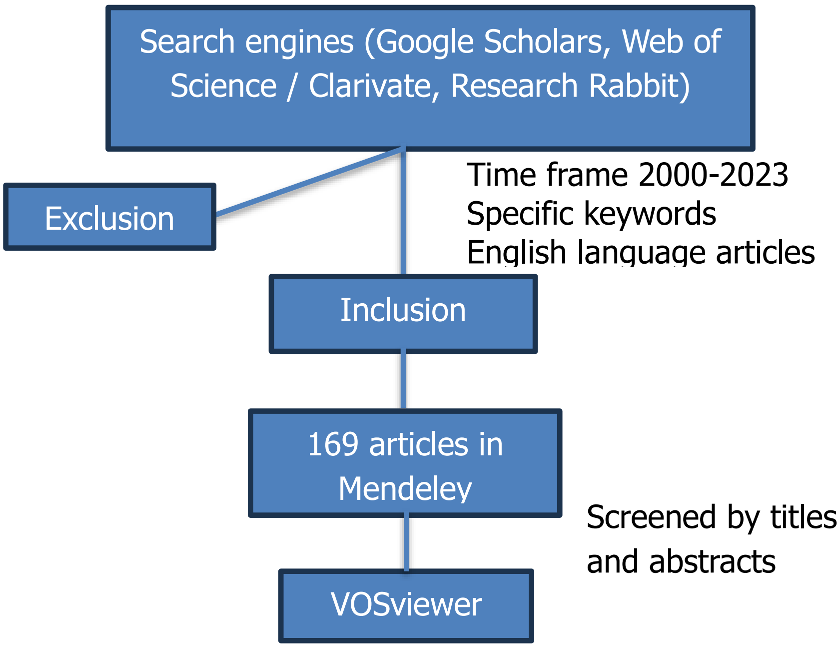


Figure 1. Flow chart of the methods (Source: author's creation).

**3. Result**

Average seasonal methane emissions from different Asian countries indicate that Indonesia, North Korea, and South Korea have the highest seasonal methane emissions in the region, with a range of 275 to 290 $kgCH_4ha^{-1}$ (Figure 2). Despite the relatively small rice field areas, methane emissions on the Korean peninsula are higher than in other regions. Regarding the variation of rice ecosystems in Asia, irrigated rice fields cover the largest area compared to other types, with $78x10^6$ Ha in total (Figure 3a). However, irrigated and rainfed rice fields in South and Southeast Asia appear almost equal. For example, in South Asia, the comparison between irrigated and rainfed rice fields has the same value of 40.91%, while in Southeast Asia, the proportion is 42.31 and 40.38%, respectively. In East Asia, rainfed rice fields are less common than irrigated ones (Figure 3b). According to Wasmann *et al.* (2000) and Rao *et al.* (2017), various rice production systems are classified based on climate and water availability, geography and topography, agriculture infrastructure, and socioeconomic factors. Some East Asian countries, such as China, South Korea, and Japan, have temperate climates with less predictable rainfall. Therefore, they use modern technology to solve the problem of water limitations. Most Southeast Asia countries such as Indonesia, Thailand, and the Philippines generally have tropical climates with high rainfall prediction supporting rainfed and irrigation rice fields. Like South Asia, which depends on the monsoon rains, Southeast and South Asia rainfed and irrigated rice fields coexist more evenly than in East Asia. Table 1 describes the difference in rice field types.

Table 1. Description of rice field types that are commonly found in Asia

| Rice field type | Locations | Water sources | Flooding pattern | Methane emissions |
|---|---|---|---|---|
| Irrigated | Lowlands, valleys, and deltas | Rivers, reservoirs, canals | Consistent shallow flooding | High |
| Deepwater | River basins, flooded-prone areas | Natural flooding, monsoon | Flooding >50 cm | High |
| Rainfed | Southeast Asia and parts of India | Rainfall | Seasonal with variable depths | Moderate |
| Upland | Hilly or mountainous areas | Rainfall | No standing water (well-drained) | Low |

Source: modified from Wassman (2000); Yuan *et al.* (2022); FAO (2024).

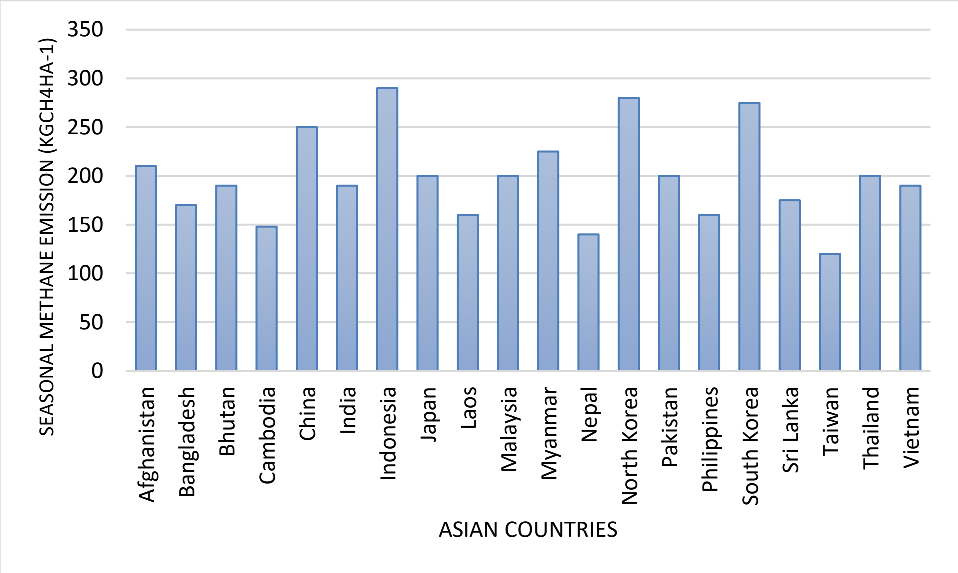


Figure 2. Average seasonal methane emissions from several Asian countries (Source: modification from Yan, *et al.* 2003).

### 3.1. Methane emissions in East Asia region

Many reports and publications on methane emissions from rice field ecosystems have been produced in some East Asian countries. Most of these publications are in English, but some are in local languages, such as Chinese, Japanese, or Korean. China, the world's largest rice producer, has been the leading source of methane emissions from rice field ecosystems since the 1980s (Yan *et al.*, 2003). A significant proportion (91.4%) of China's methane emissions come from anthropogenic sources, including agriculture (Ito *et al.*, 2019). According to a model-based assessment by Ito *et al.* (2022), methane emissions from Chinese paddy fields between 2005 and 2015 they were ranged from 2.0 to 13.7 $TgCH_4$ $yr^{-1}$, with the highest emissions occurring in central and southern China.

Although Japan has lower methane emissions than China, it is still the second-largest contributor in East Asia (Ito *et al.*, 2022). This is probably because, in 1995, about 99.1% of Japanese rice fields were irrigated, resulting in an average seasonal emission of 21 g $CH_4$ $m^{-2}$ across 47 prefectures (Yan *et al.*, 2003). The methane budget of Japan's agricultural sector alone has been estimated to be about 0.84 Tg $CH_4$ $yr^{-1}$ (Ito *et al.*, 2019).

Reports from the Korean peninsula highlight water management as a key factor in controlling methane emissions, though specific water management practices are not always clearly identified. Average methane emissions from different treatments, including water regimes and rice varieties, at three sites (Suwon, Milyang, and Iksan) in South Korea ranged from 6.02 to 15.52 mg $CH_4$ $m^{-2}$ $h^{-1}$ during one growing season (Yan *et al.*, 2003).

Agriculture in North and South Korea contributed 0.14 and 0.37 Tg $CH_4$ $yr^{-1}$, respectively, accounting for 1.4% and 2.2% of total emissions (natural and anthropogenic) (Ito *et al*., 2019).

In northern China, South Korea, and Japan, methane emissions increased mainly during flooding. As most rice fields in East Asia are irrigated, rainfed and deepwater rice fields are considered negligible contributors compared to those in Southeast and South Asia (Wassmann *et al*., 2000).

Annual methane emissions from East Asian countries increased from the 1990s to 2012 due to economic and population growth and dietary changes. In Japan and South Korea, however, GDP and per capita emissions decreased between 1997 and 2012. This decrease is attributed to the implementation of greenhouse gas (GHG) and slower population growth, which has limited emissions (Ito *et al*., 2019).

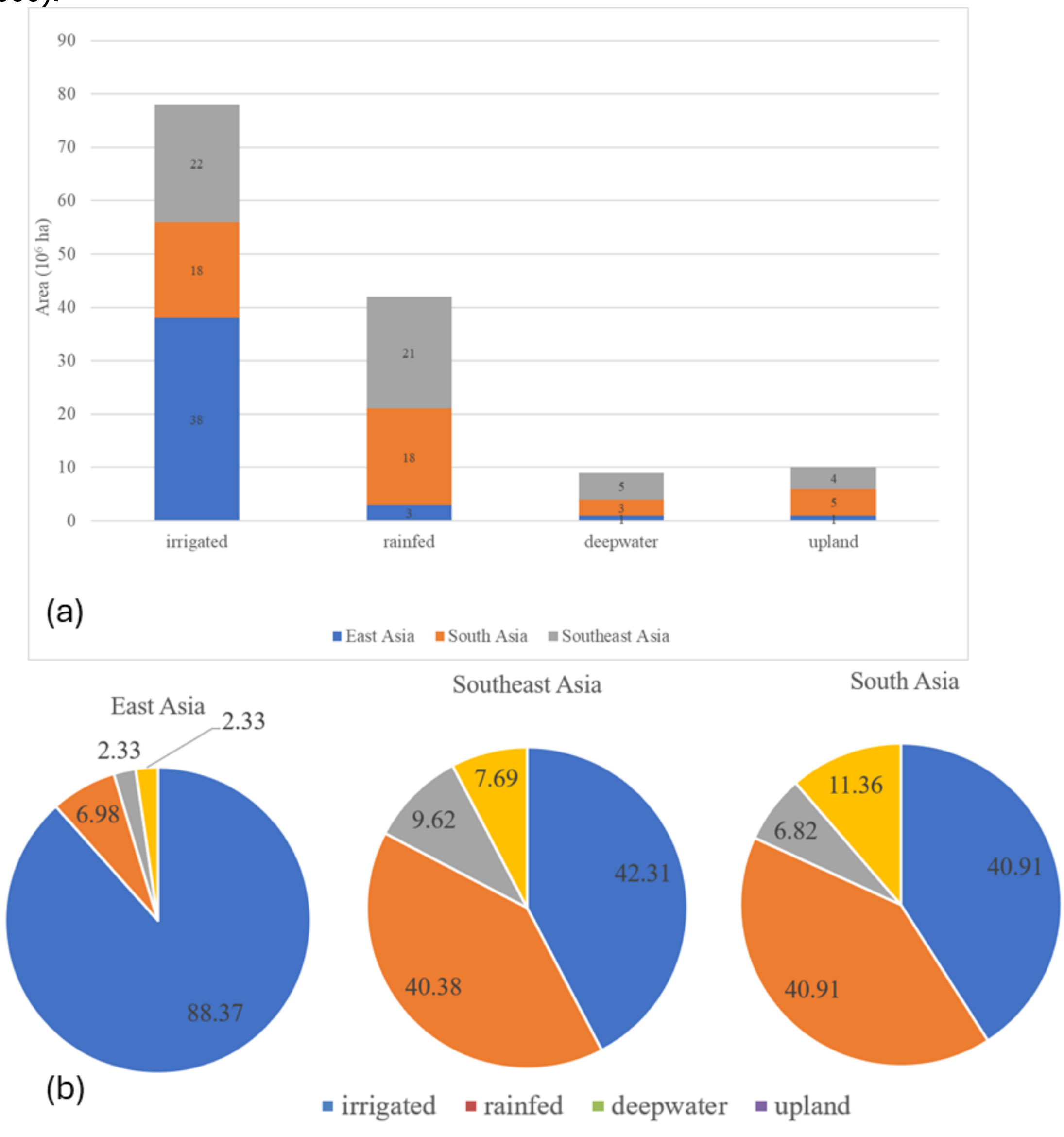


**Figure 3.** a. Area and relative emission potential of various Asian rice ecosystems; b. Percentage comparison of four types of rice ecosystems in Asia (Source: modification from Wassman *et al.* 2000).

### 3.2. Methane emissions in Southeast Asia region

Indonesia had approximately 4.8 million hectares of irrigated rice fields in 2016 (Ariani *et al.*, 2021). Total methane emissions from irrigated and rainfed rice fields were 30.74 and 20.25 mg $CH_4$ $m^{-2}$ $h^{-1}$, respectively. Research on methane emissions in Indonesia has focused on factors such as water regime, rice variety, soil type, and fertilizer use (Yan *et al.*, 2003). According to Wassmann *et al.* (2000), rainfall significantly affects methane emissions in rainfed rice fields. Methane emissions in Indonesian rice fields vary by region and cropping practices, with emissions typically higher in continuously flooded fields and lower in intermittently flooded fields (Yan *et al.*, 2003).

There is a difference between rice fields in Indonesia and Thailand. In Indonesia, rice fields are mainly irrigated or rainfed, while in Thailand, rice fields are found in three forms: rainfed, irrigated, and deepwater. Emission measurements taken in five regions of Thailand showed values of 45.98, 32.45, and 15.5 mg $CH_4$ $m^{-2}$ $h^{-1}$ in irrigated, rainfed, and deepwater rice fields, respectively (Yan *et al.*, 2003).

Along with Indonesia and Thailand, Vietnam is also a major rice producer in Southeast Asia and the third largest rice exporter in the world (Wassmann *et al.*, 2004), with a rice cultivation area of about 6.7 million hectares in 1995 (Yan *et al.*, 2003). Extensive rice cultivation in Vietnam, particularly in the Mekong Delta, is a significant source of methane emissions. Similarly, the Philippines has increased rice production to meet the needs of its growing population of 70 million people (Corton *et al.*, 2000). Yan *et al.* (2003) reported that methane emissions from irrigated rice fields in the Philippines were estimated to be 7.69 mg $CH_4$ $m^{-2}$ $h^{-1}$, while emissions from rainfed rice fields were 4.0 mg $CH_4$ $m^{-2}$ $h^{-1}$ during one growing season.

In general, methane emissions in the Southeast Asia region are primarily influenced by factors such as water management, organic matter inputs, soil type and texture, rice varieties, and fertilization. Paramitha (2023) notes that proper irrigation management, selection of rice varieties, soil types, and cultivation practices can significantly affect methane emissions from rice fields.

### 3.3. Methane emissions in South Asia region

South Asia also contributed significantly to methane emissions, with India accounting for 5.88 Tg $CH_4$ $yr^{-1}$ of the total emissions of about 25.1 Tg $CH_4$ $yr^{-1}$ for East, Southeast, and South Asia in 1995. Like Indonesia, approximately half of India's rice fields are irrigated, while the rest are rainfed or upland rice fields (Yan *et al.*, 2003). Despite having the largest cultivation area in the world, India's methane emissions are lower than China's due to less extensive irrigation and rainfall. In contrast, Bangladesh, which predominates rainfed rice fields (with only 22% irrigated), has relatively high methane emissions (Wassmann *et al.*, 2000; Yan *et al.*, 2003). With 100% of its rice fields irrigated in 1991 (Wassmann *et al.*, 2000), Pakistan emits approximately 200 kg $CH_4$ $ha^{-1}$. In Pakistan, irrigated rice fields contribute over 70% of methane emissions, while rainfed rice fields account for only 27.5% (Yan *et al.*, 2003).

## 4. Discussion

There has been extensive research on methane emissions from rice fields in Asia. The most common topic, summarized in Table 1, is the impact of water management practices. This research focuses on how different water regimes, such as continuous flooding, intermittent drainage, and pulse irrigation, affect methane emissions in various locations. Other common themes include soil types and management, cultivation techniques and crop management, and rice varieties. These recurring themes suggest that water and soil management and rice variety selection are key areas of focus for methane research related to rice production in Asia. Table 1 provides an overview of some of this research.

In Japan and Indonesia, extensive research has been conducted on water management strategies to reduce methane emissions. Studies ranging from one crop cycle to three years have shown that intermittent drainage significantly reduces emissions (Setyanto and Bakar, 2005; Hadi *et al.*, 2010; Itoh *et al.*, 2011; Nishimura *et al.*, 2020).

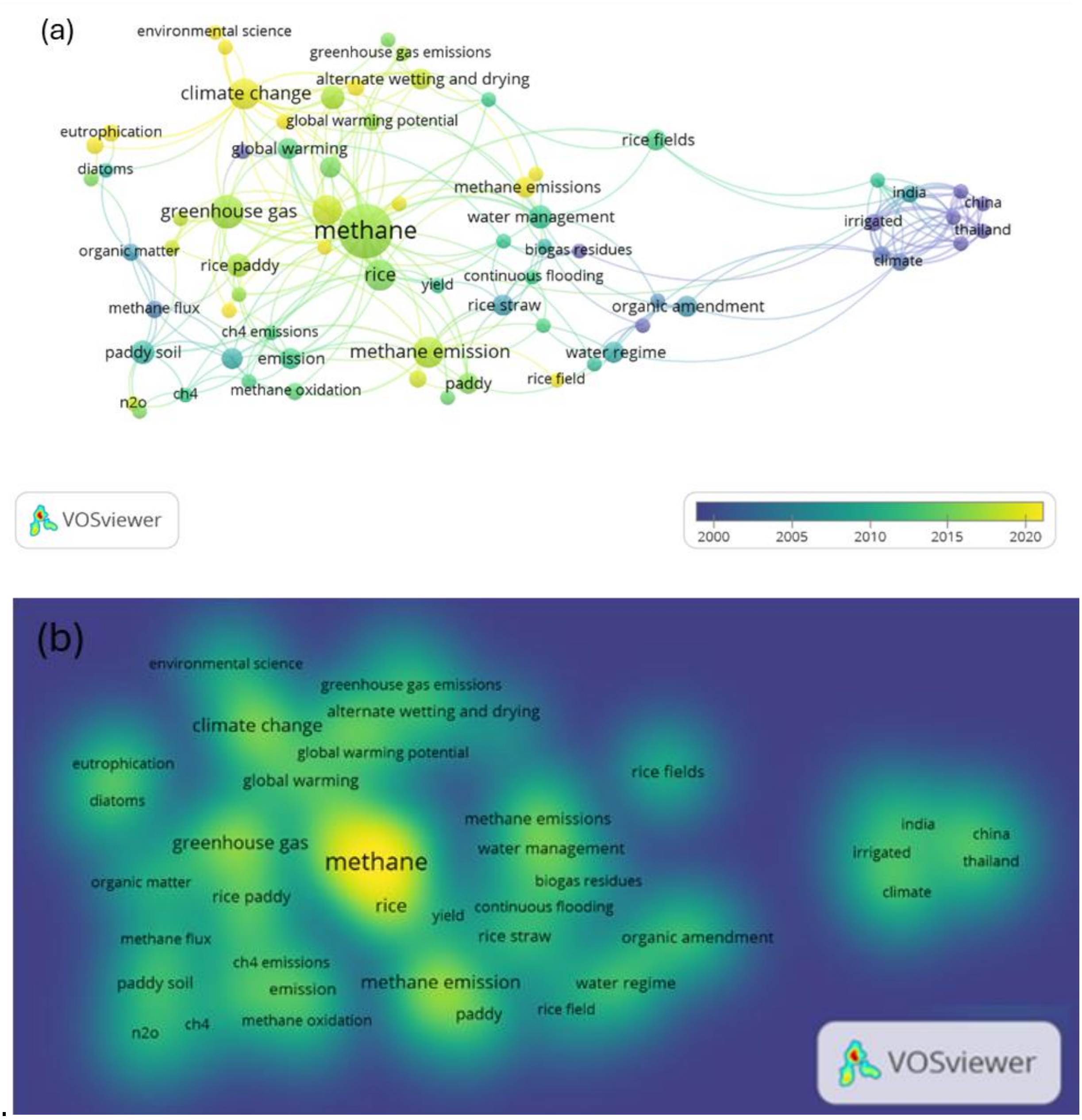


**Figure 3.** (a). Methane research topic overlay visualization; (b). Methane research topics density visualization through VOS viewer.

Variation in cultivation techniques has been extensively studied in South Korea, China, India, and Indonesia. The studies ranged from 250 days in South Korea to 25 years in China. Results indicate that in South Korea, practices such as avoiding plowing, applying rice straw during cultivation, and using conventional tillage during the fallow period significantly reduce methane emissions (Choi *et al*., 2019; Gwon *et al*., 2022). In Indonesia, direct seeding and rainfed rice fields have lower emissions, whereas alternating water and dry irrigation reduce emissions in India. However, continuous irrigation increases emissions in Indonesia and India (Setyanto *et al*., 2000; Oo *et al*., 2018). Research on rice varieties conducted in India, China, South Korea, and Indonesia, ranging from one cultivation cycle to two years, also contributes to this body of knowledge (Setyanto, 2006; Gogoi *et al*., 2008; Qin *et al*., 2015; Lim *et al*., 2021; Chandrasekaran *et al*., 2022)

**Table 2. Several methane research in several Asian countries**

| Factors | Location | Peak of methane emission | | Research Period | References |
|---|---|---|---|---|---|
| | | Highest (kg $ha^{-1}$) | Lowest (kg $ha^{-1}$) | | |
| **Water regime/ water management** | Japan | **786** (Conventional without intermittent drainage) | **31** (Front-loaded Midseason Drainage) | 2 years (2008-2009) | (Itoh *et al.*, 2011) |
| | Japan | **186** (Continuous flooding in light clay soils) | **1** (Intermittent draining in heavy clay soils) | 3 years (2016-2018) | (Nishimura *et al.*, 2020) |
| | Indonesia | **254** (Continuous flood) | **96** (Pulse irrigation) | 70 days (March-June) | (Setyanto and Bakar, 2005) |
| | Indonesia and Japan | **In Indonesia:** **1,585** (Continuous flood + local rice) **In Japan:** **634.2** (Continuous flood in alluvial soils) | **In Indonesia:** **1,065** (Intermittently drained + local rice) **In Japan:** **167.0** (Intermittently drained in peat soils) | One cultivation period (142 days in Indonesia; 125 days in Japan) | (Hadi *et al.*, 2010) |
| | Indonesia | **303.08** (Wet season with continuous flooding + normal tillage) **255.24** kg $h^{-1}season^{-1}$ (Dry season with continuous flooding + normal tillage) | **61.54** (Wet season with saturated + no tillage 3 L $h^{-1}$ sulfosate) **23.69** kg $h^{-1}season^{-1}$ (Dry season with intermittent + no tillage 3 L $h^{-1}$ paraquat) | 85 days per season Wet season (November-March) Dry season (April-July) | (Naharia *et al.*, 2018) |
| | China | **556.8** (Continuous flooding) **182.6** (Modern Japonica single crop cultivation) **179** (Pig manure) | **216.6** (Intermittent) **89.1** (Japonica hybrid early cultivation) **52.5** (Biogas residue) | 3 years (April-July and July-November 1995-1998) | (Lu *et al.*, 2000) |
| **Cultivation technique/ crop management** | South Korea | **1071.7** (spring plowing after spring spreading rice straw) | **206.5** (without plowing and rice straw application) | 2 years (May-October, each year) | (Choi *et al.*, 2019) |
| | China | **457.74** (Single crop rice) | **276.6** (Double crop rice) | 25 years (1990-2015) | (Jiang *et al.*, 2023) |

***Table 2. Cont.***

| | | | | | |
|---|---|---|---|---|---|
| | India | **0.06** (Old seedlings, narrow spacing, and continuous flooding) | **0.021** (In between two planting methods- alternate wetting and drying irrigation) | 9 months (May-January) | (Oo *et al.*, 2018) |
| | Indonesia | **2.17** (Dry season, irrigation, prilled urea) | **0.19** (Wet season, rainfed, IR-64, direct seeded) | 6 years (1993-1998) | (Setyanto *et al.*, 2000) |
| | Japan (in mineral soil over peatland) | **1160** (Single drainage + 751 g m$^{-2}$ rice straw application) | **253** (Continuous flood + 277 g m$^{-2}$ soybean stover) | 5 months (May-September) | (Naser *et al.*, 2018) |
| | Indonesia | **0.00063** (Steel slag + compost, 15 cm depth) | **0.00007** (Steel slag + compost, 35 cm depth) | 1 month (March) | (Susilawati *et al.*, 2016) |
| **Rice varieties** | India (in rice field) | **0.055** (IR-36 cultivar) | **0.083** (Monohar Sali cultivar) | 6 months (June-November) | (Gogoi *et al.*, 2008) |
| | India (in rice field) | **0.446** (vegetative stage CO 45 cultivar) | **0.001** (maturity stage of ADT 39 and ADT 45 cultivar) | 120 days for ADT 39 and ADT 45; 135 days for CO 45 (1 cultivation season). | (Chandrasekaran *et al.*, 2022) |
| | China (in rice field) | **0.82** (Huangxiuzhan cultivar) | **0.0245** (Qihuazhan cultivar) | 2 years | (Qin *et al.*, 2015) |
| | South Korea (in rice field) | **475** (Junam cultivar) | **318** (Ilmi cultivar) | 130 days (1 cultivation season). | (Lim *et al.*, 2021) |
| | Indonesia (in irrigation and rainfed rice fields) | **218** (Cisadane cultivar) | **74** (Dodokan cultivar) | 100 days for dodokan. 130 days for Cisadane. (1 cultivation season). | (Setyanto, 2006) |
| **Soil types** | China (in peatland and gley marsh) | **0.607** (peatland) | **0.375** (gley marsh) | 4 months (June-October) | (Zhu *et al.*, 2018) |
| | Indonesia | **135** (Inceptisol during dry season)<br>**335** (Inceptisol during rainy season) | **4.99** (Vertisol during dry season)<br>**3.10** (Vertisol during rainy season) | February -July (dry season)<br>October – January (rainy season) | (Susilawati *et al.*, 2015) |

2

According to the bibliometric analysis using the VOSviewer, which analyzed various journals with keywords related to methane, climate change, rice, greenhouse gases, and other relevant topics, the main research focus from 2000 to 2024 is on methane emissions in rice production about climate change. In the visualization, yellow nodes represent more recent research topics (around 2020-2024) and explore specific mitigation practices like alternate wetting, drying, and organic amendments, suggesting that research is evolving toward solution-oriented approaches. Blue nodes indicate older topics (early 2000) and seem focused on methane flux, greenhouse gases, and climate change. Larger nodes correspond to terms that are mentioned more frequently in the dataset (Fig. 3a). Strong links between methane, rice, and greenhouse gases reflect their conceptual interdependence. The connections between organic amendment and methane emissions reflect the growing interest in sustainable agricultural practices to reduce emissions. This map suggests that the field is evolving from basic emission measurements to applied research focusing on mitigation strategies, with a strong regional focus on major rice-producing countries in Asia.

The second image (Fig. 3b) represents a density visualization where bright yellow areas indicate high research activity, while green and blue areas represent less focus according to the database. Methane and rice are shown as the densest areas, marking them as key research topics. Methane emission and greenhouse gas also have high densities, reflecting their critical importance in methane-related studies. Climate change, water management, and alternate wetting and drying are clustered nearby, suggesting that water management practices (like alternate wetting and drying) are actively evolving and climate-related aspects are integral to the research. Conversely, organic matter, methane oxidation, eutrophication, diatoms, and $N_2O$ have lower densities, indicating that these topics, while still important, are less central compared to others. Some potential insights such as water management, sustainability practices such as biogas residues and organic amendment, multi-GHG interactions, and geographical research represent promising opportunities for further research.

## 5. Conclusion

This comprehensive review focuses on current research trends related to methane emissions from rice fields in East, Southeast, and South Asia, where most rice is grown. Extensive irrigated rice fields dominate East Asia, whereas Southeast Asia has a greater diversity of rice production systems. The study aimed to examine the role of diverse agricultural practices, such as irrigation methods and rice variety selection, in influencing methane emissions, with particular attention to major contributors like China and Indonesia. Practices such as intermittent drainage and pulse irrigation have been shown to be effective in reducing emissions, whereas continuous irrigation tends to increase emissions. Other critical factors influencing emissions dynamics include rice variety and soil management.

While this review highlights effective mitigation practices such as intermittent drainage and pulse irrigation, it also acknowledges the limitations of current research. Significant gaps remain in understanding the full impact of emerging factors such as alternative rice varieties, innovative organic soil amendments, and less commonly studied rice ecosystems such as rainfed and peatland fields. Addressing these gaps is critical for developing comprehensive strategies for managing methane emissions from rice fields. The integration of methane research with climate change studies reveals a growing emphasis on how climate variability affects methane emissions. This intersection highlights the need for adaptive management practices to mitigate emissions under changing climate conditions.

Despite these challenges, this review emphasizes the need for continued and expanded research in underexplored areas and highlights the importance of targeted strategies to reduce methane emissions from rice fields. The findings also highlight the critical need for localized and regionally specific approaches to managing methane emissions in rice fields, particularly in areas where variability in agricultural systems and climate conditions persist.

Future research should focus on innovative practices and technologies and address regional variability in emission factors. The broader application of such research will be crucial for reducing methane emissions and aligning rice production with global climate goals, thereby contributing to a more sustainable agricultural future. In addition, it will help develop more effective and localized mitigation strategies.

## Data availability statement

Data will be made available on request.

## Funding statement

This review received no external funding.

## Conflict of interest

The author claimed there is a conflict of interest.

## Author contribution

**IGAAP**: Conceptualization, Writing-original draft, Methodology, Investigation, Data curation, and Writing – review & editing.

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# Estimating Paddy Field Water Requirements Using CROPWAT 8.0: A Case Study in Anai Irrigation Area, West Sumatra, Indonesia

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**Abstract:** The accurate estimation of crop water requirements is critical for efficient water resource management, particularly in regions with limited irrigation resources. This study aims to evaluate the water requirements for rice crops using the CROPWAT 8.0 model and compare the results with the Penman Modification Calculation method, as specified in the Irrigation Planning Standards (KP-01). This research uses climatological data from the Kandang IV Station near the Batang Anai Irrigation area, focusing on key factors such as effective precipitation, air temperature, humidity, wind speed, sunshine duration, and topography. The representative Soil of the local area was incorporated into the analysis. The study finds that the average evapotranspiration (ETo) using CROPWAT 8.0 was 3.09 mm/day, with the peak water demand for rice occurring at the end of August, reaching 1.51 L/s·ha. These findings align with the study's objective of assessing irrigation demand for rice crops and offer a comparison of methodologies used to estimate water requirements. The results emphasize the need for improvements in the default crop and soil data used by CROPWAT 8.0 to better align with local agricultural conditions in Indonesia. This study contributes to developing more accurate models for water requirement estimation and highlights the importance of region-specific calibration in irrigation planning. Further research is needed to enhance the model's functionality and to explore alternative methods for improving water use efficiency in rice farming.

**Keywords:** CROPWAT 8.0, Eto, Batang Anai, Paddy Field Irrigation, Water Requirements Estimation.

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## 1. Introduction

Effective water management in agriculture ensures sustainable crop production, especially for water-intensive crops such as rice. In regions like Indonesia, where rice is a staple food, optimizing water use for paddy fields is essential to meet both agricultural demands and environmental sustainability goals. However, accurate estimation of water requirements for rice crops presents a challenge, particularly in areas with fluctuating climate patterns and limited water resources. Addressing these challenges requires reliable models supporting precise irrigation planning and management.

CROPWAT 8.0, 'Crop Water and Irrigation Requirements Program,' is a widely recognized tool developed by the United Nations Food and Agriculture Organization (FAO). This Windows-based software facilitates precise crop water and irrigation requirements calculations by integrating soil, climatic, and crop-specific data. Detailed information on CROPWAT 8.0 is available on the FAO Land & Water website. Based on the Penman-Monteith method (Smith, 1992), CROPWAT 8.0 provides a decision support system to estimate crop water requirements and develop irrigation schedules for various conditions (Tumiar *et al*., 2012). It allows users to input specific climate, soil, and crop data to simulate irrigation needs and assess crop performance under rainfed and irrigated conditions. CROPWAT 8.0 has been widely applied across agricultural regions globally, supporting effective water resource management. However, its application in specific local contexts, such as the Anai irrigation area of West Sumatra, Indonesia, remains underexplored.

Previous research has shown that irrigation water requirements calculated using CROPWAT 8.0 often yield different results than traditional methods like the KP-01 standard commonly used in Indonesia, which tends to overestimate water needs for rice (Shalsabillah *et al*., 2018). While CROPWAT offers potential benefits, understanding its performance in localized settings, such as West Sumatra, is critical to determining its reliability and practical utility for irrigation planning. This study addresses this gap by evaluating water availability and demand for paddy fields in the Anai irrigation area using CROPWAT 8.0, intending to provide insights into optimal water use strategies tailored to local agricultural conditions.

Recent studies underscore the importance of incorporating advanced models for effective water management under changing climate conditions (Jain & Singh, 2020; Sunil *et al*., 2021) CROPWAT 8.0 has been applied globally for estimating crop water requirements and has shown significant potential when tailored to local conditions (Poonia *et al*., 2021; Kumar *et al*., 2022). However, accurate input data, particularly for crops and soil characteristics, are critical for its successful application (Gabr, 2021).

This research is highly relevant to the field of agricultural water management as it explores the application of CROPWAT 8.0 in a specific regional context, contributing valuable data on the model's accuracy and effectiveness in Indonesia. By analyzing seasonal water requirements and exploring alternative irrigation schedules, this study supports sustainable water use practices and provides a replicable model for other regions facing similar water management challenges. The findings have potential implications for policymakers, agricultural engineers, and local farmers, offering practical guidelines for more efficient water use in rice cultivation.

Several studies have demonstrated the advantages of the Penman-Monteith method (Monteith, 1965) in calculating evapotranspiration with minimal error for reference crops, especially in tropical and subtropical climates (Pinos, 2022). However, studies also indicate that CROPWAT 8.0's effectiveness depends on accurate input data, particularly for crop and soil characteristics, which can impact water demand estimates if not carefully calibrated (Prastowo *et al*., 2016; Dasril *et al*., 2021). This study leverages recent advancements in CROPWAT 8.0 to assess its potential for optimizing irrigation practices, considering local environmental conditions in West Sumatra.

The CROPWAT 8.0 model was selected due to its flexibility in simulating various cropping systems and its robust estimation of evapotranspiration, which is essential for determining water requirements. This study incorporated climate data specific to the Anai irrigation area to provide accurate seasonal demand estimations and evaluate different planting dates for optimal water use. Limitations included the potential for user error in data input, which could affect results, particularly in areas where detailed soil and crop data may be limited.

Despite extensive use in various agricultural regions, CROPWAT's application in specific local contexts, such as the Anai irrigation area of West Sumatra, Indonesia, remains underexplored. This study addresses this gap by evaluating water availability and demand for paddy fields in the Anai irrigation area using CROPWAT 8.0, providing insights into optimal water use strategies tailored to local agricultural conditions. The findings contribute to sustainable water management practices and offer practical guidelines for policymakers and agricultural engineers (Kumar *et al*., 2022; Agrawal *et al*., 2023).

This research aims to assess the effectiveness of the CROPWAT 8.0 model in estimating irrigation water availability and requirements for the Batang Anai Irrigation Area, compared to manual calculations based on the Irrigation Planning Standards (KP-01).

## 2. Methods

This study employs an observational comparative design to evaluate irrigation water requirements in the Batang Anai Irrigation Area, utilizing both manual calculations following the Irrigation Planning Standards (KP-01) and the CROPWAT 8.0 software. The choice of this approach is informed by the need to test the robustness and accuracy of CROPWAT 8.0, a decision support tool developed by FAO, against traditional methods (Tumiar *et al*., 2012).

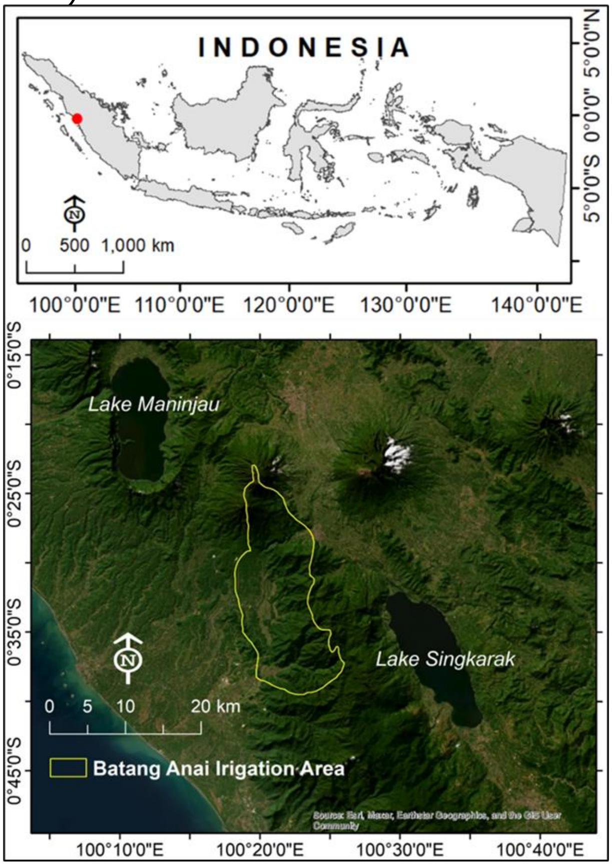


Figure 1. The study area map of Batang Anai Irrigation Area, in Padang Pariaman City, West Sumatera Province, Indonesia

### 2.1 Research Location

The research was conducted in the Batang Anai Irrigation Area in Padang Pariaman City, encompassing 8,421 hectares with a catchment area of 233 km². This site was selected for its significance in supporting regional agriculture and its comprehensive historical hydrological data, which is essential for robust analysis (Dasril *et al*., 2021). Figure 1 illustrates the layout of the catchment area.

### 2.2 Data Collection

*Hydrological and Climatological Data:*

Daily Rainfall Data (mm/day): Sourced from the Kandang IV station, covering 10 years from 2012–2021. This extended period ensures a representative sample for calculating average and effective rainfall (Prastowo *et al*., 2016).

*Climatological Data:*

They were collected from nearby meteorological stations, including Air Temperature (°C), Humidity (%), Sunlight (radiation in MJ/m²), and Wind Speed (m/s). These variables were chosen based on their relevance for evapotranspiration calculations and their impact on irrigation needs (Pinos, 2022).

### 2.3 Data Analysis

The analysis involved multiple stages to determine irrigation water requirements:

Evapotranspiration (ETo) Calculation: Conducted using the Penman-Monteith equation, integrated into CROPWAT 8.0. This method is recommended by FAO for its accuracy in a variety of climates and its comprehensive consideration of meteorological data (Allen *et al*., 1998). The equation inputs temperature, humidity, radiation, and wind speed to provide a reliable estimate of ETo.

Effective Rainfall Calculation: Rainfall data from Kandang IV station were analyzed using the R80 method, where effective rainfall was calculated as 70% of the R80 value (Tumiar *et al*., 2012). The formula used is:

$$Re = \frac{0.7\ x\ R80}{10} \qquad ...(1)$$

$$\mathrm{R80} = \frac{N}{5} + 1\mathrm{R} \qquad ...(2)$$

Planting Schedule and Crop Coefficients (Kc): Inputs were determined based on local cropping patterns and growth stages of rice, which is crucial for accurately assessing crop water needs (FAO, 2009).

Soil Type Data were assessed to determine water retention and infiltration characteristics. The soil type, such as sandy loam or clay, impacts water-holding capacity, a critical factor in irrigation planning (Wardana & Saputra, 2019). Manual Calculations with KP-01: Manual irrigation requirements were calculated following KP-01 standards, which involve empirical methods to estimate evapotranspiration and effective rainfall. The

KP-01 approach was a benchmark for evaluating the CROPWAT 8.0 outputs (Shalsabillah *et al*., 2018).

**2.4 Statistical Analysis**

A comparative analysis was conducted to evaluate discrepancies between the results obtained from CROPWAT 8.0 and KP-01 calculations. To ensure the **study's validity and reliability**, data sources were cross-referenced with regional meteorological records to confirm accuracy. Second, pre-processing involved data cleaning to remove anomalies or outliers, ensuring consistent analysis. Lastly, a pilot test was conducted with a subset of data to validate the CROPWAT 8.0 setup before full-scale analysis.

## 3. Result

### 3.1. Irrigation Water Requirements with Penman Modification (KP-01)

*Potential evapotranspiration with Penman Modification (KP-01)*

Table 2 illustrates the fluctuations in monthly Evapotranspiration Potential (Eto) values calculated using the Penman Modification (KP-01) method, which represents the atmospheric water demand and is crucial for understanding crop water requirements. In the Batang Anai Irrigation Area, the lowest Eto was recorded in July at 3.07 mm/day (approximately 95.3 mm/month), while the highest was observed in March at 3.57 mm/day (approximately 110.6 mm/month). The average evapotranspiration over the study period was 3.26 mm/day. These values reflect the impact of varying climatic conditions, such as temperature and solar radiation, directly influencing crops' water needs.

The seasonal variation in Eto indicates how crop water requirements shift throughout the year. For instance, March's highest monthly Eto value corresponds to increased solar radiation and higher temperatures, leading to greater evaporation and water demand. On the other hand, the lowest values in February and September, and notably in July, coincide with periods of reduced solar radiation and cooler temperatures, resulting in lower evapotranspiration rates. Such data underscores the necessity of incorporating climatic conditions into irrigation planning to optimize water use, reduce crop stress, and enhance water-use efficiency.

As shown by these results, the monthly distribution of Eto highlights the importance of adapting irrigation practices according to seasonal trends. Months with high Eto, such as March, August, and October, indicate the need for more intensive water management to meet crop requirements. Meanwhile, months with lower Eto suggest a potential reduction in irrigation needs. Further, the data reveals the importance of monitoring factors like temperature, solar radiation, and humidity, which can influence these variations. A comprehensive understanding of these trends allows for better irrigation scheduling and resource management, ensuring efficient water use throughout the year. Cross-referencing Eto values with local climate data could further refine irrigation strategies and support adaptation to potential climate changes.

Table 1. Potential evapotranspiration with Penman Modification (KP-01)

| Parameter | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature ($^0$C) | 25.5 | 25.5 | 25.7 | 26.0 | 26.0 | 25.8 | 25.6 | 25.6 | 25.9 | 25.2 | 25.8 | 25.9 |
| Humidity (%) | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Solar-Radiation (%) | 31.1 | 31.3 | 37.3 | 32.0 | 33.8 | 44.1 | 37.8 | 35.4 | 29.4 | 32.9 | 30.9 | 30.7 |
| Wind speed (m/s) | 1.1 | 1.2 | 0.9 | 0.8 | 0.7 | 0.8 | 0.8 | 0.7 | 0.7 | 0.6 | 0.6 | 0.6 |
| Eto/day (mm/day) | 3.2 | 3.3 | 3.6 | 3.3 | 3.1 | 3.3 | 3.1 | 3.4 | 3.2 | 3.3 | 3.3 | 3.3 |
| Number of days | 31 | 28 | 31 | 30 | 31 | 30 | 31 | 31 | 30 | 31 | 30 | 31 |
| Eto/month (mm/month) | 98.4 | 92.0 | 110.6 | 97.6 | 96.0 | 97.6 | 95.3 | 104.4 | 94.6 | 103.0 | 100.3 | 101.2 |

### 3.2. Irrigation Water Availability with F.J. Mock

Irrigation water availability is crucial in determining optimal cropping patterns, as it directly impacts the ability to meet crop water requirements. This availability is influenced by climatic conditions, with effective rainfall serving as a primary source. The F.J. Mock method estimates water contributions from rainfall, accounting for these climatic factors. The results outlined in Figure 2 and the data presented on half-monthly water availability provide a comprehensive view of how seasonal and annual rainfall variations impact irrigation planning.

The analysis shows significant variability in irrigation water availability throughout the year, with peaks in early December (31.39 units), late November (28.41 units), and early April (26.93 units), indicating periods of abundant water resources likely due to increased rainfall. Conversely, the lowest water availability is seen in late June (2.97 units), with other low points in early February (5.99 units) and early October (6.59 units), suggesting drier weather and potential water shortages. These fluctuations underscore the importance of understanding seasonal rainfall patterns to align irrigation strategies with periods of high and low water availability, ensuring crop water needs are met efficiently while avoiding water wastage.

Effective rainfall can complement or exceed irrigation requirements, particularly when it aligns with crop water demand. Recognizing these seasonal trends in water availability enables better irrigation scheduling and resource conservation, especially during periods of surplus rainfall. For example, during high water availability periods like November, December, and April, farmers may reduce reliance on supplemental irrigation, conserving resources and minimizing costs. On the other hand, proactive water management strategies, such as using stored water or adjusting planting cycles, become essential during low-availability periods like late June and early February. This adaptive approach to water management can optimize water use throughout the growing season, promoting sustainable agriculture and resilient irrigation practices.

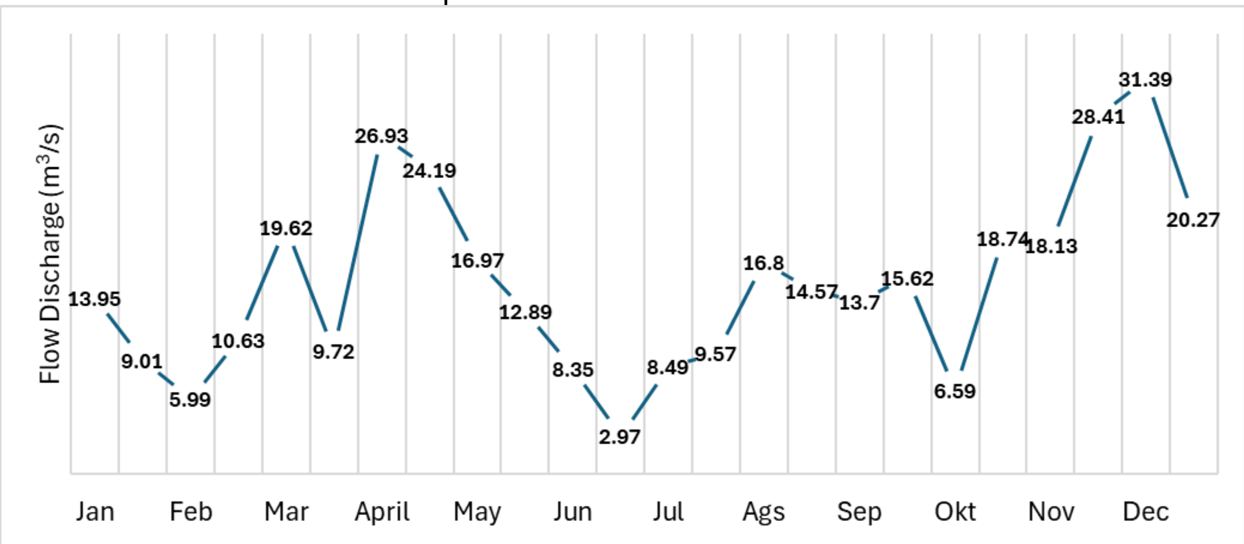


Figure 2 Irrigation Water Availability calculated using F.J. Mock method

### 3.3. Irrigation Water Requirement using Penman Modification

Estimating irrigation water requirements is essential for managing water resources in paddy fields. Using the Penman Modification (KP-01), the water demand is calculated for each half-month period, considering climatic variables and crop water consumption. This empirical approach, incorporating weather data and crop growth stages, helps ensure accurate irrigation planning. The irrigation water demand fluctuates during the study period based on climatic conditions, as shown in Figure 3. For example, in the first half of

January, the irrigation demand is relatively low (0.4 mm). Still, it increases significantly in February (7.2 mm for the first half and 13.9 mm for the second half), highlighting the impact of temperature and precipitation variations on crop water requirements.

In the seasonal analysis, three distinct cropping periods are observed: October to February, February to May, and June to September. The water requirement during these periods reflects the changing weather conditions and the growth cycle of the rice crop. The irrigation demand varies from 2.9 mm to 3.5 mm from February to May, indicating a stable water requirement during this phase. However, from June to September, the demand significantly drops, with negative values such as -6.2 mm in the second half of September, indicating periods of reduced water needs, possibly due to rainfall or lower evaporation rates during cooler weather conditions. Such variations emphasize the need for adaptive irrigation strategies that respond to seasonal fluctuations and maintain crop health without excessive water usage.

The results underscore the importance of adjusting irrigation practices based on seasonal patterns and daily climatic conditions. For instance, the irrigation demand in May (around 3.4 mm) is relatively moderate, yet there is a considerable increase in the later months. These seasonal variations in water demand highlight the need for well-managed irrigation systems to optimize water use throughout the cropping season. Ensuring water availability during peak demand periods and conserving it during lower-demand phases is key to sustainable water resource management in rice cultivation. As illustrated by the seasonal water demand data, this calculation provides valuable insights for optimizing irrigation scheduling and promoting efficient water use in paddy fields.

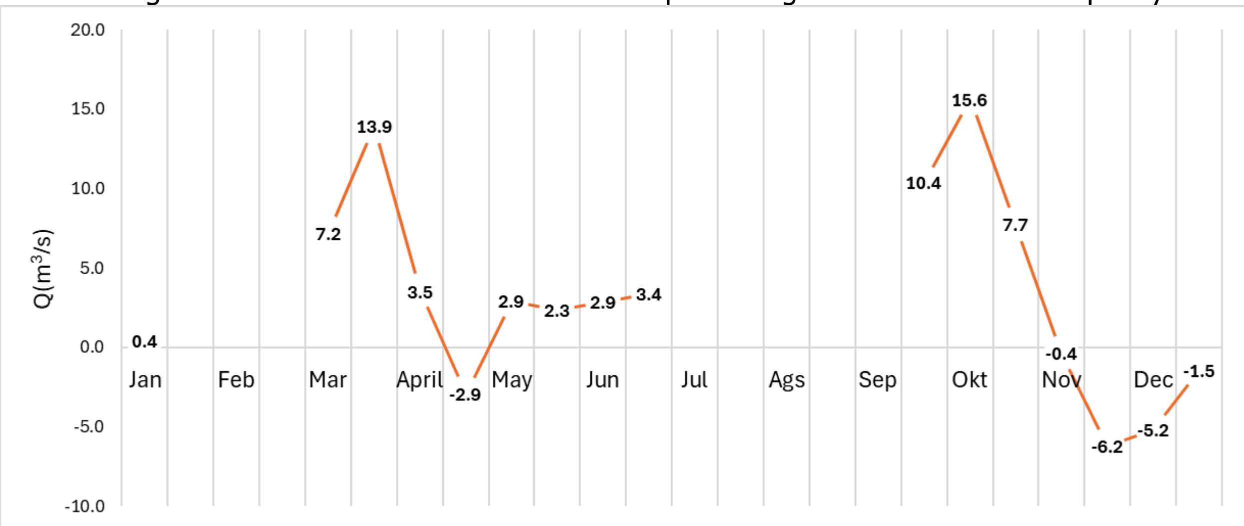


Figure 3. Irrigation Water Requirement calculated using Penman Modification (KP-01)

### 3.4. Irrigation Water Requirements with CROPWAT 8.0

The CROPWAT 8.0 model is another widely used tool for estimating irrigation water requirements. This study assumed that three planting cycles occur annually, each with specified start and harvest dates. This study assumes that planting cycles occur three times per year, each with specific start and harvest dates. Planting Season I (MT1) commences on January 1 and continues until the harvest on April 30. Planting Season II (MT2) follows, beginning on May 1 and concluding with harvest on August 28. Finally, Planting Season III (MT3) starts on September 1 and is harvested on December 29. The analysis indicates that the peak water demand is observed in August, with a maximum requirement of 12.75 m³/second. In specific periods, the irrigation water demand reaches 0 m³/second, signifying that the effective rainfall during these times is sufficient to meet the irrigation needs without additional water input. This finding emphasizes the importance of accurately assessing rainfall and integrating it into irrigation scheduling to reduce water consumption when natural resources are adequate.

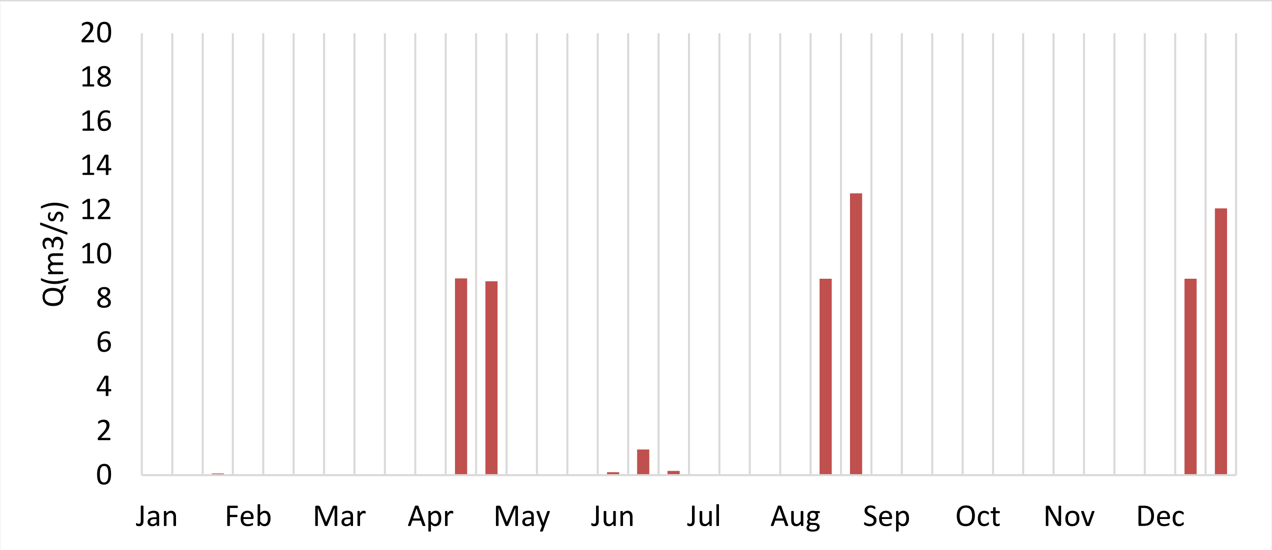


Figure 4. Irrigation Water Requirement calculated using CROPWAT 8.0

### 3.5. Statistical analysis

#### 3.5.1. Comparison of Potential Evapotranspiration Results of Penman Modification Method and CROPWAT 8.0 Calculation

The comparison results show that the potential evapotranspiration (ETo) value using the Penman modification method is higher, at 3.26 mm/day, compared to the value obtained with the CROPWAT 8.0 method, which is 3.01 mm/day. Both methods used the same climatological data from the Kandang IV Station over 10 years, from 2012 to 2021. The climatological data used includes temperature (°C), wind speed (km/day), humidity (%), and solar radiation duration (%). The differences in the results between the two methods can be attributed to using different albedo values, representing the ratio of incoming solar radiation to the radiation reflected into the atmosphere (Purnomo, 2003). In the Penman modification method, the albedo value is 0.25, while in the CROPWAT 8.0 method, the albedo value is 0.23 (Anggraeni and Kalsim, 2013). These variations in albedo values lead to differences in the estimated potential evapotranspiration (ETo) values obtained by each method.

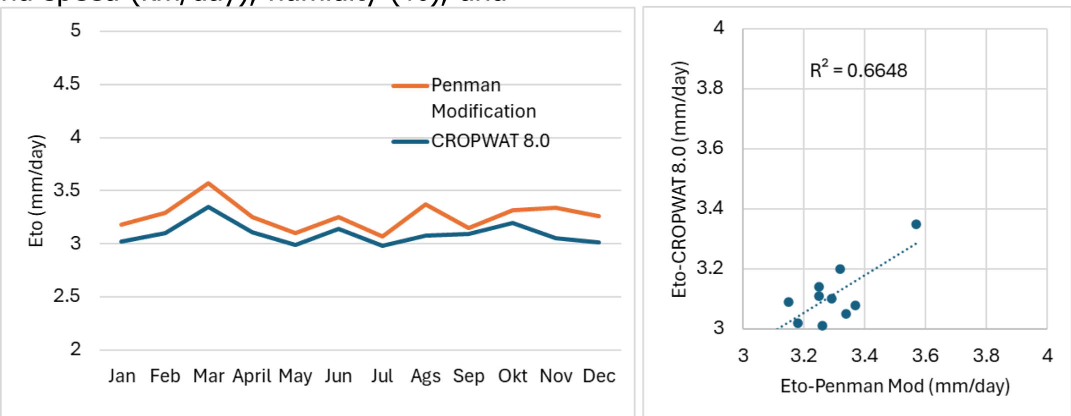


Figure 5. Comparison and scatterplot of Potential Evapotranspiration (Eto) calculated using Penman modification method and CROPWAT 8.0

### 3.5.2. Comparison of Irrigation Water Requirement by Penman Modification Method and CROPWAT 8.0 Calculation

The comparison of irrigation water requirements calculated using the Penman Modification Method and CROPWAT 8.0 (Table 2) reveals that the CROPWAT 8.0 method estimates a higher water requirement, at 1.51 L/day/ha, compared to the 0.73 L/day/ha estimated by the Penman modification method. This discrepancy in results can be attributed to differences in the handling of soil characteristics and crop data between the two methods. While both methods utilize general soil data, particularly clay soil, applying soil data in CROPWAT 8.0 can be further refined by using more localized data derived from specific field studies, which would more accurately reflect the natural conditions of agricultural land. Additionally, CROPWAT 8.0 considers various factors, such as soil saturation depth, irrigation scheduling, and the volume of irrigation water applied, all of which contribute to more precise and realistic water requirement estimations for rice crops. In contrast, the Penman method does not incorporate these additional factors, leading to lower water demand estimates. Therefore, the results suggest that the CROPWAT 8.0 method offers a more detailed and adaptable approach for calculating irrigation needs, potentially providing more reliable data for efficient irrigation management.

Table 2. Comparison of Irrigation Water Requirement

| Calculation Method | Irrigation Water Requirements (L/s/ha) |
|---|---|
| Penman Modification | 0.73 |
| CROPWAT 8.0 | 1.51 |

While both methods use general soil data (clay soil), CROPWAT 8.0 can be further refined by incorporating site-specific soil data from field studies. Moreover, CROPWAT 8.0 considers additional factors such as soil saturation depth, irrigation timing, and water application volume, contributing to more precise water requirement estimations. In contrast, the Penman method does not account for these factors, leading to lower water demand estimates. As such, the results suggest that CROPWAT 8.0 provides a more detailed and adaptable approach to estimating irrigation needs, which may be more suitable for real-world irrigation management and optimization.

The findings of this study highlight the importance of selecting appropriate models and methods for estimating irrigation water requirements. The comparison between the Penman Modification method and CROPWAT 8.0 reveals similarities and differences, with CROPWAT 8.0 providing more detailed estimations due to its incorporating additional factors. This study emphasizes the need for accurate modeling of evapotranspiration and irrigation water requirements to optimize water resource management and ensure efficient irrigation practices, particularly in regions with fluctuating climatic conditions like Batang Anai.

This study's findings are consistent with previous research indicating that CROPWAT 8.0 often estimates higher irrigation water requirements than traditional methods such as the Penman Modification method. However, the current study’s use of specific soil data and cropping patterns provides a more tailored approach that reflects the unique conditions of the Batang Anai Irrigation Area, offering insights that could be applied to similar agricultural regions.

The results of this study have significant implications for irrigation management in regions with varying climatic conditions. The ability to accurately estimate water requirements using models like CROPWAT 8.0 can help optimize irrigation schedules, reduce water wastage, and improve crop yields. Integrating effective rainfall data further enhances the model's applicability by reducing the reliance on irrigation when natural water resources are sufficient.

One limitation of this study is the use of general soil data, which may not fully capture the variability of soil properties across different Batang Anai Irrigation Area areas. Future studies should consider incorporating more localized soil data to refine the estimations of water requirements. Additionally, the analysis assumes a constant cropping pattern, which may not always align with real-world agricultural practices.

Future research could explore the use of remote sensing and soil sensors to gather more accurate data on soil moisture and evapotranspiration, improving the precision of irrigation water requirement models. Additionally, further studies could investigate the impacts of climate change on water availability and irrigation needs, particularly in regions vulnerable to extreme weather events.

In conclusion, this study provides valuable insights into estimating irrigation water requirements using the Penman Modification method and CROPWAT 8.0. The results underscore the importance of incorporating accurate soil and climatic data and considering local agricultural practices when planning irrigation strategies. The findings contribute to the ongoing efforts to optimize water use in agriculture and enhance sustainable farming practices.

## 4. Conclusion

This study evaluated the irrigation water needs of the Batang Anai Irrigation Area using the Penman Modification and CROPWAT 8.0. The Penman Modification estimated a Potential Evapotranspiration (PET) of 3.26 mm/day, while CROPWAT 8.0 calculated 3.09 mm/day, with irrigation requirements of 0.73 l/dt/ha and 1.51 l/dt/ha, respectively. CROPWAT 8.0 also identified months with no irrigation need, indicating sufficient rainfall. The findings underscore the importance of accurate water requirement estimations for optimizing water management in areas with variable rainfall. While both models provide valuable insights, CROPWAT 8.0 offers more detailed results by incorporating localized data. Future research should refine these models with more specific data and advanced technologies like remote sensing to improve irrigation efficiency, particularly in regions with climate variability.

## Data availability statement

Data will be made available on request.

## Conflict of interest

The authors claimed there is no conflict of interest.

## Author contribution

**SIS**: conceptualization, methodology-investigation-validation, writing—original draft, **N** and **J**: supervising, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

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# Comparative Water Quality Assessment of Cascade Urban Lakes: Dori, Walini, and Dora in Bogor Regency, Indonesia

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**Abstract:** Urban lakes are critical in flood mitigation, providing fresh water, and offering green spaces in urban environments. However, many urban lakes face increasing ecological pressures from various human activities. This study assesses the water quality and morphometric conditions of three urban lakes—Dori, Walini, and Dora in the Cibinong Botanical Garden Complex in Bogor Regency, located in a high-rise residential area. The study aims to evaluate the current water quality, identify contributing factors to water quality degradation, and compare conditions across the three lakes. Data was collected between January and April 2023, utilizing a Geographic Information System (GIS) for morphometric analysis and water quality measurements. Water quality was assessed in accordance with the standards set by Government Regulation No. 22 of 2021. Results indicate that Lake Dori, Walini, and Dora have surface areas of 3,406.96 $m^2$, 7,668.37 $m^2$, and 13,599 $m^2$, respectively. Based on water quality classifications, both Lake Walini and Lake Dori meet Class III standards (suitable for water use), while Lake Dora, though also in Class III, exhibits milder pollution indicators. These findings provide important insights for managing urban lakes and can guide future environmental policies.

**Keywords:** urban lakes, water quality, water status, environmental management, Indonesia

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## 1. Introduction

Urban lakes are unique ecosystems, natural or artificial water resources surrounded by urbanized or anthropogenically influenced areas. These lakes typically enhance comfort and beauty, fulfilling functions such as flood mitigation and fresh water supply (Ribbe *et al.*, 2023). Urban lakes and ponds are commonly found in parks or surrounded by green belts and vegetation, making them "green spaces" and "blue spaces" that provide essential facilities for urban residents (White *et al.,* 2010; Mishra *et al.,* 2020; Mitroi *et al.*, 2022). However, these lakes frequently experience environmental pressures from urbanization, leading to issues such as (1) poor inflowing water quality, (2) significant water level variations, (3) continuous stratification, (4) prolonged rainwater residence time, and (5) high organic carbon content (Walker and Lucke, 2018). As a result, urban lakes, often shallow and hyper-eutrophic, experience rapid degradation, with water quality challenges linked to urban activities (Birch and McCaskie, 1999).

In densely populated areas, such as Indonesia, urban lakes face significant ecological challenges. Increased population and urban development create greater demand for land and water resources, often without the infrastructure or policies to manage pollutants effectively. Consequently, urban lakes have become pollution hotspots (Wagner and Erickson, 2017; Jadeja *et al.,* 2022). Population and economic growth lead to increased development, increasing demand for residential, business, and industrial land, increasing wastewater, and adding ecological pressure on urban lakes (Vasistha and Ganguly, 2020).

In Indonesia, many urban lakes have undergone degradation, including sedimentation, shoreline change, and severe pollution. For instance, Lake Gintung has become heavily polluted according to the Indonesian government's water quality standards, making it unsuitable for consumption, fisheries, recreation, or irrigation (Maresi *et al.,* 2020). The Jakarta metropolitan area, known as Jabodetabek (Jakarta, Bogor, Depok, Tangerang, and Bekasi), is home to numerous urban lakes, many of which have been adversely affected by reduced water volumes, shrinking shorelines, and pollution. Bogor, a highland region within Jabodetabek, has also seen its urban lakes come under pressure from rapid population growth and increasing housing demand. A study by Henny and Meutia (2014) found that approximately 5% of Jakarta's urban lakes have shrunk by more than 50%, while 10-30% have experienced a reduction in area by less than 50%.

In Bogor, several urban lakes, including Lakes Dori, Walini, and Dora, form a cascading system within the densely populated Cibinong Botanical Garden Complex at the Cibinong Science Center – Botanical Garden (CSC-BG) operated by BRIN in Bogor Regency. These artificial lakes, with an average depth of approximately 1 meter for visitor safety, serve as tourist attractions and are fed by 23 spring sources (BRIN, 2024). These lakes are situated in high-rise residential areas, typically surrounded by tall buildings such as office complexes (Henny and Meutia, 2014). This area has a low risk of shoreline change, moderate sedimentation, and low to moderate algal bloom and pollution levels.

A previous study on Lake Dora in 2018 showed total nitrogen (TN) values of 0.556 mg/L and total phosphorous (TP) of 0.038 mg/L (Sulastri *et al.,* 2020), still below Class III of Indonesia's Government water quality standards. However, with accelerating urban development, there is a risk that environmental conditions around the lakes could further impact water quality.

This study aims to evaluate the current water quality of Lakes Dori, Walini, and Dora, identify parameters driving any observed degradation, and compare these across the three lakes. Findings from this research will contribute insights to inform management strategies for these essential urban ecosystems. Furthermore, the study also intends to provide a foundation for ongoing monitoring, pollution source identification, and sustainable lake management, benefiting government agencies, researchers, and the public in efforts to preserve and protect urban lakes.

## 2. Materials and Methods

### 2.1 Data Collection and Analysis

This study was conducted at Lakes Dori, Walini, and Dora from January to April 2023. These lakes form an inlet and outlet cascade system, with Lake Dora as the upstream lake and Dori as the downstream lake, while Walini is in between (Figure 1). Water sample collection and quality measurements were performed at the water surface twice weekly between 09:00 and 11:00.

The water quality parameters measured in this study include temperature, pH, dissolved oxygen (DO), turbidity, total dissolved solids (TDS), nitrate ($NO_3^-$), ammonia ($NH_3$), and phosphate ($PO_4^+$). The first five parameters were measured on-site using a water quality checker (HORIBA U-52). The device was immersed in the water surface to obtain real-time readings of these physical and chemical properties. Additionally, 1L surface water samples were collected monthly at each sampling point using a water dipper to analyze $NO_3^-$, $NH_3$, and $PO_4^+$.

All water samples were stored in a chiller for preservation and analyzed within two days of collection. Samples were filtered using 0.45 μm filter paper to remove organic matter before nutrient analysis. Nutrient concentrations were determined using a Hach DR 3900 Spectrophotometer with specific reagents for each parameter. $NO_3^-$ was measured via the cadmium reduction method (method 8192, detection limit: 0.01–0.05 mg/L), $NH_3$ with the salicylate method (method 8155, detection limit: 0.01–0.05 mg/L), and $PO_4^+$ with the ascorbic acid method (method 8048, detection limit: 0.02–2.50 mg/L).

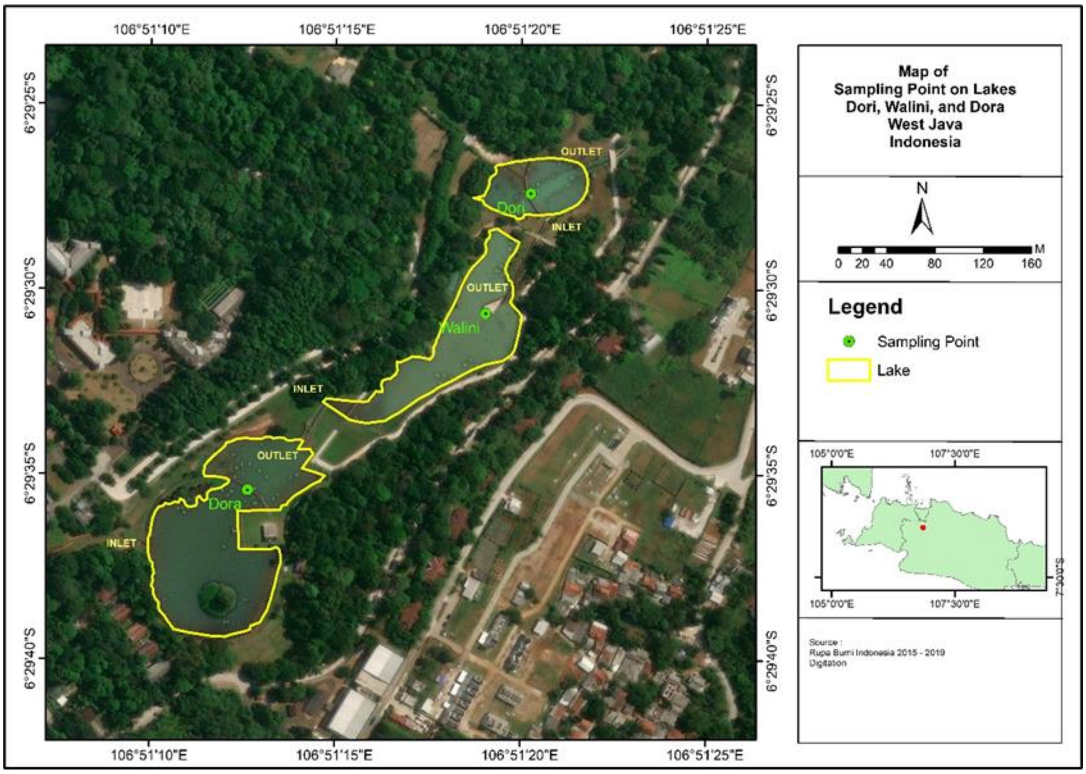


Figure 1. Sampling location of Lake Dori, Lake Walini, and Lake Dora.

## 2.2 Data analysis

The morphometric conditions of the lake surface were analyzed using free and open-source QGIS 3.22 software. Surface parameters to be analyzed include maximum length, effective maximum length, maximum width, average width, effective maximum width, surface area, shoreline perimeter length, and shoreline development index (Wetzel 2001) based on the extracted Google Maps in 2023 (Figure 1). The morphometric surface analysis method involves digitization on-screen using base maps available in QGIS.

A variance analysis (ANOVA) was conducted to determine whether there were any significant differences among the three lakes based on the measured parameters. A similarity index was also applied to assess how alike or different the lakes are. This index helps quantify the similarity or dissimilarity between entities, in this case, based on water quality data. The similarity results are visualized in a dendrogram. Hierarchical cluster analysis (HCA), ANOVA, and the Tukey HSD test were performed using Python.

The STORET Index calculation in this study includes several key physical and chemical parameters: pH, DO, turbidity, TDS, $NO_3^-$, $NH_3$, and $PO_4^+$. The collected data were evaluated by comparing each measured parameter to the corresponding standard outlined in Government Regulation No. 22 of 2021 on Implementation of Environmental Protection and Management. This assessment categorizes water quality status based on its suitability for different usage classes. This study performed STORET calculations for water quality classes I, II, and III, providing a detailed view of the lakes' compliance with regulatory standards.

## 3. Result and Discussion

### 3.1. Physical Condition

The quantification of the morphometric study revealed distinct surface morphological characteristics between the lakes. During the study, these three lakes were overgrown with aquatic plants. Lotus species covered the entire surface of Lake Dora, Lake Walini had aquatic plants in the water column, while aquatic plants covered the surface of Lake Dori but not as many as Lake Dora (Figure 2).

The quantification of the morphometric study revealed distinct surface morphological characteristics between the lakes. The result of the quantification of morphometric analysis is shown in Figure 3 and Table 1. These lakes have areas of 3,406.96 m² (Lake Dori), 7,668.37 m² (Lake Walini), and 13,599 m² (Lake Dora), Lake Dora was the largest from the other.

When analyzed using the Shoreline Development Index, all three lakes have a value greater than 1, indicating irregular lake shape. A value closer to or equal to 1 would suggest a more regular, circular lake morphology. A higher Shoreline Development Index value reflects greater human influence on the lake's shoreline, which can negatively impact the lake ecosystem and the sustainability of the surrounding natural environment (Wetzel, 2001).


Figure 2. The surface of Lake Dora (A), Lake Walini (B), and Lake Dori (C).

Table 1. Morphometric of Lakes Dori, Walini, and Dora.

| No. | Parameter | Lake Dori | Lake Walini | Lake Dora |
|---|---|---|---|---|
| 1 | Maximum Length (m) | 97.78 | 178.33 | 176.16 |
| 2 | Effective Maximum Length (m) | 94.78 | 178.33 | 176.16 |
| 3 | Maximum Width (m) | 46.24 | 61.36 | 111.70 |
| 4 | Average Width (m) | 35.94 | 43.00 | 77.20 |
| 5 | Effective Maximum Width (m) | 46.24 | 61.36 | 111.70 |
| 6 | Surface Area ($m^2$) | 3405.96 | 7668.37 | 13599.75 |
| 7 | Shoreline Perimeter Length (m) | 242.62 | 545.26 | 664.66 |
| 8 | Shoreline Development Index | 2.35 | 3.51 | 3.22 |

### 3.2. Physicochemical characteristics

The average temperature of the three lakes during the observation was 28°C (Table 2). The surface temperatures of the three lakes range from 26-32°C. Lake Dori ranges from 26-29°C, with the maximum temperature in April at 29.79°C and the minimum in June at 24.85°C. On the other hand, Lake Walini has a temperature range of 27 to 30°C, with the lowest temperature recorded in February at 27.2°C and the highest in April at 30.45°C. Meanwhile, Lake Dora has a temperature range of 27 to 29°C, with the lowest temperature in February and the highest in January at 29.94°C (Figure 4A).

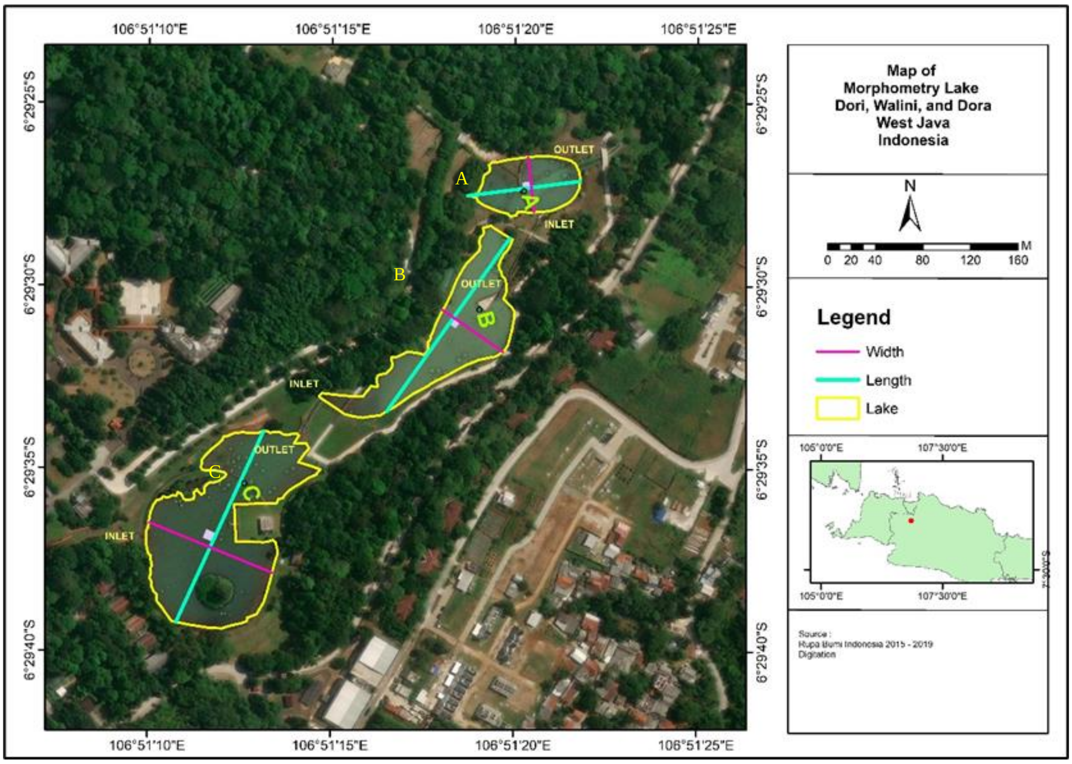


Figure 3. Morphometric of Lake Dori (A), Walini (B), and Dora (C).

Table 2. Average Water quality from Lake Dora, Lake Walini, and Lake Dori.

| No. | Parameters | Dori | Walini | Dora |
|---|---|---|---|---|
| 1 | Temperature(°C) | 28.38 | 28.93 | 28.78 |
| 2 | pH | 7.37 | 7.06 | 6.39 |
| 3 | DO | 6.29 | 6.00 | 4.79 |
| 4 | Turbidity | 89.27 | 110.36 | 45.70 |
| 5 | TDS | 0.04 | 0.04 | 0.045 |
| 6 | $NH_3$ | 0.34 | 0.30 | 0.30 |
| 7 | $NO_3^-$ | 0.15 | 0.06 | 0.06 |
| 8 | $PO_4^+$ | 0.07 | 0.04 | 0.04 |

Temperature stratification can occur in lakes deeper than 20 meters in the water column, whereas in shallow lakes, the water column temperatures tend to undergo continuous mixing (Brönmark and Hansson, 2017). When observing the water temperatures of the three lakes, they appear to exhibit a similar pattern or trend. Although the graph (Figure 4A) shows differences in temperatures among the three lakes, the average temperature from the data is 28°C, ranging from 27-30°C. This range represents typical lake water temperatures in tropical regions (Fakhrudin *et al.*, 2019; Jasalesmana *et al.,* 2019). The temperature dropped from January to mid-February because it was the rainy season. Fluctuations in temperature can be caused by factors such as sunlight intensity, wind strength and speed, air temperature, and rainfall (Magee and Wu, 2017; Jasalesmana *et al.,* 2019). In addition, from January to April 2023, it was the rainy season, and the light intensity was low because clouds covered the sun.

Photosynthesis of phytoplankton and submerged aquatic plants at Lake Dora was hampered by dense floating plants, which increased $CO_2$ production from its respiration process. Hence, the DO concentration decreases and simultaneously lowers the pH value. The pH value in water can fluctuate due to various factors, including the DO content. Oxygen can increase water pH by reducing its acidity. This occurs because oxygen reacts with compounds like carbon dioxide to form less acidic substances. pH changes are influenced by photosynthesis and respiration activities within the ecosystem in waters. Photosynthesis consumes carbon dioxide, which autotrophic organisms convert into sugars, reducing carbon dioxide levels and increasing water pH.

Conversely, ecosystem respiration increases carbon dioxide levels, decreasing water pH (Haghi *et al.*, 2017). Comparing the pH and DO graphs (Figure 4B – 4C), pH generally decreases from January to April, corresponding inversely with the DO graph, which also shows a decrease. pH fluctuations are also influenced by water hardness, organic matter content, and oxygen levels. In this study, the observed pH values remain within the normal range, although Lake Dori shows relatively high values. DO levels also affect nutrient content; under toxic conditions, nutrients like nitrogen, sulfur, and carbon compounds exist in oxidized forms, such as nitrate, sulphate, and carbonate. These various forms of nutrients are relatively non-toxic under moderate conditions but still favor the growth of phytoplankton so the impact of phytoplankton accumulation (algae blooming) can cause water quality degradation (Li *et al*., 2020).

The measurement results of DO values in the three lakes show a 2-9 mg/L range. The lowest DO level was recorded in Lake Dori, at 3.74 mg/L in April, while the highest was in January at 9.33 mg/L. Conversely, Lake Walini recorded its lowest DO level in May at 3.64 mg/L and its highest in February at 7.44 mg/L. On the other hand, Lake Dora had its lowest DO level in April at 2.01 mg/L and its highest in February at 7.02 mg/L (Figure 4C).

Various factors, such as temperature, influence the oxygen content in water; as water temperature rises, it facilitates the release of oxygen from water (Wetzel, 2001). The chart (Figure 4A) shows that the temperature decreases from January to April. Lakes deeper than 50 m experience stratification, resulting in oxygen-deprived or anoxic layers (Subehi *et al*., 2021). In contrast, urban lakes or ponds with depths less than 10 m allow light to reach the bottom, and mixing processes occur from the surface to the bottom, preventing the formation of anoxic layers in urban lakes. However, DO levels in surface water are influenced by other factors such as wind speed, air temperature, and rainfall (Magee and Wu, 2017; Jasalesmana *et al.,* 2019). The decrease in DO concentration since February is related to the rainy season and the decrease in light intensity, which leads to a decrease in photosynthesis and, therefore, a decrease in oxygen levels in the water. Lake Dora has a lower DO concentration than the other because aquatic plants have covered the surface water of Lake Dora, this plant can reduce the light intensity to penetrate the water column and reduce the photosynthesis, which means only respiration happens.

Additionally, according to Henny (2009), sulfide can significantly impact dissolved oxygen levels in the water. Sulfide can potentially deplete the toxic layer at the lake surface and may eliminate it entirely if the DO concentration in that layer cannot counteract the sulfide levels. This situation threatens aquatic organisms due to inadequate respiration oxygen and sulfide toxicity to biota (Putri *et al.,* 2024).

The result of the turbidity measurement shows that Lake Walini has the highest turbidity value than the other. This is because the water from Lake Dora (upstream) always flows to Lake Walini (middle stream), and carries the material from Lake Dora to Lake Walini. On the other hand, water from Lake Walini to Lake Dori (downstream) had water gates to separate them, and the water did not always flow to Lake Dori. This gives Lake Walini the highest turbidity value.

Generally, turbidity values in the three lakes range from 20.5 to 149.5 NTU. In Lake Dori, turbidity ranges from 57.5 to 116.45 NTU, with the lowest observed in January and the highest in April. Conversely, Lake Walini shows the lowest turbidity of 72.9 NTU in January and the highest of 149.5 NTU in March. Lake Dora has the lowest and highest turbidity values in April, at 20.3 and 82.8 NTU, respectively (Figure 4D).

Turbidity values significantly impact aquatic biota, as research shows that high turbidity can affect shrimp survival by disrupting respiration (Suhendar, 2020). Turbidity exceeding 50 NTU is considered high, and levels above 25 NTU can disturb aquatic organisms (Cech, 2005). Turbidity in lake ecosystems results from suspended particles such as sediments and phytoplankton, which reduce water clarity and light penetration (Çako *et al.,* 2013).

Elevated turbidity levels can have diverse ecological impacts, affecting visibility, foraging behavior, and prey detection in visual-hunting predators like fish (Lunt and Smee, 2020). Furthermore, turbidity can alter benthic macroinvertebrate communities, with different species thriving in pristine, semi-transparent, and turbid lakes, underscoring its influence on biodiversity and community composition (Sosa-Aranda and Zambrano, 2020).

The observations show that the three lakes' TDS values fluctuate monthly. Generally, TDS values tend to increase in March and experience significant decreases in May. The highest TDS values in Lakes Dori, Walini, and Dora occurred in January, at 0.048 g/L, 0.047 g/L, and 0.049 g/L, respectively, while the lowest values in May were 0.039 g/L, 0.044 g/L, and 0.038 g/L (Figure 4E).

Variations in TDS levels in natural water bodies typically arise from industrial discharge and changes in water balance, such as reduced inflow, increased water consumption, or heightened precipitation (Weber-Scannell Duffy, 2007). In this study, human activities and surrounding vegetation cover dominantly influence the cascade lake.

### 3.3. Nutrient Characteristics

Laboratory testing of water quality in the three lakes indicates $NH_3$ concentrations ranging from 0.25 to 0.30 mg/L in January. Lake Dori peaks in $NH_3$ levels in February at 0.47 mg/L, followed by stabilization from March to April. Lake Walini shows steady from January to February, then rises to its highest in March. Meanwhile, Lake Dora's $NH_3$ values increase until March before dropping to 0.16 mg/L in April (Figure 5A). Compared to Lake Gintung, one of the urban lakes near Jakarta (Maresi *et al.,* 2020), the $NH_3$ value in Lakes Dora, Walini, and Dori is relatively low.

$NH_3$ is a nitrogen compound that occurs naturally and dissolves in water, as the $NH_4^+$, with its form affected by pH and temperature. Under low pH conditions, $NH_4^+$ converts to ammonia, which is toxic to aquatic life, particularly fish (Edwards *et al.*, 2024). Urban lakes, including Lake Dori, often exhibit higher $NH_3$ ranges due to anthropogenic factors like vehicle emissions, industrial activities, and runoff (Edwards *et al.*, 2024).

$NO_3^-$ is another essential water quality parameter, as excessive concentrations can disrupt aquatic ecosystems (Arnanda, 2023). Observations of $NO_3^-$ levels in Lakes Dora, Walini, and Dori reveal a similar fluctuation pattern across all three lakes, with concentrations increasing from January to February and then decreasing through April. Lake Dori $NO_3^-$ levels increased significantly from 0.14 mg/L in January to 0.34 mg/L in February. The availability of inorganic nutrients ($NH_3$, $NO_2^-$, and $NO_3^-$) in these lakes supports aquatic life, with nutrients typically declining during the rainy season.

Nitrogen assimilation naturally occurs in waters as the water ecosystem utilizes it to grow. For instance, nitrogen plays a role in bacteria, such as cyanobacteria growth as their medium. Excessive amounts of ammonia, nitrate, and nitrite in water can trigger them to thrive. Continued nitrate levels increase in Lakes Dora, Walini, and Dori could lead to algal blooms, particularly cyanobacteria or blue-green algae (Luthfiani *et al.*, 2021). Algal blooms can disrupt aquatic ecosystems by depleting oxygen, producing neurotoxins, and harming fish gills (Sosa-Aranda and Zambrano, 2020).

Based on Figure 5, $PO_4^+$ values in Lake Dori remained stable from January to March and decreased from March to April. At the beginning of the observation period, the $PO_4^+$ value in Lake Walini was 0.05 mg/L, decreasing to 0.03 mg/L in February. Similarly, Lake Dora also experienced the same $PO_4^+$ values from February to April (Figure 5C).

Lake Dori has higher nitrate and PO4 levels because it is downstream, so nutrients flow and accumulate. In January and February, the nitrate and PO4 levels increased due to the rainy season, so a lot of water flowed into Lake Dori and the sluice gates from Lake Walini were opened, causing an increased water flow into Lake Dori, which gradually decreased after February.

The fluctuation in $PO_4^+$ content in each lake is influenced by the quality of incoming water and the specific conditions of each lake, such as location, land use, biogeochemistry condition, water inlet source quality, and so on. The decrease observed in Lake Walini and Lake Dora could be due to $PO_4^+$ being absorbed by phytoplankton (Boyd and Musig, 1981) or aquatic plants. Additionally, $PO_4^+$ in water can easily bind with particulates and precipitate into lake sediments (Wang *et al.,* 2022).

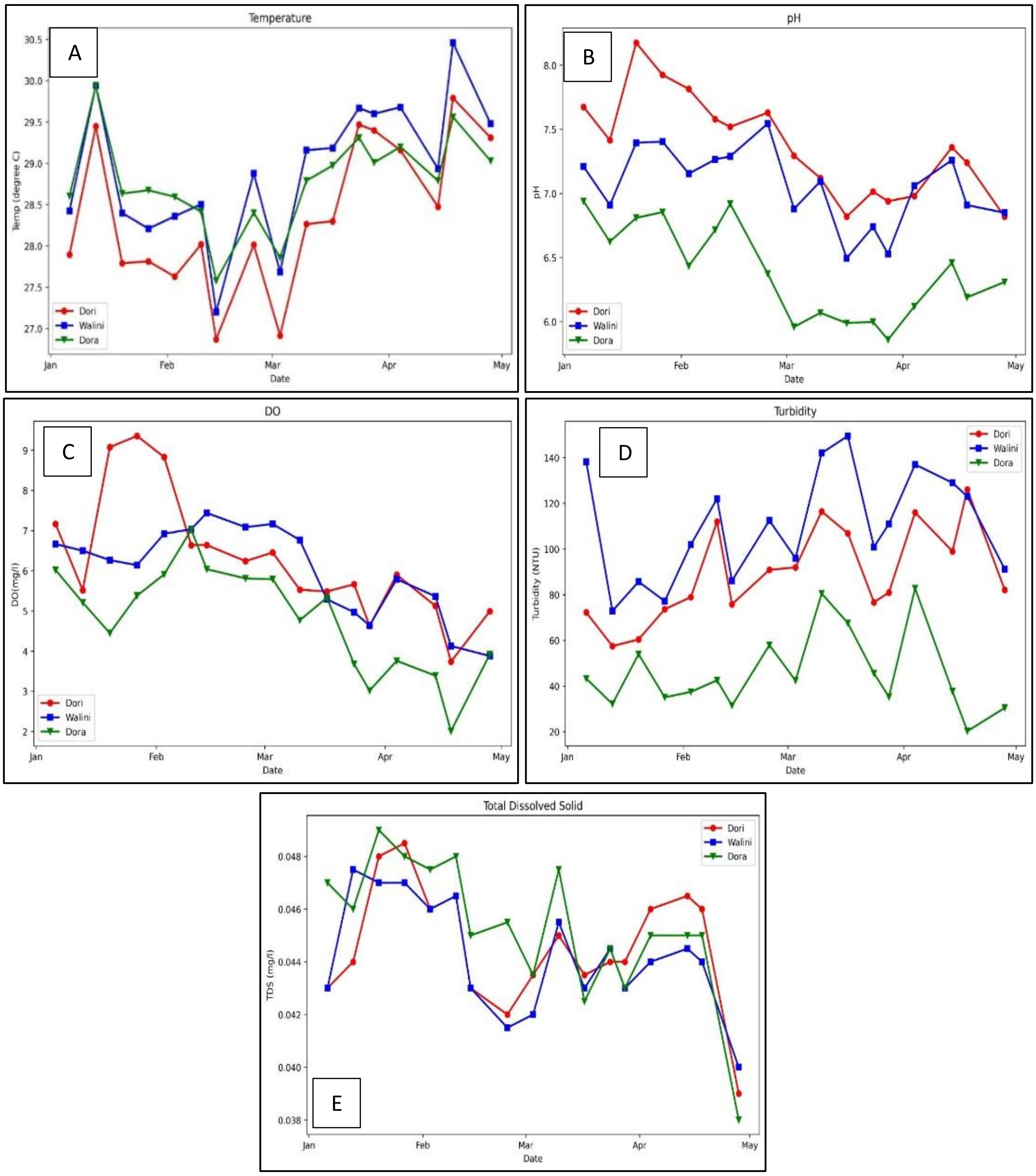


Figure 4. Graphic Water quality of Lakes Dora, Walini, and Dori. (A) Temperature; (B) pH; (C) Dissolved Oxygen; (D) Turbidity; (E) Total Dissolved Solid.

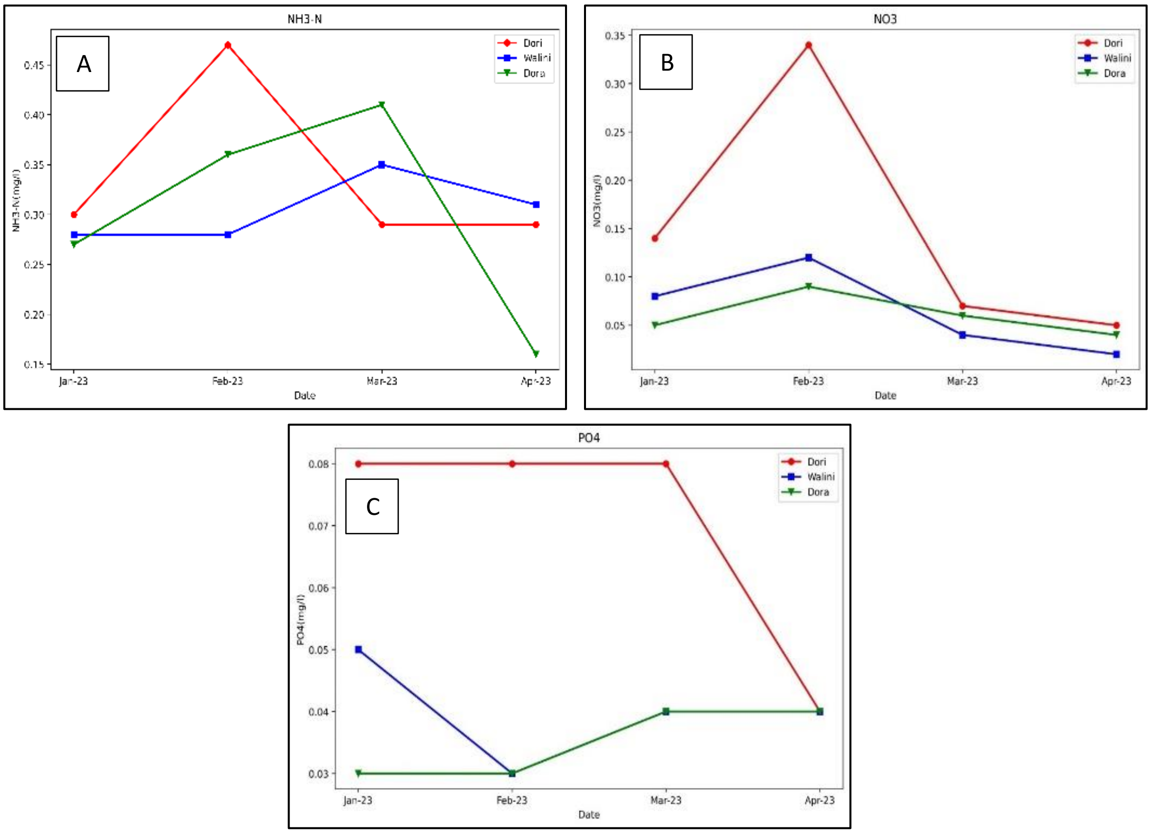


Figure 5. Graphic Nutrient of Lakes Dora, Walini, and Dori (A) $NH_3$; (B) $NO_3^-$; (C) $PO_4^+$.

### 3.4. A distinct characteristic between lakes

The similarity index analysis and dendrogram results indicate that the water quality at the Lakes Dori and Walini sites is more closely aligned, while Lake Dora shows more distinct characteristics (Figure 6). This distinction is illustrated by the shorter Euclidean distance between Lakes Dori and Walini, approximately 4, signifying a relatively high level of similarity. In contrast, Lake Dora connects to the Lake Dori-Walini group at a greater Euclidean distance of about 12, highlighting Lake Dora's substantial difference from the other two lakes.

ANOVA analysis results indicate no significant differences among the lakes for most measured parameters, except for the dissolved oxygen and turbidity parameters, which had a p-value below 0.05 (Table 3). This indicates that DO and turbidity levels varied significantly across the lakes.

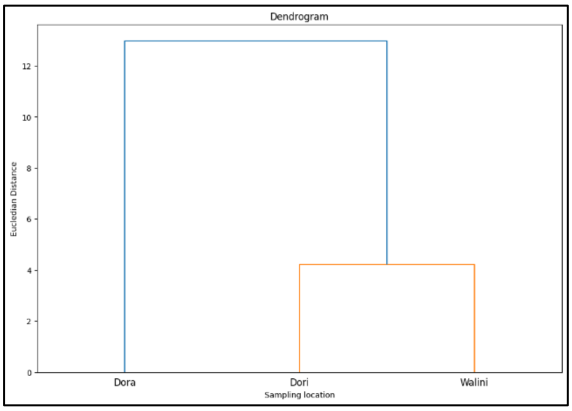


Figure 6. The similarity index from Lakes Dora, Walini, and Dori.

### 3.5. Lake Management Strategies Based on The Water Quality Status

Although the three lakes' physical and chemical water quality remains within the acceptable range of water quality standards, an analysis of lake management strategies based on the water quality status is essential. This analysis will help determine appropriate uses for these lakes in alignment with water class classifications established by regulations. Water quality status and class classifications guide pollution control or management strategies in aquatic environments (Ratnaningsih *et al.,* 2018). In urban lakes, class classification specifies the type of water use that is suitable for each lake's water quality.

Determining water pollution status and class classification follows Government Regulation No. 22 of 2021 on Implementation of Environmental Protection and Management, while the water quality status of the three lakes is evaluated using the STORET method (Canter, 1977), as it enables analysis of multiple water quality parameters. Additionally, measurements were conducted temporally at specific locations over a defined period, allowing water quality status to be assessed for each water class as specified in the regulations. The STORET calculations result for each water class in each lake are presented in Table 4.

Table 3. The result of the ANOVA analysis for the three urban lakes.

| Parameter | Temperature | pH | DO | Turbidity | TDS | $NH_3$ | $NO_3^-$ | $PO_4^+$ |
|---|---|---|---|---|---|---|---|---|
| p-Value | 0.1222 | 5.5644 | 0.0040 | 1.60E-12 | 0.4289 | 0.8339 | 0.3678 | 0.0978 |
| Significancy (Yes/No) | no | no | yes | yes | no | no | no | no |

Determining water pollution status and class classification follows Government Regulation No. 22 of 2021 on Implementation of Environmental Protection and Management, while the water quality status of the three lakes is evaluated using the STORET method (Canter, 1977), as it enables analysis of multiple water quality parameters. Additionally, measurements were conducted temporally at specific locations over a defined period, allowing water quality status to be assessed for each water class as specified in the regulations. The STORET calculations result for each water class in each lake are presented in Table 4.

Table 4. Water Quality Status as per Water Classification of Lakes Dori, Walini, and Dora.

| Lake Name | Water Quality Status | | |
|---|---|---|---|
| | **Class I** | **Class II** | **Class III** |
| Dora | C | C | B |
| Walini | C | C | A |
| Dori | C | B | A |

A: Meet quality standard
B: Slightly polluted
C: Moderately polluted

Table 4. shows none of the three lakes meet Class I or Class II water standards, as their water quality status falls within Class C or B, indicating moderate to slightly polluted. The source of contamination may originate from NH3 nutrients with relatively high concentrations, which is evident from the presence of aquatic plants on the lake surface. Besides temperature conditions, DO levels also influence nutrient concentrations in water bodies (Arnando *et al.,* 2023). For instance, higher temperatures increase nitrate and phosphate levels due to enhanced water evaporation, impacting nutrient concentrations in the water. Additionally, low oxygen levels in water affect nitrate content, as inadequate oxygen disrupts or diminishes nitrification processes in aquatic environments.

In contrast to Lake Walini and Lake Dori, which contain several submerged aquatic plant species, Lake Dora is densely populated with emergent plants that majority extend above the water surface. While emergent plants help reduce excess nutrients in the water, the morphology and other factors, such as weather, dissolved oxygen levels, and biological activities within the water influence nutrient dynamics (Kreuzwieser and Gessler, 2010; Trombetta *et al.,* 2019). Due to differences in plant density and nutrient level, Lake Walini and Lake Dori meet Class III standards, which are classified as Class A (meeting water quality standards). These lakes are suitable for uses such as freshwater fish farming, livestock farming, and irrigation in compliance with Government Regulation No. 22 of 2021 on Implementation of Environmental Protection and Management. However, Lake Dora, classified as Class B (slightly polluted) under Class III criteria, is not recommended for maintaining aquatic organisms but may be used for other purposes, such as gardens or farmland.

## 4. Conclusion

This study found that the downstream lakes have a better water quality index than the upstream lakes, with Lake Dora acting as a buffer for further downstream lakes. Therefore, improving water quality for recreational purposes may best focus on Lake Dora. Further research is needed to assess the conditions of the surrounding watershed areas and their impact on fluctuating water quality in these lakes. This research is essential for enhancing our understanding of water quality dynamics and for developing more effective management strategies to preserve urban lake ecosystems.

### Data availability statement

We state that the source of all required data has been written in the manuscript

### Conflict of interest

All authors have declared that there is no conflict of interest in the writing and submission of the manuscript.

### Contributor Statement

**AR, AYA, DR**, and **RN** are the main contributors for the work. They were developed the concept for the study, as well as conducted field investigation, formal analysis, and writing the manuscript. **AD** and **DV** as the co-authors contributed on formal analysis. While **AD** give substantial analysis for data interpretation, **DV** contributed on graphical data preparation and its analysis, and editing. All authors and co-authors declare that they reviewed and consented to the final version of the manuscript.

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# Plankton Community Structure in the Estuaries of Banten Bay, Banten Province, Indonesia

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**Abstract:** Estuaries in Banten Bay support fisheries activities by serving as critical habitats for plankton communities, which form the foundation of the aquatic food web. This study aims to determine the structure of plankton communities, both phytoplankton and zooplankton, as baseline data for fisheries management in Banten Bay estuaries. Fieldwork was conducted in April and October 2021 at four estuaries in Banten Province: Karangantu, Wadas, Cengkok Estuary, and Pamong. Water samples were collected for plankton identification and analysis of physical and chemical water quality parameters in situ and laboratory. Key structural attributes, including Shannon - Wiener diversity index (*H'*), evenness index (*E*), dominance index (*C*), trophic status, and canonical correspondence analysis (CCA), were also assessed. The highest abundance of phytoplankton was found in the Karangantu estuary, while the highest abundance of zooplankton was observed in the Pamong estuary, with overall abundance higher in April. Phytoplankton diversity was highest in Karangantu in October, whereas zooplankton diversity peaked in April. Plankton diversity indicated slight to moderate pollution levels, and trophic status analysis revealed eutrophic to hypertrophic conditions across the estuaries, suggesting high nutrient levels that support fish productivity. CCA revealed significant correlations between environmental variables and plankton composition and abundance. Mitigation strategies are recommended to monitor the growth of *Chaetoceros* sp. and *Bacteriastrum* sp., especially during the dry season. Long-term monitoring of water quality and plankton dynamics is essential in other estuaries of Banten Bay to assess nutrient loading impacts and develop strategies to mitigate harmful algal blooms. These efforts are critical to ensuring sustainable fisheries management in the region.

**Keywords:** phytoplankton; zooplankton; plankton community structure; estuary; Banten Bay

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## 1. Introduction

Estuaries, the regions where a river meets the sea, are brackish water environments with highly variable salinity levels ranging from 10 to 32‰, influenced significantly by tidal cycles (Ducklow and Shiah, 1993). In Banten Bay, estuaries such as Karangantu, Wadas, Cengkok, Pamong, and Terate serve as important fishing grounds, supporting the livelihoods of local fishermen. However, the intense human activities surrounding these estuaries necessitate regular monitoring of water quality and plankton populations, which are vital as fish feed, to ensure the sustainability of capture fisheries in the region. Understanding species' presence or notable absence in relation to shifts in physico-chemical conditions offers crucial insights into how environmental changes influence or limit the functioning of estuarine ecosystems (Downie *et al*., 2024). Phytoplankton is found globally and exhibits diversity that varies along latitudinal, longitudinal, and altitudinal gradients. Their community structure is primarily shaped by bottom-up factors, including nutrient availability, temperature, and light. Consequently, shifts in land use and climate that alter local environmental conditions significantly threaten the ecological balance of phytoplankton communities (Mancuso *et al*., 2021).

Plankton exhibits a wide range of body sizes with significant ecological and physiological implications. Their size influences their ability to assimilate dissolved nutrients from the environment and to position themselves at optimal depths with suitable light and suitable for growth (Peters, 1983). Phytoplankton, often referred to as algae, are simple autotrophic organisms and represent one of the largest groups of photosynthetic organisms in aquatic ecosystems. Meanwhile, zooplankton are heterotrophic, unicellular, or multicellular organisms that act as consumers in the food web of microorganisms (Bathmann and Marine Zooplankton Colloquium, 2001).

Plankton is essential as a biological indicator of water quality and tropical status because they respond quickly to environmental changes. Phytoplankton acts as an energy transducer that converts solar energy into chemical energy (food) and as a mediator, sharing the cycle of elements such as carbon, nitrogen, and sulfur. Meanwhile, zooplankton passes this energy to a higher tropic to link energy from producers to other consumers (Rissik *et al*., 2008). Phytoplankton serve as valuable indicators of environmental shifts, offering insights into how ecosystems respond and adapt to climate change. Additionally, research on phytoplankton can guide the development of strategies to mitigate and adapt to the adverse impacts of a changing climate (Eker-Develi *et al*., 2022).

Each group of plankton has specific environmental requirements to survive, making them highly sensitive to any physical, chemical, and biological changes in the environment. Phytoplankton size variation (pico- <2 μm, nano- ≥2–20 μm, micro- ≥20–200 μm, and macroplankton- > 200 μm) is related to environmental conditions and plays a vital role in carbon cycling and ecological functions, such as energy transfer through the aquatic food chain. The size distribution also affects their survival by influencing their sinking rate and stability. This distribution, in turn, is shaped by the intricate hydrodynamic processes within estuarine ecosystems (Wai New *et al*., 2022).

Several studies have examined plankton in various locations within Banten Bay. Mulyadi (1989) investigated fluctuations and the composition of the phytoplankton community in the mangrove waters of Dua Island. Alianto *et al*. (2008) analyzed the primary productivity of phytoplankton and its relationship with nutrients and light intensity in Banten Bay. Farchan *et al*. (2008) explored the phytoplankton community and water quality in Bojonegara, while Ronauli *et al*. (2022) focused on phytoplankton biodiversity and their role as pollution bioindicators in the coastal waters of Bojonegara. However, information on the plankton community structure in Banten Bay's estuaries remains limited. This gap underscores the importance of the current study, which aims to analyze species composition and abundance, integrate statistical data analysis, and examine water quality factors influencing plankton composition. The findings will serve as baseline data and provide recommendations for sustainable fisheries management in Banten Bay estuaries.

## 2. Materials and Methods

### 2.1. Sampling sites

This study was conducted at four estuaries in Banten Bay, located in the city and district of Serang, Banten Province (Figure 1): Karangantu Estuary (station 1), Wadas Estuary (station 2), Cengkok Estuary (station 3), and Pamong Estuary (station 4). These sampling stations were designated to represent fishing grounds locations in Banten Bay, each with different characteristics of the environmental conditions around the estuary. Iron processing industries, sugar industries, fish auctions, and residential areas surround Wadas Estuary. Karangantu Estuary features fishing ports, residential areas, and tourist attractions. Cengkok Estuary is characterized by aquaculture, agriculture activities, and fishermen's settlements. Pamong Estuary is dominated by agricultural and residential areas. Sampling was conducted in April and October 2021 to capture variability in environmental conditions.

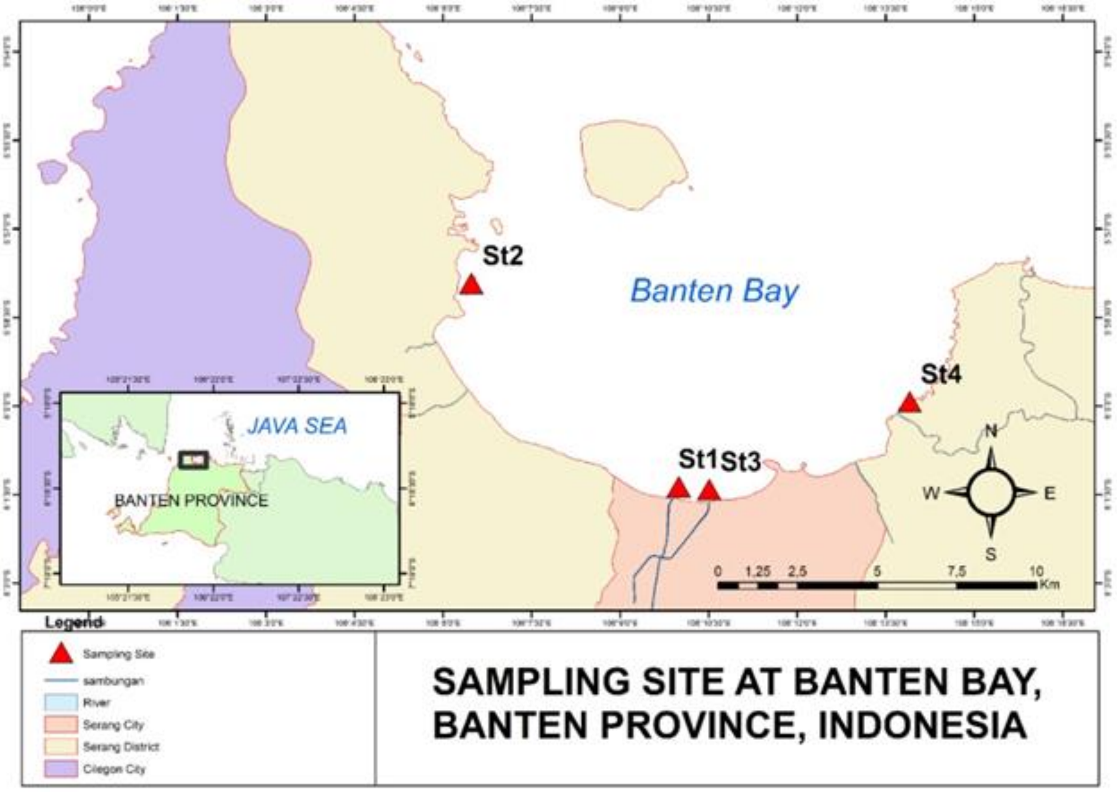


Figure 1. Map of sampling locations in the estuaries of Banten Bay.

## 2.2. Samples collection and analysis

Water samples were collected as composite samples from three depths: the surface, the Secchi depths level, and near the bottom of water bodies. Water transparency was measured using a Secchi disk. Physical and chemical parameters, including pH, dissolved oxygen, water temperature, total dissolved solids (TDS), turbidity, conductivity, salinity, and oxidation-reduction potential (ORP), were measured in situ using the Water Quality Checker (WQC) Horiba. Water currents were assessed using a Flowatch current meter.

Laboratory analyses were performed for parameters such as total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS), and chlorophyll-a, following APHA (2017) standard methods. Composite water samples (250 mL) for TN and TP analysis were preserved with sulfuric acid ($H_2SO_4$) to adjust the pH to 2, and then TN was analyzed using the destruction and brucine method. At the same time, TP was analyzed using the destruction and ascorbic acid method. TSS was determined by filtering 250 ml of composite water samples through GF/A filter paper, followed by the gravimetric method. Chlorophyll-a was measured by filtering 250 mL of water through GF/F filter paper and analyzing the filtrate spectrophotometrically. Plankton samples, including phytoplankton and zooplankton, were collected using a plankton net with a mesh size of 25 µm. Two liters of surface water from each station were filtered through the net, transferred into a 10 mL plankton bottle, and preserved with Lugol's solution. Plankton enumeration was performed using a microscope and a Sedgewick Rafter Counting Cell (SRCC) following APHA (2017). Species identification was based on Davis's (1955) and Smith's (1977) manuals. Plankton abundance was calculated with the formula as follows:

$$N = \frac{1}{V} \, x \, \frac{Ja}{Jb} \, x \frac{Vt}{Vs} \, x \, n \quad ....(1)$$

where;

$N$ = number of plankton abundance (cell/mL)

$V$ = volume of filtered water sample (mL)

*Vt* = volume of water sample (L)
*Vs* = volume of sample in Sedgewick Rafter (mL)
*Ja* = number of box in Sedgewick Rafter
*Jb* = number of identification box in Sedgewick Rafter
*n* = number of plankton obtained (cell)

## 2.3. Data Analysis

### 2.3.1 Shannon -Wiener diversity index (*H'*)

Species diversity shows the number of types of organisms found in an area. The most widely used relative diversity index is the Shannon-Wiener diversity index (H') or the Shannon diversity index or Shannon index (Dash and Dash, 2009). The Shannon-Wiener diversity index is a measure of diversity that combines species richness and relative abundance. The Shannon – Wiener index value is obtained by the following formula (Wilhm and Dorris, 1968):

$$H' = \sum_{i=1}^{S} p_i \ln p_i \qquad ....(2)$$

where;
H' = Diversity index
pi = ni /N (proportion of the total number of individuals that belong to i - th species)
ni = the number of individuals of species i
S = total number of species

The Species diversity based on the Shannon-Wiener diversity index is divided into three criteria, namely:

*H'* < 1 = low species diversity
1< *H'* <3 = moderate species diversity
H' >3 = high species diversity

In addition, the Shannon-Wiener diversity index was applied to evaluate water quality, with the resulting values indicating the level of pollution level in the water (Lee *et al.,* 1978):

*H'* > 2.0 = not polluted
*H'* 2.0 - 1.5 = slightly polluted
*H'* 1.5 - 1.0 = moderately polluted
*H'* < 1.0 = heavy polluted

### 2.3.2. Evenness index (E)

Evenness represents the relative abundance of species within a community and reflects the distribution pattern of biota. Pielou's Evenness Index (1966) was used to assess this metric:

$$E = \frac{H'}{ln\, S} \qquad ....(3)$$

where;
*E* = Evenness index
*H'* = Diversity index
*S* = The number of species in the community

The evenness index value is between 0 – 1. An index value of 0 indicates low evenness, signifying the dominance of a single species within the community. Conversely, an index value of 1 indicates high evenness, where all species in the community have an equal number of individuals.

### 2.3.3. Simpson dominance index (*C*)

Evenness represents the relative abundance of species within a community and reflects the distribution pattern of biota. Pielou's Evenness Index (1966) was used to assess this metric:

$$D = \sum (n_i/N)^2 \qquad ....(4)$$

where;
D = Simpson Diversity Index
Ni = the number of individuals in species i
N = The total number of species

This index is used to assess the complexity of a community. Simpson's diversity index ranges from 0 to 1, with a value of 1 indicating maximum dominance, which occurs when only a single species is present.

### 2.3.4. Trophic Index

Estimation of water fertility level as Trophic Index (TRIX) applying equation from Vollenweider *et al*. (1998):

$$TRIX = \frac{k}{n} \sum_{i}^{n} \frac{(log\, M - log\, L)}{(log\, U - log\, L)} \qquad ....(5)$$

where;
*K* = Scaling factor
*N* = The amount of four parameters, including phosphorous, chlorophyll-a, dissolved oxygen, and nitrogen
*M* = Parameter value
*Log U* = Upper limit (average of log M+ 2SD)
*Log L* = Lower limit (average of log L– 2SD)
*SD* = Standard Deviation

The Trophic index criteria is :

*TRIX* < 2 = Oligotrophic
2 ≤ *TRIX* <4 = Mesotrophic
4 ≤ *TRIX* <6 = Eutrophic
*TRIX* ≥ 6 = Hypertrophic

### 2.3.5. Canonical Correspondence Analysis (CCA)

The relationship between plankton species and aquatic environmental factors was tested using canonical correspondence analysis (CCA), a multivariate statistical analysis. CCA identifies the "best" synthetic gradients from field data by forming maximal linear combinations of environmental variables and biological community responses. This approach is particularly effective in elucidating how multiple species respond simultaneously to environmental factors, whether based on observational data or experimental designs (Ter Braak and Verdonschot, 1995). The analysis was performed using PAST version 4.04 software.

## 3. Result

The abundance of phytoplankton in the estuaries of Banten Bay is presented in Table 1 and illustrated in Figure 2 - 5. The phytoplankton community indices for the four estuaries are detailed in Table 2.

Table 1. Phytoplankton abundance in the estuaries of Banten Bay

| Infor-mation | Station 1 | | Station 2 | | Station 3 | | Station 4 | |
|---|---|---|---|---|---|---|---|---|
| | April | October | April | October | April | October | April | October |
| Number of species | 20 | 22 | 17 | 16 | 15 | 21 | 14 | 18 |
| Abundance (cell/mL) | 136,971,014 | 3,297,103 | 37,00,001 | 37,756,523 | 96,313,045 | 9,721,741 | 14,073,914 | 5,727,540 |

Table 2. Phytoplankton community indices in the estuaries of Banten Bay

| Index | Station 1 | | Station 2 | | Station 3 | | Station 4 | |
|---|---|---|---|---|---|---|---|---|
| | April | October | April | October | April | October | April | October |
| Diversity | 1.34 | 2.58 | 1.17 | 0.84 | 1.02 | 2.26 | 1.50 | 1.82 |
| Evenness | 0.45 | 0.84 | 0.41 | 0.30 | 0.38 | 0.74 | 0.57 | 0.63 |
| Dominance | 0.40 | 0.10 | 0.51 | 0.61 | 0.5 | 0.16 | 0.33 | 0.24 |

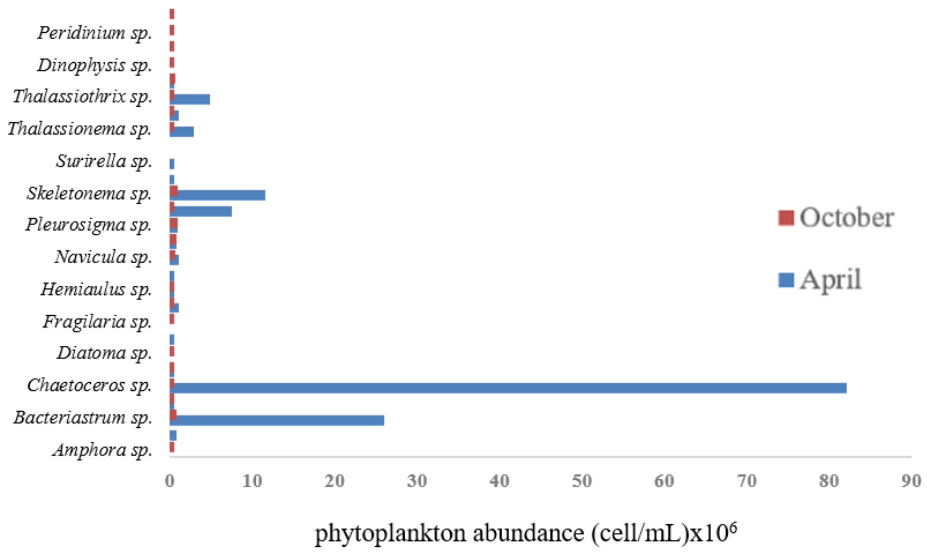


Figure 2. Phytoplankton abundance at Station 1

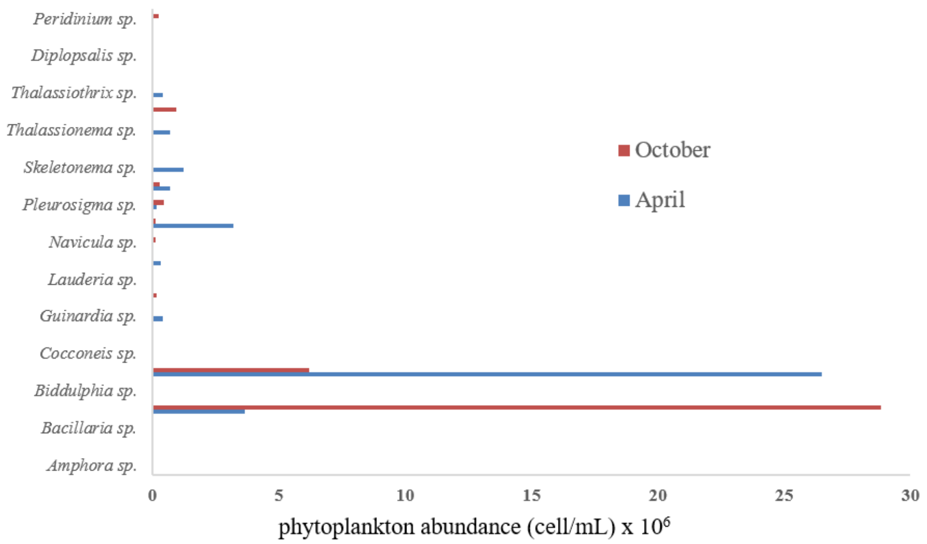


Figure 3. Phytoplankton abundance at Station 2

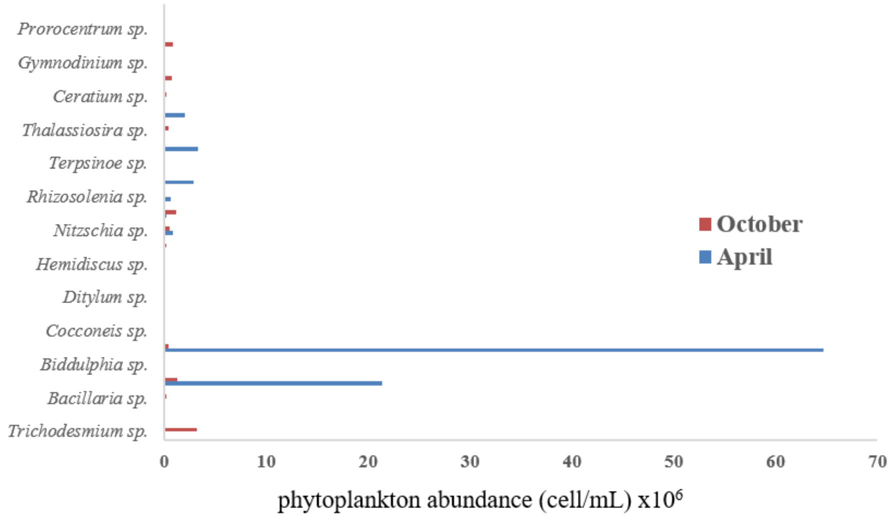


Figure 4. Phytoplankton abundance at Station 3

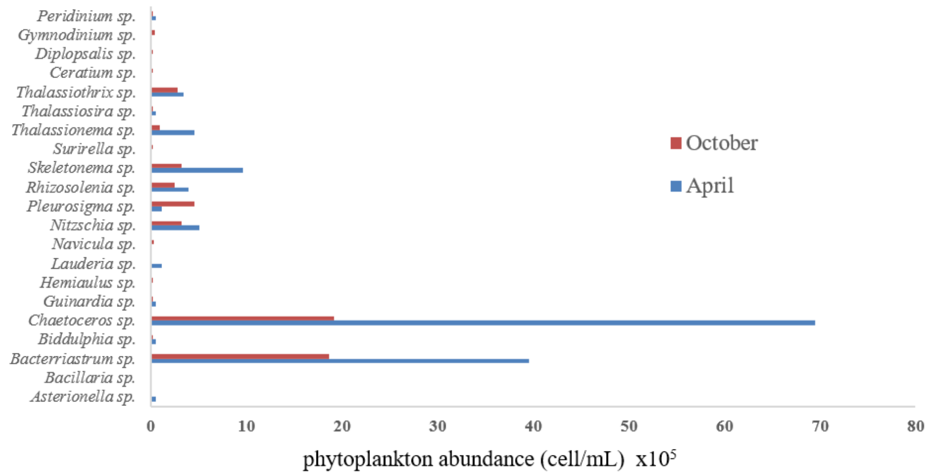


Figure 5. Phytoplankton abundance at Station 4

The abundance of zooplankton in the estuaries of Banten Bay is presented in Table 3 and Figures 6–9, while the zooplankton community indices for the four estuaries are detailed in Table 4. The result of physical and chemical analysis can be seen in Table 5. The result of the trophic index can be seen in Table 6. The result of CCA can be seen in Figure 10.

Table 3. Zooplankton abundance in the estuaries of Banten Bay

| Information | Station 1 | | Station 2 | | Station 3 | | Station 4 | |
|---|---|---|---|---|---|---|---|---|
| | April | October | April | October | April | October | April | October |
| Number of species | 11 | 7 | 4 | 6 | 8 | 8 | 7 | 6 |
| Abundance (number/mL) | 203,478 | 118,695 | 104,65 | 135,651 | 79,130 | 251,522 | 262,825 | 98,912 |

Table 4. Zooplankton community indices in the estuaries of Banten Bay

| Index | Station 1 | | Station 2 | | Station 3 | | Station 4 | |
|---|---|---|---|---|---|---|---|---|
| | April | October | April | October | April | October | April | October |
| Diversity | 1.99 | 1.22 | 1.22 | 1.24 | 1.81 | 0.9 | 1.51 | 1.12 |
| Evenness | 0.83 | 0.63 | 0.88 | 0.69 | 0.87 | 0.43 | 0.78 | 0.63 |
| Dominance | 0.18 | 0.44 | 0.32 | 0.40 | 0.20 | 0.61 | 0.26 | 0.4 |

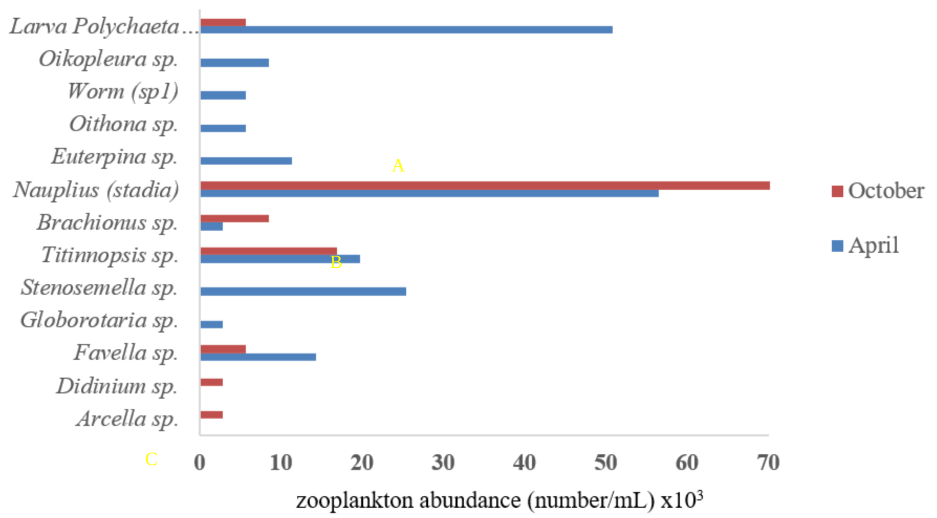

Figure 6. Zooplankton abundance at Station 1

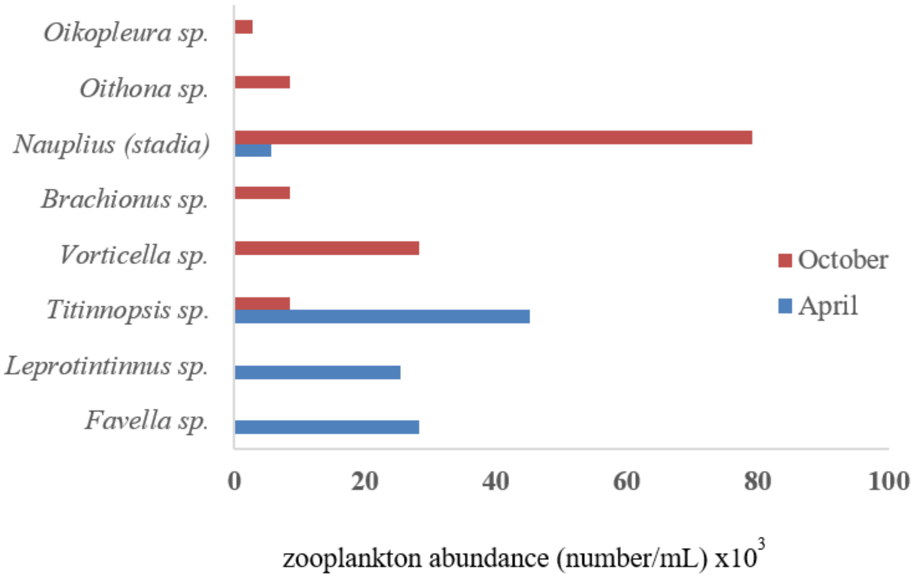


Figure 7. Zooplankton abundance at Station 2

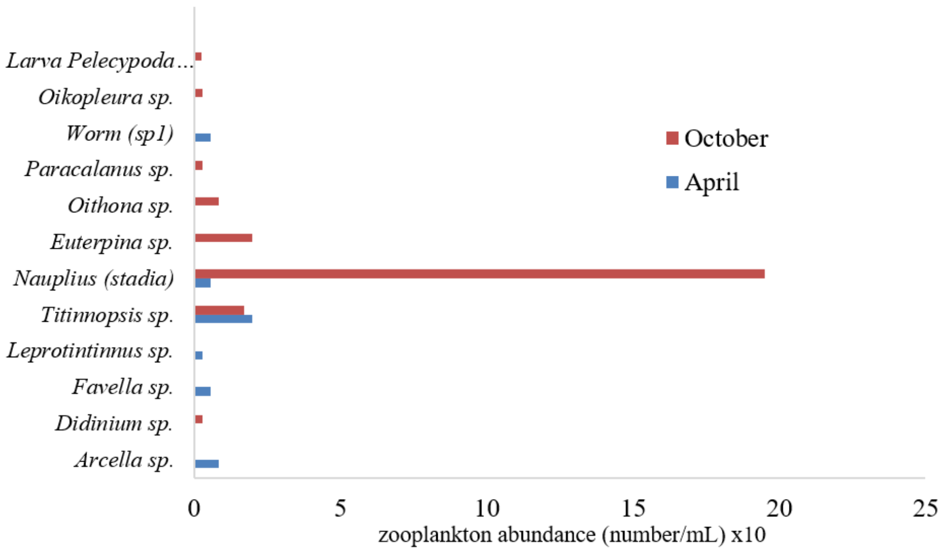


Figure 8. Zooplankton abundance at Station 2

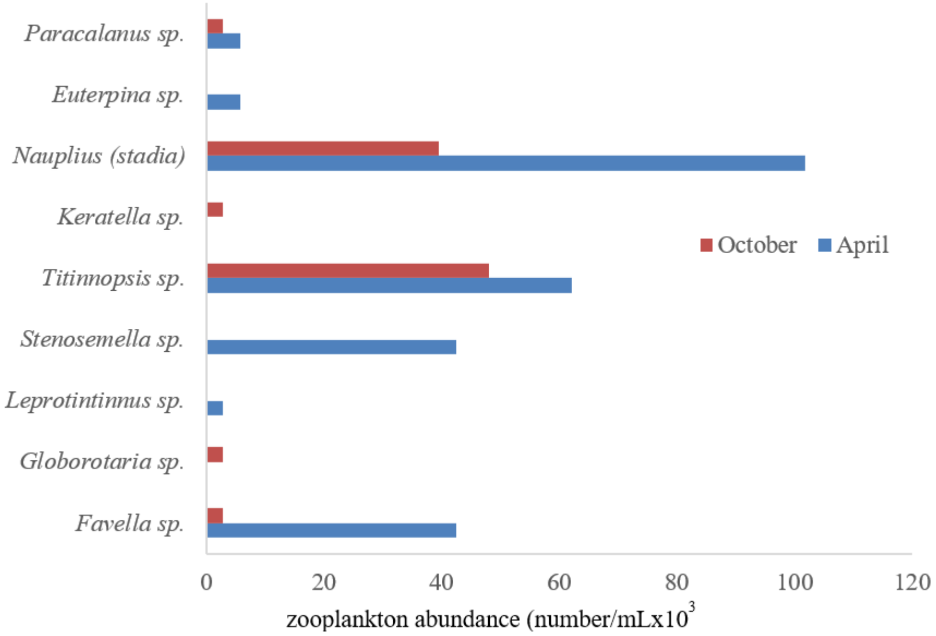


Figure 9. Zooplankton abundance at Station 4

Table 5. Result of physical and chemical analysis on estuary water samples in Banten Bay

| **Parameter** | **Analytical Result** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Station 1** | | **Station 2** | | **Station 3** | | **Station 4** | |
| | April | October | April | October | April | October | April | October |
| Water Depth (cm) | 120 | 138 | 135 | 151 | 140 | 156 | 80 | 120 |
| Sechi Depth (cm) | 10 | 26 | 88 | 69 | 20 | 42 | 60 | 20 |
| Water Current (m/s) | 0.2 | 0.9 | n.d | 0.2 | 0.6 | 0.5 | 0.1 | 0.9 |
| pH | 7.99 | 7.81 | 8.00 | 6.90 | 6.97 | 6.58 | 7.87 | 7.78 |
| DO (mg/L) | 6.31 | 6.89 | 6.37 | 4.85 | 5.67 | 5.91 | 7.22 | 7.79 |
| ORP (ohm) | 113 | 179 | 103.5 | 254.3 | 200.5 | 266.3 | 166.0 | 177.0 |
| Water-Temp (°C) | 30.93 | 32.96 | 30.64 | 31.45 | 27.98 | 31.14 | 31.03 | 32.93 |
| Turbidity (NTU) | 168.0 | 66.7 | 36.6 | 391.3 | 114.0 | 21.7 | 79.3 | 15.9 |
| Conductivity (mS/cm) | 23.8 | 50.8 | 46.0 | 51.0 | 13.7 | 28.3 | 45.9 | 50.4 |
| Salinity (%) | 14.4 | 33.4 | 29.8 | 33.4 | 29.7 | 28.3 | 7.9 | 33.0 |
| TDS (g/L) | 14.8 | 30.5 | 28.1 | 30.6 | 8.5 | 26.7 | 28.0 | 30.2 |
| TSS (mg/L) | 208.0 | 657.2 | 61.6 | 642.8 | 78.0 | 510.6 | 41.6 | 469.0 |
| Chl-*a* ($mg/m^3$) | 22.095 | 6.925 | 1.506 | 3.210 | 1.927 | 3.555 | 2.208 | 5.757 |
| TN (mg/L) | 33.463 | 28.450 | 31.495 | 25.777 | 33.092 | 29.193 | 32.127 | 27.634 |
| TP (mg/L) | 0.213 | 0.077 | 0.054 | 0.130 | 0.206 | 0.117 | 0.072 | 0.041 |

Table 6. Result of trophic index analysis on estuaries water sample in Banten Bay

| Station | Sampling Time | TRIX value | Trophic Status |
|---|---|---|---|
| 1 (Karangantu) | April 2021 | 7.506 | hypertrophic |
| | October 2021 | 7.513 | hypertrophic |
| 2 (Wadas) | April 2021 | 5.027 | eutrophic |
| | October 2021 | 5.077 | eutrophic |
| 3 (Cengkok) | April 2021 | 7.504 | hypertrophic |
| | October 2021 | 7.512 | hypertrophic |
| 4 (Pamong) | April 2021 | 8.775 | hypertrophic |
| | October 2021 | 8.788 | hypertrophic |

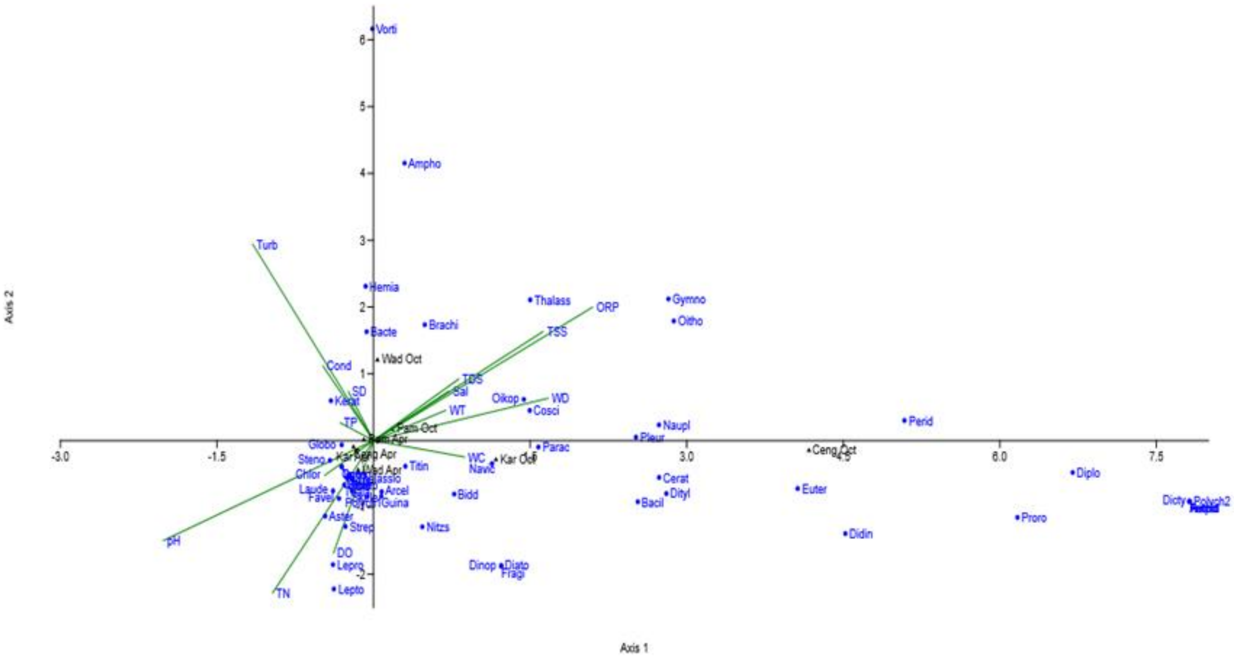


Figure 10. The result of CCA analysis found that aquatic environmental variables have a significant correlation with the composition and abundance of plankton at the study location

## 4. Discussion

Phytoplankton abundance in Karangantu during April 2021 was higher compared to other stations, with species diversity also showing greater values. This is likely attributed to the high nutrient availability, primarily from anthropogenic sources such as transportation, tourism, fishing activities, and residential waste. The trophic status of Karangantu was classified as hypertrophic (Table 6), consistent with findings by Sugiarti *et al*. (2022), who reported a hypertrophic status for the Karangantu estuary in 2013. The chlorophyll - a, TN, and TP concentrations were higher in Karangantu than in other stations in April 2021 (Table 5). Phytoplankton growth in metabolism requires nutrients from the environment, with elevated nitrogen and phosphorus inputs from human activities triggering biomass production and eutrophication (Oduor *et al*., 2024).

In October, the highest phytoplankton abundance was recorded at Wadas (Station 2), although its diversity and evenness indices were lower than at other stations. The trophic status of Wadas in October was eutrophic, differing from different stations (Table 6). Chlorophyll-a, TN, and TP concentrations in Wadas were also lower than those observed elsewhere (Table 5). However, the dominance index value in Wadas was higher, with *Bacteriastrum* sp. (a genus within the Bacillariophyceae division) as the dominant species. This phytoplankton is known for its broad distribution in freshwater and saline habitats (Rosada and Sunardi, 2021). Environmental conditions in Wadas, including higher turbidity, dissolved oxygen (DO), and total suspended solids (TSS) concentrations, likely contributed to the dominance of *Bacteriastrum* sp. in October.

The Bacillariophyceae division, known as diatoms, is the dominant plankton group observed at the four stations. Their widespread occurrence can be attributed to their cosmopolitan nature, allowing them to thrive in diverse environments, including polluted waters (Nastiti and Hartati, 2013; Yulianto *et al.*, 2014; Adriana *et al.*, 2017). Diatoms' silica-based cell walls make them prone to sinking, but they exhibit competitive advantages under various nutrient concentrations, cooler temperatures, and low light conditions. They particularly benefit from frequent water column mixing, which reduces sedimentation risk through turbulent resuspension, moderates temperatures in the photic zone, and redistributes nutrients. Additionally, nutrient enrichment, decreasing temperatures, and strong mixing events, such as those caused by storms, further support diatom proliferation by enhancing nutrient availability and disrupting thermal stratification (Mancuso *et al.*, 2021).

Another phytoplankton found in abundance across the four estuaries is *Chaetoceros* sp. According to Nurfadilah *et al.* (2020), similar conditions were observed in the Pangkep estuary, South Sulawesi. *Chaetoceros* sp. is a diatom known for tolerating extreme water conditions, including high turbidity levels, as Mancuso *et al.* (2021) described. Turbidity levels in the four estuaries of Banten Bay were found to be relatively high, ranging from 15.9 to 391.3 NTU (Table 5). This suggests that, in addition to the influence of nutrients that can trigger plankton blooms, turbidity significantly affects the community structure of plankton.

The abundance of *Chaetoceros* sp. and *Bacteriastrum* sp. peaked in April during the dry season but decreased in the rainy season (October). This decline may result from dilution effects during the rainy season when larger currents transport diverse phytoplankton from rivers to estuaries. Water current in April (dry season) across the four stations ranges from 0.1 to 0.55 m/s, whereas during the rainy season, they increase significantly, ranging from 0.23 to 2.88 m/s (Table 5). Estuaries are highly dynamic ecosystems that undergo significant changes along various environmental gradients, such as salinity, temperature, nutrients, and turbulence, due to the mixing of freshwater and seawater during tidal cycles (Bilbao *et al.*, 2023).

*Chaetoceros* sp. serves as a food source for larvae and juveniles of various aquaculture species. *Chaetoceros* sp., form chains of cells with long, spiny protuberances called setae, which can clog fish gills and cause mortality, although they do not produce toxins (Medlin *et al.*, 2013). Their spiny structure can adhere to fish gills, causing irritation that stimulates excessive mucus production, obstructing the respiratory system and potentially leading to fish deaths. Such impacts have been observed in regions like the Pacific Northwest of Canada and the United States (Weliyadi, 2013). Fish mortality can significantly reduce the economic value of salmon fisheries, as demonstrated by blooms of *Chaetoceros* sp. between 1980 and 1990 in British Columbia, Canada, and Washington, United States, which caused losses estimated at 35 million USD (Trainer and Yoshida, 2014). The maximum growth of *Chaetoceros* sp. occurs at pH levels between 7.9 and 8.5 (Indarmawan *et al.*, 2012). Similarly, Nastiti and Hartati (2013) note that optimal pH conditions for phytoplankton growth are generally ≤8.5. During the rainy season, the abundance of *Chaetoceros* sp. is lower, likely due to the reduced pH range observed in four estuaries (6.58–7.99; Table 5), which may inhibit its growth.

The phytoplankton species diversity across four stations in Banten Bay was generally moderate (1 < H' < 3). The diversity was higher in October than in April (Table 2). The evenness index in October was generally higher across all four estuaries, while the dominance index was lower in October than in April. However, phytoplankton abundance in the estuaries tended to decrease in October. During the rainy season, dilution occurs due to stronger currents, transporting a greater variety of phytoplankton from the river to the river mouth, resulting in higher phytoplankton diversity. Conversely, the decrease in total nitrogen (TN) and total phosphorus (TP) concentrations (Table 5) led to a reduction in phytoplankton abundance in the estuaries. The availability of nutrients in estuaries is strongly influenced by freshwater input and seawater exchange. Freshwater inputs, including rivers, groundwater, and runoff, supply nutrients to the estuaries, while seawater exchange through tides dilutes nutrient concentrations (Nybakken, 1992).

The highest zooplankton abundance was observed in Pamong in April and Cengkok in October, where hypertrophic trophic status was observed during these periods. This suggests that high nutrient levels may have influenced zooplankton abundance. The greatest species diversity of zooplankton in April occurred in Karangantu, likely due to the abundance of nutrients from human activities, such as transportation, tourism, fishing, and residential areas. Cengkok had the highest species diversity in October, corresponding with its peak zooplankton abundance (Station 3). The evenness index ranged from 0.43 to 0.88, while dominance values varied from 0.18 to 0.61, indicating that no single zooplankton species dominated the communities across the four estuaries in Banten Bay.

The abundance of zooplankton in the four estuaries of Banten Bay generally decreased in October (rainy season), likely due to reduced nutrient levels in the region, as indicated by lower concentrations of TN and TP (Table 5). Conversely, the dominance index in these estuaries tended to increase. Jiang *et al*. (2024) suggest that reductions in nitrogen and phosphorus can lead to declines in species abundance, including both phytoplankton and zooplankton, while favoring the dominance of specific species.

*Nauplius calanoid,* the larval stage of Crustacea, was commonly found at all four estuaries in Banten Bay. Crustaceans often hatch their eggs in coastal areas like estuaries, where food availability is high. According to Huys and Boxshall (1991), many *Nauplius calanoids* prefer estuaries for incubation due to their safer environment and abundant food resources. Besides *Nauplius calanoid*, another zooplankton species, *Titinnopsis* sp., was found abundantly at four stations. This species belongs to the protozoa group commonly observed in marine and freshwater environments. Tintinnid species within the Ciliophora group are categorized as micro-zooplankton due to their size. These organisms, recognized as critical consumers of nano-plankton at the trophic level in pelagic ecosystems, played an essential role in the food chain by serving as prey for heterotrophic organisms capable of digesting them (Durmus *et al*.,2023)

According to the diversity values for phytoplankton and pollution criteria from Lee *et al*. (1978), the water quality at station 1 ranges from non-polluted to moderately polluted, station 2 is classified from moderately polluted to heavily polluted, station 3 is non-polluted to moderately polluted, and station 4 is slightly polluted. Meanwhile, based on the zooplankton diversity values and pollution criteria from Lee *et al*. (1978), station 1 falls between slightly to moderately polluted, station 2 is moderately polluted, station 3 ranges from slightly to heavily polluted, and station 4 is moderately polluted.

Overall, the water quality across the four estuaries in Banten Bay varies from slightly polluted to moderately polluted, which is still conducive to fish growth. The trophic status of these estuaries spans from eutrophic to hypertrophic, indicating that high nutrient concentrations support a diverse range of plankton, which serve as a food source for fish.

The ordination results from CCA are shown in Figure 10. The plot displays the distribution of plankton species, with the distance between points reflecting the similarity of species. Green arrows represent key aquatic environmental variables, including water depth, Secchi depth, water current, pH, DO, ORP, water temperature, turbidity, conductivity, salinity, TDS, TSS, chlorophyll-a, TN, and TP. The first axis (CCA1) explains 53.7% of the variation in the data, while the second axis (CCA2) accounts for 21.0%. The analysis shows a significant correlation between these environmental variables and the composition and abundance of plankton in the study area. Akrimi and Gatot (2012) noted that phytoplankton growth is influenced by factors such as turbidity, photosynthesis processes, and nutrient availability. High turbidity negatively impacts water quality by reducing oxygen concentration, primarily due to limited photosynthetic activity and increased oxygen demand from organic matter degradation. Elevated turbidity also affects biota by disrupting predator-prey interactions, food availability, visibility, and overall health (Megina *et al*., 2023).

The majority of plankton species were found at all sampling stations in both April and October, with the exception of the Wadas station in October. These species were associated with environments characterized by high water temperature, pH, DO, TN, salinity, water depth, water current, TP, turbidity, TSS, and TDS. Notably, species such as *Bacteriastrum* sp. and *Chaetoceros* sp. thrived in high turbidity and low DO conditions, suggesting they are more abundant under these conditions.

Mitigation strategies should be developed to monitor the growth of *Chaetoceros* sp. and *Bacteriastrum* sp., especially during the dry season. Ongoing water quality and plankton monitoring in other estuaries within Banten Bay is recommended. Investigating the long-term effects of nutrient loading on plankton communities and exploring mitigation strategies for harmful algal blooms are crucial for supporting fisheries management in the estuaries of Banten Bay.

## 5. Conclusion

The highest phytoplankton abundance was observed in the Karangantu Estuary, while the highest abundance of zooplankton peaked in the Pamong Estuary. Both phytoplankton and zooplankton reached their highest abundance in April. The highest phytoplankton diversity occurred in Karangantu in October, while zooplankton diversity was greatest in Karangantu in April. Based on plankton diversity, both phytoplankton and zooplankton, the study sites were generally classified as slightly to moderately polluted. The trophic status across all four estuaries ranged from eutrophic to hypertrophic, indicating that high nutrient concentrations continue to support fish growth. CCA revealed a significant correlation between aquatic environmental variables, plankton composition, and abundance at the study locations. Mitigation strategies should focus on monitoring the growth of *Chaetoceros* sp. and *Bacteriastrum* sp., particularly during the dry season. Ongoing monitoring of water quality and plankton in other estuaries of Banten Bay is recommended. Investigating the long-term effects of nutrient loading on plankton communities and developing mitigation strategies for harmful algal blooms are crucial for supporting sustainable fisheries management in Banten Bay estuaries.

## Data availability statement

All data included and used in the study is open and contains no confidential and ethically private information.

## Conflict of interest

This manuscript has no identified and possible conflicts of interest among authors.

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## Contributor Statement

**S** contributed design the topic and research methods, data collection and analysis, wrote the manuscript. **AW** and **DR** contributed data collection and analysis, wrote the manuscript. **AR**, **RN**, **SA** and **R** contributed data analysis and wrote the manuscript.

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