

E-ISSN 2618-6365 Vol. 9 Issue 2 2026

AQUATIC RESEARCH

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AQUATIC RESEARCH
E-ISSN 2618-6365

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Aims and Scope

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Abbreviation: **Aquat Res**

e-ISSN: 2618-6365

Journal published in one volume of four issues per year by

<http://aquatres.scientificwebjournals.com> web page

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Determination of changes in biodiesel production by *Gloeocystis vesiculosa* cultured in wastewater with the artificial sweetener aspartame and its metabolites

Melih ONAY

Cite this article as:

Onay, M. (2026). Determination of changes in biodiesel production by *Gloeocystis vesiculosa* cultured in wastewater with the artificial sweetener aspartame and its metabolites. *Aquatic Research*, 9(2), 78-89. <https://doi.org/10.3153/AR26008>

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Submitted: 31.10.2025

Revision requested: 05.12.2025

Last revision received: 21.01.2026

Accepted: 21.01.2026

Published online: 16.03.2026

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ABSTRACT

Microalgae can grow and form biomass in wastewater to produce various end products. In this study, the biomass, lipid content, and lipid yield changes of *Gloeocystis vesiculosa* cultured in wastewater containing different concentrations of the artificial sweetener APM and its metabolites DKP and PHE were evaluated. The highest biomass amount was 1080 ± 20 mg/L at 250 mg/L of DKP. Conversely, the highest lipid percentage was $31 \pm 2\%$ at 250 mg/L APM. There was no significant difference in lipid yield due to the lower biomass content relative to the higher lipid percentages. APM acts as a stress trigger. In addition, the antioxidant enzyme activities of *Gloeocystis vesiculosa* in wastewater with artificial sweeteners were determined. At 250 mg/L APM, the highest levels of SOD, CAT, APX, and MDA activity were 91 ± 4 U/mg protein, 78 ± 4 U/mg protein, 18 ± 2 U/mg protein, and 3.9 ± 0.2 nmol/mg protein. Future studies using a large-scale photobioreactor will investigate the effects of *Gloeocystis vesiculosa* on biodiesel production and wastewater treatment in wastewater contaminated with APM, DKP, and PHE.

Keywords: Biodiesel, *Gloeocystis vesiculosa*, Aspartame, Wastewater, Antioxidant activity



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Introduction

Microalgae are the subject of research in the scientific community because of their rapid growth and the ease with which they may be manipulated to create the product of interest. In a medium known as a nutrient medium, which contains a variety of elements, microalgae can thrive (Jeffers et al., 2025). Alternatively, they can grow in municipal wastewater, which can supply them with all of the elements or minerals they require. This media typically contains a source of carbon, either organic or inorganic, that microalgae are able to harvest and absorb. If the microalgae are autotrophic, the carbon source that they use is typically carbon dioxide, which they then convert into an organic compound such as sugar (Duan et al., 2020). However, if the microalgae are heterotrophic, they are able to consume an organic molecule like glucose or a derivative in an anabolic cycle to make use of the carbon (Morales-Sánchez et al., 2013). In addition, if the microalgae are facultative, they are able to utilise any carbon source that they recognise, depending on the conditions of the surrounding environment (Abiusi et al., 2021). Microalgae not only require carbon for growth but also utilise high amounts of nitrogen and phosphorus (Salgado et al., 2023). Generally, increasing nitrogen levels accelerates microalgae growth and increases biomass. This process is a common manipulation method used in studies to increase microalgae biomass. However, increasing nitrogen levels and biomass also result in a decrease in microalgae metabolic products such as carbohydrates, lipids, or proteins (Maltsev et al., 2023). One strategy for increasing lipid content and biodiesel yield is to use a nitrogen-deficient medium. This allows for manipulation of lipid levels and changes in FAME content (Oğuz et al., 2024). The growth of microalgae and the production of lipids can be enhanced by adjusting the concentrations of key components in the culture media, including nitrogen, carbon, and phosphorus. Microalgae cultivated in various media, such as BG-11, TAP medium, and BBM, exhibit fluctuations in both their growth rates and lipid content based on the different sources and concentrations of these components (Vishwakarma et al., 2019). If the process negatively impacts the synthesis of products used as precursor molecules, it is undesirable for industrial production. For this purpose, increasing the desired precursor molecule and applying appropriate manipulation methods to increase the amount of the final product are more suitable for industrial studies. Another important issue in microalgae growth is the high cost of chemicals in artificially prepared media, which creates additional costs on an industrial scale. At this point, growing microalgae in wastewater may be considered (Dias et al., 2025). Wastewater provides a large portion of the nitrogen, phosphorus, and often carbon required by microalgae. This process allows for both

wastewater treatment and microalgae growth, enabling the desired product to be obtained from the resulting biomass (Nur & Buma, 2019). If the wastewater is municipal or industrial, using proper microalgae can clean it while also producing a larger volume of product, as previously stated. The effluent from municipal facilities may contain significant quantities of artificial sweeteners (Shen et al., 2023). The metabolites of aspartame, which are known as (2S,5S)-3,6-Dioxo-5-(phenylmethyl)-2-piperazineacetic acid (DKP) and phenylalanine (PHE), can be discovered in wastewater when products that contain aspartame (APM) are released directly into wastewater without being used or mixed with wastewater after being metabolised. APM is commonly used as a sweetener in the pharmaceutical and food industries. Because of its expiration date or disposal in the bath and kitchen water, it directly enters municipal wastewater. Furthermore, when humans consume aspartame, the body converts it into PHE, which is expelled in urine. When exposed to harsh conditions such as high temperatures, acidic or neutral pH, and long-term storage, APM undergoes chemical reactions that result in DKP formation. Thus, DKP and PHE can be found in wastewater. DKP and PHE are referred to as APM metabolites since they are formed in the human body as a result of APM metabolism (Wawryk et al., 2023). It is possible for the artificial sweetener APM, as well as its metabolites DKP and PHE, to simultaneously promote the growth of microalgae that are suited for wastewater and to eliminate them from it. This investigation made use of the microalgae *Gloeoecystis vesiculosa*, which had been cultured in the past on a variety of different media. This particular microalgae is classified as a member of the phylum Chlorophyta and the genus *Gloeoecystis* (Capek et al., 2023). Any industrial product can be produced by microalgae that have been cultured in any medium or effluent. Microalgae have emerged as a highly influential actor in the energy industry. Microalgae have the potential to be recycled into biofuels. In this category, biodiesel, bioethanol, biogas, and biohydrogen are considered the most significant (Sharma et al., 2025). To manufacture biodiesel, the first step is to cultivate them for the generation of biomass, the acquisition of lipids, and finally the transesterification of the lipids (Geng et al., 2025). In addition, manipulation of any environmental component in microalgae may increase or decrease the metabolic product produced by stress factors. The status of antioxidant enzyme systems formed as a result of stress factors in microalgae can be examined. The most important antioxidant activity systems are superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and malondialdehyde (MDA). Their levels can vary depending on the microalgal stress level (Fal et al., 2022).

In this study, the microalgae *Gloeocystis vesiculosa* were cultivated in wastewater containing varying concentrations of artificial sweeteners (APM) and their metabolites (DKP and PHE). The purpose of this research was to investigate the biomass, lipid, and biodiesel yields of *Gloeocystis vesiculosa* grown in wastewater with APM, DKP, and PHE. Additionally, the study aimed to analyse the concentrations of aspartame and its metabolites present in wastewater, which microalgae can utilise. The study also aimed to investigate whether the stress generated by these sweeteners affected the levels of metabolic products in *Gloeocystis vesiculosa*.

Materials and Methods

Growth of Microalgae

The microalga *Gloeocystis vesiculosa* was obtained from the Culture Collection of Autotrophic Organisms (CCALA), Czechia. The microalgae were first cultivated in a medium containing 50% (v/v) Tris-Acetate-Phosphate (TAP) and 50% (v/v) Blue-Green-11 (BG-11). 80% (v/v) of a 1:1 TAP and BG-11 mixture was then combined with 20% (v/v) municipal wastewater for use in the studies. The final mixture consisted of 40% TAP, 40% BG-11, and 20% municipal wastewater (v/v). The wastewater used in this study had a COD value of 715 ± 23 , a TN value of 68 ± 5 , and a TP value of 21 ± 2 . The final pH was adjusted to 7.2. A prior study provided detailed information on prepared wastewater (Onay, 2020). Microalgae were cultured at a speed of 60 rpm, a temperature of $24 \pm 1^\circ\text{C}$, and a light intensity of $90 \mu\text{mol m}^{-2} \text{s}^{-1}$. Then, experiments were carried out on a 1 L flat photobioreactor. Experimental setups were carried out in three parallel samples by adding different concentrations (25 mg/L, 75 mg/L, 125 mg/L, and 250 mg/L) of the artificial sweetener aspartame (APM) and its metabolites (2S,5S)-3,6-Dioxo-5-(phenylmethyl)-2-piperazineacetic acid (DKP) and phenylalanine (PHE) to the medium. Microalgae were grown for 12 days until they reached the stationary phase at OD 680, after which their dry weights were measured gravimetrically. For harvesting, the samples were then centrifuged at 3000g for 10 minutes. After harvesting, the samples were washed three times with distilled water. Then, they were filtered through filter paper (1.2 μm GF/C; 47 mm). The samples were lyophilised. Then, they were placed in a desiccator to remove moisture, and they were weighed gravimetrically for biomass analysis. The samples were stored at -20°C for further analysis.

Determination of Lipid Percentage

The Folch method was used to determine the lipid content of *Gloeocystis vesiculosa* samples (Folch et al., 1957). 1g of mi-

croalgae samples was used for lipid extraction, and the samples were prepared by combining chloroform and methanol in a 2:1 ratio, evaporating the chloroform, and measuring the residual oil gravimetrically.

Determination of Antioxidant Enzyme Activities

Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and malondialdehyde (MDA) activities were determined spectroscopically, and the procedures were detailed in a previous study (Onay, 2020).

Biodiesel Production

Methanol, together with 0.1 M potassium hydroxide, was used to transesterify the lipids of microalgae extracts to produce biodiesel. A period of 4 h was spent incubating the mixture at 60°C . Thereafter, it was stored at room temperature for the entire night. The glycerol and biodiesel phases were separated with the help of a flask separator, and the biodiesel was rinsed three times with distilled water to make sure that any undesirable residues were removed. Biodiesel content was determined gravimetrically (Onay & Ayas, 2024).

Statistical Analyses

The experimental groups contained three parallel samples. All statistical analyses were performed by one-way analysis of variance (ANOVA) and Tukey's test. The confidence level was higher than 95%. In this report, the results are expressed as the mean \pm standard deviation (SD). When $P < 0.05$, * shows significant, and ** shows very significant.

Results and Discussion

This study focused on how *Gloeocystis vesiculosa* microalgae, grown in wastewater with aspartame (APM), (2S,5S)-3,6-Dioxo-5-(phenylmethyl)-2-piperazineacetic acid (DKP), and phenylalanine (PHE), affected their metabolic content and contribution to biodiesel production by examining changes in biomass concentrations. The control group comprised microalgae cultivated in wastewater that did not include APM, DKP, or PHE. The biomass of the control was 827 ± 15 mg/L. The lowest biomass content was 663 ± 15 mg/L at 250 mg/L of APM, while the maximum was 1080 ± 20 at 250 DKP. The study demonstrated that when the APM concentration grew, the biomass concentration fell. The biomass concentration at 25 mg/L APM was 830 ± 15 mg/L, but at 75 mg/L APM, it was 753 ± 6 mg/L. Furthermore, as the APM concentration grew, the biomass decreased. The biomass concentration at 125 mg/L APM was 720 ± 10 mg/L, with the lowest concentration at 250 mg/L. Unlike APM, DKP showed a different growth pattern. As DKP concentra-

tion increased, biomass concentration also increased. The increase in biomass concentration was slow at 25 mg/L (837 ± 6) and 75 mg/L (880 ± 10 mg/L), but it became rapid at 125 mg/L DKP (963 ± 15 mg/L), with the maximum biomass (1080 ± 20 mg/L) reached at 250 mg/L DKP. PHE showed a different effect than APM and DKP. At low PHE concentrations, the biomass amount was close to the control. Accordingly, at 25 mg/L and 75 mg/L PHE, biomass amounts were 827 ± 6 mg/L and 853 ± 15 , respectively. Similarly, at 125 mg/L and 250 mg/L PHE concentrations, the biomass concentrations were 900 ± 10 mg/L and 920 ± 10 , respectively. As a result, APM and its metabolite products, DKP and PHE, which are structurally similar, had distinct effects on microalgae development in wastewater. When the results are evaluated statistically, biomass concentrations of 125 and 250 mg/L for APM, DKP, and PHE are highly significant. In contrast, biomass at 75 mg/L for DKP and PHE is considered significant, while APM also shows high significance.

Although studies on *Gloeocystis vesiculosa* are scarce in the literature, a few publications exist, mostly about carbohydrate and sugar production. Exopolysaccharide from *Gloeocystis vesiculosa* was found to contain α -D-Glcp residues, the majority of which were found to be 1,4-linked. To a lesser extent, they were found to be the terminal sugar. It can be inferred from this that β -D-xylo- α -D-mannan was slightly contaminated with amylose, around 10 weight per cent (Capek et al., 2023). In another study, the microalgae *Gloeocystis vesiculosa* was grown in BBM medium, and its biomass yield was examined under different light sources. The highest biomass was obtained under red light (0.62 g/L), while under white light, it was found to be 0.59 g/L (Çeliktaş, 2020). The impacts of altering the amounts of carbon, nitrogen, sulfate, sodium, and calcium in wastewater were explored for *Gloeocystis vesiculosa* in another study. That study focused on the concentration of biomass. It was discovered that the highest concentration of biomass was 0.63 g/L (Al-Badri, 2021). The findings of these studies were comparable to ours, although they were distinct from our own. Differences in biomass concentrations were observed because of the utilisation of various light wavelengths, as well as various media and wastewater ingredients. Figure 1 depicts the fluctuation in the biomass amount of *Gloeocystis vesiculosa* growing in wastewater containing APM, DKP, and PHE.

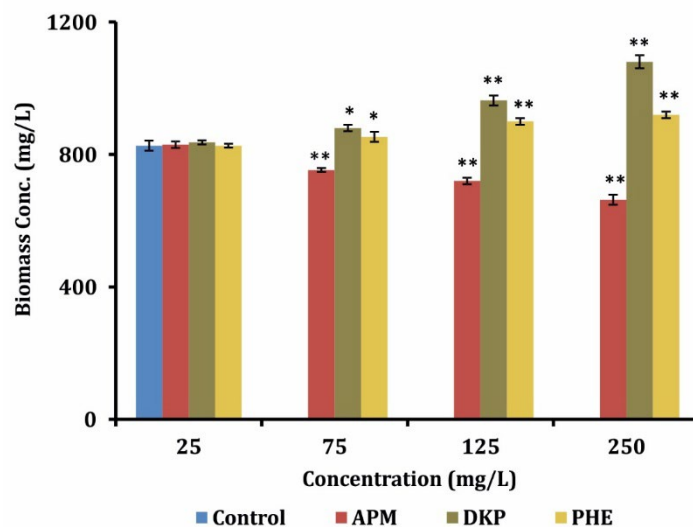


Figure 1. The changes in the biomass amount of *Gloeocystis vesiculosa* growing in wastewater containing APM, DKP, and PHE

Then, lipid content changes were investigated after biomass change in *Gloeocystis vesiculosa* cultured in wastewater containing APM, DKP, and PHE. The control had a lipid content of $25\% \pm 1\%$. Furthermore, the maximum lipid percentage was $31 \pm 2\%$ at 250 mg/L APM, and the lowest was $20 \pm 1\%$ with 250 mg/L DKP. According to the results, as the APM increased, the amount of lipids also increased. At 25 mg/L APM, the lipid percentage was $26 \pm 1\%$, while at a 75 mg/L APM concentration, the lipid percentage ($27 \pm 1\%$) was close to this value. The increase in lipid percentage accelerated after this value. At a 125 mg/L APM concentration, the lipid percentage was $28 \pm 1\%$, and at a 250 mg/L APM concentration, it reached a maximum value of $31 \pm 2\%$, as mentioned above. DKP, on the other hand, exhibited the opposite behaviour to APM. As the amount of DKP increased, the amount of lipid decreased. At low concentrations such as 25 mg/L DKP, the lipid percentage was the same as the control ($25 \pm 1\%$). At 75 mg/L DKP, the lipid percentage was $24 \pm 2\%$, and at 125 mg/L DKP, it was $22 \pm 1\%$. However, at high DKP concentrations such as 250 mg/L, the lipid percentage was $20 \pm 1\%$. PHE showed similar behaviour to DKP. As PHE concentration increased, the lipid percentage decreased slightly but steadily. At a 25 mg/L PHE concentration, the lipid percentage was the same as the control ($25 \pm 1\%$). At 75 mg/L PHE, it was $24 \pm 1\%$. The decrease continued as the concentration increased. At a 125 mg/L PHE concentration, it was $23 \pm 1\%$, and at a 250 mg/L PHE concentration, it was $21 \pm 1\%$. This indicates that while higher levels of APM lead to greater lipid accumulation, the presence of DKP and PHE influences the lipid composition, causing variability in lipid

percentages. Consequently, the interplay between these compounds affects the overall lipid profile differently. The statistical interpretation of the results indicated that the differences in lipid percentages were most pronounced at a concentration of 250 mg/L. At this concentration, there were very significant differences observed in APM and DKP, while PHE was noted to be significant as well.

There are articles in the literature showing changes in lipid amounts by manipulating the environmental properties of microalgae. In a study with *Chlamydomonas reinhardtii*, fatty acid yield increased by 150% when nitrogen and phosphorus were depleted from the medium, and 4 g/L sodium acetate was utilised as the carbon source (Yang et al., 2018). In another study, *Chlamydomonas reinhardtii* microalgae were cultivated with *Azotobacter chroococcum* in nitrogen-deficient medium, and the lipid content increased from 29% to 66% when compared to the control (Xu et al., 2018). This showed that, in addition to removing nitrogen from the environment, the use of biological material could increase the amount of lipid. Additionally, *Chlorella sp.* have demonstrated the ability to increase lipid levels. Under zinc stress, *Chlorella sp.* cells created stress conditions, increasing lipid levels to 23% (Mihiraj et al., 2022). These results showed that any stress factor or change in the concentration of the chemical can cause changes in the lipid content of microalgae. Figure 2 shows how the lipid percentage of *Gloeocystis vesiculosa* changes when it grows in wastewater with APM, DKP, and PHE.

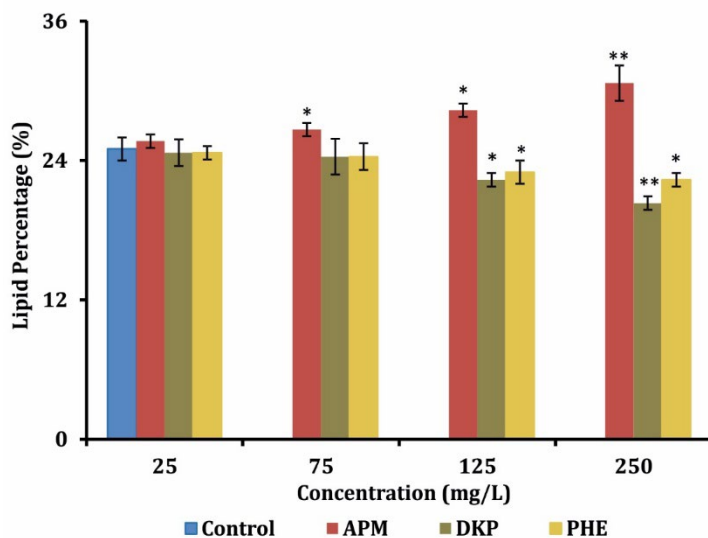


Figure 2. The lipid percentage of *Gloeocystis vesiculosa* grown in wastewater with APM, DKP, and PHE

In addition to changes in biomass and lipid content, the antioxidant capabilities of *Gloeocystis vesiculosa* cultured at various doses of APM and its metabolites were assessed. Changes in biomass and lipid levels can influence antioxidant activity; hence, a stress state can be described by these changes. For this purpose, SOD activities were first examined. The highest SOD activity was 91 ± 4 U/mg protein at 250 APM. The lowest SOD activity was 32 ± 2 U/mg protein at 25 DKP. The SOD value of the control group was 35 ± 3 U/mg protein. When we evaluated the results in terms of APM, SOD activity also increased as the APM amount increased. At a 25 mg/L APM

concentration, SOD activity was 36 ± 2 U/mg protein, while at a 75 mg/L APM concentration, it was 44 ± 4 U/mg protein. When the APM was increased to 125 mg/L, SOD activity increased to 69 ± 5 U/mg protein. Although the results for DKP seemed similar, the increases were more limited. Probably, the decreases in lipid levels corresponded with a lesser effect on SOD activity. As mentioned above, SOD activity at a 25 mg/L DKP concentration was 32 ± 5 U/mg protein, while at a 75 mg/L DKP concentration it was around 36 ± 2 U/mg protein. At a 125 mg/L DKP concentration, it was 48 ± 4 U/mg protein, and at a 250 mg/L DKP concentration, it was 46 ± 4 U/mg protein. This condition showed that SOD activity remained constant at high concentrations of DKP. PHE's SOD activities were shown to be opposing those of DKP. SOD activity was unaltered at low PHE amounts but increased at high PHE concentrations. SOD activity was 34 ± 1 U/mg protein at PHE concentrations of 25 mg/L and 35 ± 3 U/mg protein at 75 mg/L. Then, at 125 mg/L and 250 PHE concentrations, SOD activity was 45 ± 2 U/mg protein and 49 ± 4 , respectively. The SOD activities of the microalgae samples were statistically analysed. At a concentration of 250 mg/L, the SOD activities of the APM and PHE samples were found to be highly significant compared to the control, while the DKP samples were noted as significant. At a concentration of 125 mg/L, APM was highly significant, whereas DKP and PHE were deemed significant.

There are literature articles showing changes in SOD activity in microalgae exposed to varying heavy metal concentrations. In one of these, *Chlorella sorokiniana* and *Scenedesmus acuminatus* were exposed to 1 mM and 0.6 mM zinc concentrations. Due to its antioxidant properties, *Chlorella sorokiniana* was less exposed to stress, while SOD activity increased 2.2-fold compared to the control (Hamed et al., 2017). An additional investigation was conducted in which *Coelastrella sp.* was grown in swine wastewater and subjected to different doses of zinc ranging from 0 to 8 mg/L. The metabolic contents of the organism were then evaluated. It was discovered

that a zinc concentration of 8 mg/L (63 U/mg protein) produced the maximum SOD activity measured. As the zinc concentration grew, so did the activity of SOD, which is evidence of the harmful effects of zinc (Li et al., 2020). The application of physical elements is another method by which microalgae can be subjected to stress. The degrees of stress experienced by *Scenedesmus obliquus* and *Nannochloropsis gaditana* were evaluated in one of these experiments, which involved the animals being subjected to a magnetic field. SOD activity of *Scenedesmus obliquus* increased by 60% when it was subjected to a magnetic field, but the SOD activity of *Nannochloropsis gaditana* increased by 115% when it was subjected to stress (Serrano et al., 2021). Microalgae may experience an increase in SOD activity and changes in metabolic content when they are subjected to any stress, as may be deduced from these investigations, which are conducted in parallel with our research. Figure 3 demonstrates the changes in *Gloeo-cystis vesiculosa* SOD enzyme activity as it grows in wastewater containing APM, DKP, and PHE.

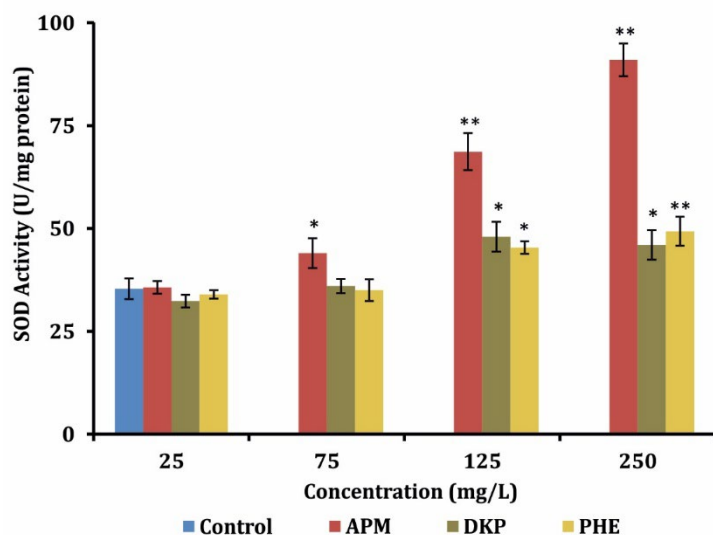


Figure 3. The changes in SOD enzyme activity for *Gloeo-cystis vesiculosa* grown in wastewater containing APM, DKP, and PHE

CAT activity was also investigated, in addition to the assessment of SOD activity. Comparing the results of the CAT activity to those of the SOD activity revealed comparable findings. The variation is most likely the result of the interrelated nature of the two enzymes and their relatively minor impact on the various enzyme systems. The system likely produced oxygen radicals and hydrogen peroxide in proportionate amounts. The highest CAT activity was observed at 250 mg/L APM, with a protein concentration of 78 ± 4 U/mg. On the contrary, the lowest CAT activity was observed at 25 mg/L

DKP, with 26 ± 3 U/mg. In the control group, the CAT activity was 29 ± 3 U/mg protein. CAT activities were evaluated at low amounts of 25 mg/L and 75 mg/L, with 31 ± 3 U/mg protein and 44 ± 3 , respectively, at the relevant doses. At concentrations of 125 mg/L APM, the increase continued, and the CAT activity was determined to be 62 ± 6 U/mg protein at 250 mg/L APM. The CAT activity was also measured to be 78 ± 4 U/mg protein at these levels. At low concentrations in the medium with DKP, CAT activity did not rise noticeably. CAT activity was 26 ± 3 U/mg protein when DKP was 25 mg/L. However, when DKP was 75 mg/L, the CAT activity was 28 ± 2 U/mg protein. Additionally, aggregation took place at 125 mg/L and 250 DKP per volume. Although the CAT activity was 38 ± 4 U/mg protein when the DKP was 125 mg/L, it was 41 ± 3 U/mg protein when the DKP was 250 mg/L. The CAT activity of PHE exhibited behaviours that were comparable to those of the SOD activity. CAT activities were measured at 25 mg/L and 75 mg/L PHE, with the former exhibiting 26 ± 3 U/mg protein and the latter exhibiting 28 ± 3 U/mg protein. According to the findings of this investigation, the activity of CAT was comparable to that of the control at low values of PHE. However, when PHE was 125 mg/L, the CAT was 40 ± 4 U/mg protein. However, when it was 250 mg/L, the CAT activity was 39 ± 3 . After 125 mg/L of PHE was added, the results demonstrated that there was no change in CAT. CAT activity may change with the emergence of stress factors. When the CAT activity levels were evaluated in the microalgae statistically, we observed results similar to those for SOD activity. The samples with concentrations of 125 and 250 mg/L showed more significance. While APM samples at these concentrations were very significant, DKP and PHE samples could be considered significant.

In one study, *Scenedesmus sp.* was exposed to aluminium stress, and CAT activity was observed to decrease at the highest concentration of 100 μ M. It has been explained that this is due to the decrease in protein expression (Ameri et al., 2020). In another study, *Pseudochlorella pringsheimii* was exposed to salinity and iron stress. CAT activity increased under both stress factors, reaching a two-fold increase compared to the control (Ismail & Piercey-Normore, 2023). Similarly, in another study, CAT activities were investigated when *Phormidium ambiguum* and *Microcystis aeruginosa* were grown at different light intensities, and the maximum CAT activity for *Phormidium ambiguum* increased 12-fold at $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ after 15 hours. However, CAT activity for *Microcystis aeruginosa* increased 5-fold at $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ after 15 hours. This suggests that both microalgae species are exposed to stress at high light intensities, causing an increase in CAT activity (Muhetaer et al., 2020).

These results were parallel to our results. Figure 4 demonstrates the variations in CAT activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE.

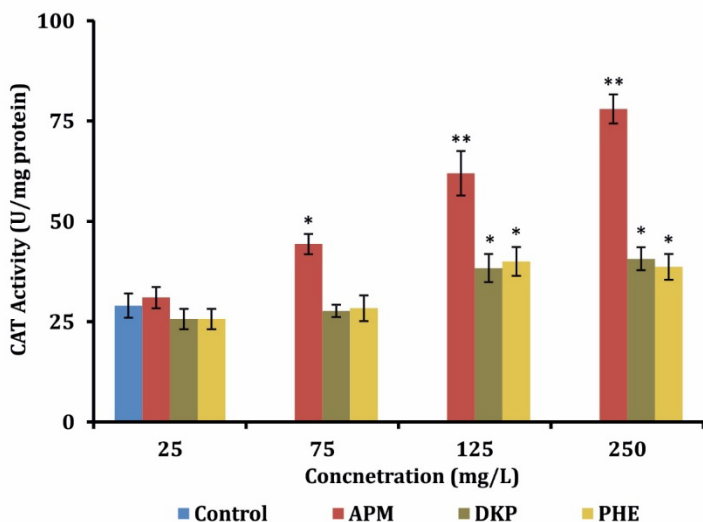


Figure 4. The variations in CAT activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE

Another antioxidant enzyme studied was APX in this study. The changes observed in APX activity were somewhat more pronounced. The APX value in the control group, which did not contain APM, DKP, or PHE, was 6 ± 1 U/mg protein. The highest APX value was 18 ± 2 U/mg protein at 250 mg/L APM, three times the control level. The lowest APX activity value was 5 ± 1 U/mg protein at 25 mg/L DKP, very close to the control level. The lower DKP concentration proved to have no effect on APX activity. The situation was similar at low APM concentrations. The APX activity value at 25 mg/L APM was 6 ± 2 U/mg protein, while the APX activity at 75 mg/L APM was 9 ± 2 U/mg protein. As the amount of APM increased, the APX activity value continued to increase. The APX activity at 125 mg/L APM was 13 ± 2 U/mg protein, while the APX activity value at 250 mg/L APM was 18 ± 2 U/mg protein, as mentioned above. The levels of APX activity at various concentrations of DKP were quite comparable to one another. When DKP was 25 mg/L, the activity of APX was measured to be 5 ± 1 U/mg protein. However, when DKP was 75 mg/L, the APX activity was 7 ± 1 U/mg protein. Without taking into account the other findings, the APX activity was 8 ± 1 U/mg protein when the DKP was 125 mg/L. At these three concentrations, the APX activities were highly comparable to each other. When DKP was increased to 250 mg/L, the activity of APX was found to be 11 ± 1 U/mg protein following this increase. There was a close relationship between the APX activity levels of DKP and the APX activity values at various concentrations of PHE. The APX value was

found to be 6 ± 1 U/mg protein when PHE was 25 mg/L. However, when PHE was 75 mg/L, the APX value was 7 ± 1 U/mg protein. After increasing PHE to 125 mg/L, the APX activity value was 8 ± 1 U/mg protein following the increase. When PHE was significantly raised to 250 mg/L, the APX activity value was found to be 10 ± 1 U/mg protein. The APX activities of microalgae samples were statistically distinct at concentrations of 125 and 250 mg/L. While 125 mg/L APM, DKP, and PHE samples showed statistically significant results, 250 mg/L APM, DKP, and PHE samples were considered highly significant.

Studies on APX in the literature were consistent with our results. In one of these, the APX activity of the microalgae *Chlamydomonas reinhardtii* grown at a high light intensity of $1400 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ increased, reaching a maximum of around 0.12 mmol/mg protein, and this increase was found to be associated with an increase in transcript levels (Kuo et al., 2020). Another study investigated how APX activity changes during various growth phases of *Chlorella vulgaris*, and it revealed that APX activity was induced in the lag phase and was 37 U/mg protein (Yusuf et al., 2022). *Chlorella sorokiniana* and *Scenedesmus acuminatus* were subjected to zinc concentrations of 1 mM and 0.6 mM, respectively. *Chlorella sorokiniana* had considerably higher APX activity (about 0.7 U/mg protein) than *Scenedesmus acuminatus* (0.25 U/mg protein); however, APX activity increased in both microalgae (Hamed et al., 2017). As a result, the APX activities of microalgae exposed to stress factors increased, as in this study. Figure 5 shows the differences in APX activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE.

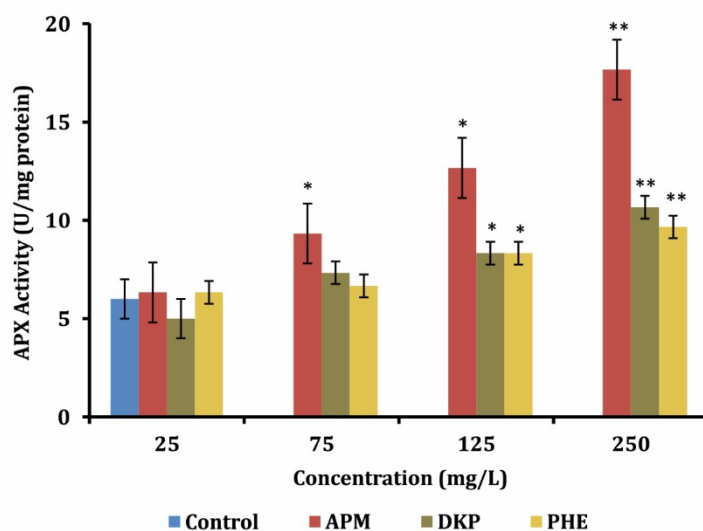


Figure 5. The differences in APX activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE

Finally, MDA activity was examined to understand the response of *Gloeocystis vesiculosa* to stress factors (APM, DKP, and PHE) in wastewater. MDA activity gave comparable results to APX activity and was consistent with other enzyme activities. The MDA activity of the control was 1.3 ± 0.1 nmol/mg protein. The highest MDA activity was found to be 3.9 ± 0.2 nmol/mg protein at 250 mg/L APM concentration. In contrast, the lowest MDA activity was 1.3 ± 0.1 at 25 and 75 DKP. In addition, the MDA value at low APM concentration was very close to the control value. MDA values at 25 and 75 mg/L APM were 1.4 ± 0.1 nmol/mg protein and 1.5 ± 0.1 nmol/mg protein, respectively. As the amount of APM increased, MDA activity increased. At an APM concentration of 125 mg/L, MDA activity was 2.8 ± 0.2 nmol/mg protein. At an APM concentration of 250 mg/L, it reached its maximum value, as mentioned above, and became 3.9 ± 0.2 nmol/mg protein. Changes in DKP concentrations also had a minor impact on MDA activity. At DKP dosages of 25 mg/L and 75 mg/L, MDA activity was 1.3 ± 0.1 nmol/mg protein, comparable to the control group. In other words, lower concentrations of DKP did not influence MDA activity, but as the concentration grew, it increased slightly. MDA activity was 1.7 ± 0.1 nmol/mg protein at 125 mg/L DKP but rose to 2 ± 0.1 nmol/mg protein at a 250 mg/L DKP concentration. PHE, in contrast to APM and DKP, did not react to a significant amount of MDA activity. When PHE was utilised, the MDA activity reached its peak at 250 mg/L, with a value of 1.3 ± 0.1 nmol/mg. At PHE concentrations of 25 and 75 mg/L, the protein concentration was found to be 1.4 ± 0.1 nmol/mg protein and 1.5 ± 0.1 nmol/mg protein, respectively, during the experiment. In addition, at 125 mg/L PHE, the MDA activity value was 1.6 ± 0.1 nmol/mg protein. MDA activity can vary depending on the nature of the microalgae and the stress conditions applied. When the MDA activities of microalgae were statistically evaluated, samples of 250 mg/L APM, DKP, and PHE were found to be highly significant. In contrast, samples of 125 mg/L DKP and PHE were noted as significant. Additionally, samples of 125 mg/L APM were also considered highly significant.

There is a wealth of information in the literature regarding varying MDA activity. One study examined the MDA changes in *Spirulina platensis* grown at different concentrations of lead, copper, and zinc. The highest MDA activity increased by almost 100% at the 0.2 mg/L copper concentration, while an almost 90% increase was observed at 0.2 mg/L zinc and lead concentrations. This demonstrates that MDA activity increased significantly at high amounts of heavy metals (Choudhary et al., 2007). In another study, *Scenedesmus vacuolatus* and *Chlorella kessleri* were exposed to copper (between 6.2 mM and 414 μ M), and their MDA activities

were examined. While the samples at 414 μ M copper concentrations of *Scenedesmus vacuolatus* had maximum activity with approximately 10 nmol 10^6 cells⁻¹ MDA activity, no significant change was observed in the MDA of *Chlorella kessleri* (Sabatini et al., 2009). *Chlorococcum sp.* was also subjected to different levels of copper and cadmium, and microalgae grew. The maximum concentration of both heavy metals was 200 mg/L, and the highest MDA activity was found at 200 mg/L copper, with a value of approximately 35 nmol/g ww. In contrast, the MDA value at 200 mg/L cadmium was approximately 29 nmol/g ww (Qiu et al., 2022). These instances demonstrate that, similar to ours, MDA activity increased in response to stress. Figure 6 illustrates the variations in MDA activities of *Gloeocystis vesiculosa* cultivated in wastewater containing APM, DKP, and PHE.

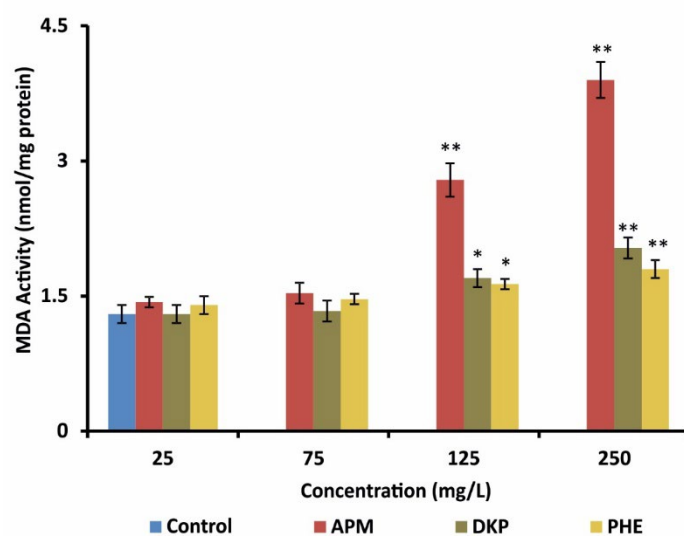


Figure 6. The variations in MDA activities of *Gloeocystis vesiculosa* cultivated in wastewater containing APM, DKP, and PHE

Finally, this study looked into the effect of *Gloeocystis vesiculosa* microalgae cultivated at various concentrations of APM, DKP, and PHE on lipid yields. Microalgae samples cultivated at a DKP concentration of 250 mg/L produced the maximum lipid yield (220 ± 3 mg/L). Microalgae samples with 75 mg/L APM had the lowest lipid yield (201 ± 5 mg/L). In fact, when the concentration of APM increased, the lipid yield decreased, but this decline was not substantial. The lipid yield of samples with 25 mg/L APM was 213 ± 7 mg/L, whereas the highest APM content yielded 203 ± 10 mg/L. In fact, APM significantly increased cellular lipid content while decreasing biomass concentration. The metabolic shift was caused by stress rather than an overall increase in lipid productivity. APM seems to shift carbon flow away from processes that help growth and toward lipid storage as a way to

survive. However, when looked at from the point of view of biofuel production, this rise in lipid percentage did not lead to a higher total lipid yield because biomass was lost at the same time. The trade-off between biomass suppression and lipid accumulation greatly limits the practical benefit of APM treatment because total lipid yield is the most important factor in determining whether biodiesel is possible. Consequently, APM ought to be regarded as a modulator of lipid metabolism at the cellular level, rather than a method to enhance overall lipid productivity, unless integrated with process optimisation techniques that can reduce biomass loss. Increases in SOD, CAT, APX, and MDA concentrations support this conclusion, strengthening the concept that it functions as a stress trigger. In samples grown in DKP, an increase in lipid yield was observed as the DKP concentration increased. This increase was gradual but small in amount. At a DKP concentration of 25 mg/L, the yield was 206 ± 8 mg/L, while at a DKP concentration of 250 mg/L, as mentioned above, the maximum value was 220 ± 3 mg/L. The yields at 75 mg/L and 125 mg/L DKP concentrations were quite close to each other. The yields at 75 mg/L and 125 mg/L DKP concentrations were 214 ± 11 mg/L and 215 ± 4 mg/L, respectively. Although PHE exhibited similar behaviour to APM and DKP, the lipid yields here were closer to each other, and increases in PHE concentration did not significantly affect lipid yield. The lowest yield was 204 ± 6 mg/L at a PHE concentration of 25 mg/L, while the highest yield was 208 ± 7 mg/L at a PHE concentration of 75 mg/L.

Most studies on biodiesel synthesis from microalgae involve changes in ambient pollutants. In one of these studies, *Chlorella ellipsoidea* was exposed to salt stress, and lipid levels were boosted to increase biodiesel yield. The maximum lipid content was 46% at an NaCl concentration of 5 g/L, resulting in a higher biodiesel output (Satpati et al., 2016). According to the findings of another study, the lipid content of *Skeletonema costatum* increased when it was grown in a medium that was limited in both nitrogen and silica. Microalgae grown in a medium with 6.8 $\mu\text{mol/L}$ of nitrogen and 0.36 $\mu\text{mol/L}$ of silicon, under N-Si restriction, demonstrated a lipid content that increased by 114% (Gao et al., 2019). In another study, the lipid content of the thermo-resistant microalga *Micractinium sp.* was examined to determine how it altered as temperature increased. The experiment indicated that the highest lipid content was 23% at a temperature of 25°C (Onay et al., 2014). Table 1 shows the amounts of biomass and lipid yield produced by *Gloeocystis vesiculosa* grown in different amounts of APM, DKP, and PHE.

Finally, *Gloeocystis vesiculosa*, when grown in various concentrations of artificial sweetener and its metabolites (APM, DKP, and PHE), responded by altering its metabolic contents,

which led to changes in biomass and lipid. However, it did not cause significant changes in lipid yield. This situation is observed as a decrease in the biomass amount and an increase in the lipid amount, and it can be said that APM, in particular, increases the lipid amount due to stress. It is a stress trigger. Conversely, in DKP, lipid percentage decreased with concentration. Variations in SOD, CAT, APX, and MDA activity levels also explained these findings. In addition, the concentrations of aspartame and its breakdown products in this study (25–250 mg/L) are higher than those in wastewaters ($\mu\text{g/L}$ – ng/L range). This study was designed to test how well the treatment system works under heavy loads. Additionally, point-source or industrial wastewaters, particularly those from the food, drink, or drug industries, may contain these high levels. Accordingly, the results should be considered a high-load toxicity test that shows how well the system can handle stress, what effects it might have on performance, and what its limits are in the worst-case scenario.

Table 1. The yield of biomass and lipid of *Gloeocystis vesiculosa* cultivated at various APM, DKP, and PHE concentrations

Biomass (mg/L)	APM	DKP	PHE
25 mg/L	830 ± 10	837 ± 6	827 ± 6
75 mg/L	$753 \pm 6^{**}$	$880 \pm 10^*$	$853 \pm 15^*$
125 mg/L	$720 \pm 10^{**}$	$963 \pm 15^{**}$	$900 \pm 10^{**}$
250 mg/L	$663 \pm 15^{**}$	$1080 \pm 20^{**}$	$920 \pm 10^{**}$
Lipid Yield (mg/L)	APM	DKP	PHE
25 mg/L	213 ± 7	206 ± 8	204 ± 6
75 mg/L	201 ± 5	214 ± 11	208 ± 7
125 mg/L	204 ± 2	215 ± 4	207 ± 10
250 mg/L	203 ± 10	220 ± 3	205 ± 4

Conclusion

When grown in wastewater containing artificial sweeteners such as APM and its metabolites, such as DKP and PHE, *Gloeocystis vesiculosa* metabolises some of these substances, altering biomass and lipid contents, and affecting antioxidant activities such as SOD, CAT, APX, and MDA. In addition, no significant change was observed in lipid yield. High levels of APM increase lipid percentage, while high levels of DKP increase microalgal biomass. APM functions as a stress trigger and induces severe stress. Increases in the antioxidant enzymes SOD, CAT, APX, and MDA provide evidence of this. In both cases, these chemicals are metabolised, benefiting wastewater treatment and leading to product formation. In

light of these results, future studies using a large-scale photobioreactor will investigate the effects of *Gloeocystis vesiculosa* on biodiesel production and wastewater treatment in wastewater contaminated with APM, DKP, and PHE.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The authors declare that this study does not involve experiments with human or animal subjects, and therefore, ethics committee approval is not required.

Data availability: The data will be made available upon request from the author.

Funding disclosure: -

Acknowledgements: -

Disclosure: -

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Coastal fish community structure at a proposed site for a new maritime port: Punta Colonet, Baja California, México (Eastern Pacific)

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Cite this article as:

Rosales-Casián, J.A. (2026). Coastal fish community structure at a proposed site for a new maritime port: Punta Colonet, Baja California, México (Eastern Pacific). *Aquatic Research*, 9(2), 90-106. <https://doi.org/10.3153/AR26009>

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Submitted: 10.10.2025

Revision requested: 02.01.2026

Last revision received: 22.01.2026

Accepted: 22.01.2026

Published online: 16.03.2026

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ABSTRACT

Punta Colonet, Baja California, México, is 240 kilometres south of California (USA), an upwelling and fishing site considered for a seaport construction. Coastal fish structure was determined through monitoring and available information, resulting in 7,010 individuals (106 species). Black perch (*Embiotoca jacksoni*) was most abundant by diving, and with hook-and-line, the Starry rockfish (*Sebastes constellatus*). Otter trawls and gillnets caught abundant Dwarf perch (*Micrometrus minimus*) and *E. jacksoni*. Artisanal fishing caught the Ocean whitefish (*Caulolatilus princeps*) abundantly in winter, summer, fall, and Vermilion rockfish (*Sebastes miniatus*) in spring; the overall mean was 67.6 ± 17.3 SE fish/boat, highest in summer (81 ± 4.6 fish/boat), and lowest during winter (49.5 ± 4.2 fish/boat). Sportfishers caught Yellowtail (*Seriola lalandi*, 32.2%), *S. miniatus*, and Lingcod (*Ophiodon elongatus*). Ensenada Seafood Market sold California sheephead (*Bodianus pulcher*, formerly *Semicossyphus pulcher* 66.4%), *C. princeps*, and *Paralabrax nebulifer*. Colonet fish in the preserved collection recorded higher numbers of Northern anchovy (*Engraulis mordax*, $n = 286$) and White croaker (*Genyonemus lineatus*). The most abundant in all series was *B. pulcher* (16%) and ordered by Index of Community Importance: *E. jacksoni* (occurrence 41.1%), *C. princeps*, *S. miniatus*, *B. pulcher*, and *M. minimus*. With different methods, a greater number of fish and species were collected, and this shows the guidelines to follow after the port's construction. Colonet highlights fish habitat, also for commercial and sportfishing, and the information will help decision-makers before port construction.

Keywords: Baseline, Fish species, Abundance, Seasonality, Occurrence, Importance, Bahía Colonet

Introduction

Many fish species from the temperate eastern Pacific have their spawning grounds off Baja California (Mexico). It is a source of eggs, larvae, and young-of-the-year (YOY) for the Southern California Bight, USA (Moser et al., 1993), by northward flow during the marine current relaxation (Shanks & Eckert, 2005). Fishes are distributed between the northern cold temperate province (Oregonian), from British Columbia (Canada) to near Point Conception in Southern California (USA), and the warm temperate San Diegan province extending south to Bahía Magdalena, Baja California Sur, Mexico (Horn et al., 2006).

In California, there is information about coastal fishes in habitats such as bays, estuaries, rocky intertidal, rocky reefs, kelp beds (*Macrocystis pyrifera*), surf to pelagic, and commercial and recreational fisheries (Allen et al., 2006). In Baja California, at 106 km south of the California border, there is information at Bahía Todos Santos and Estero Punta Banda (Hammann & Rosales-Casián, 1990; Rosales-Casián, 1997a). Also, 306 km south at Bahía and Coast of San Quintín (Rosales-Casián, 1996; 1997b; 2004a), and 366 km at Punta Baja (Rosales-Casián, 2011). Punta Banda and San Quintín, as coastal lagoons, are vital for California halibut, *Paralichthys californicus* (Kramer, 1990; Rosales-Casián, 2004b), and upwelling benefits the food web (Álvarez-Borrogo, 2004; Rosales-Casián, 2004a).

An important site 132 km south of Bahía Todos Santos is Punta Colonet, with intense upwelling and a surface plume of 12°C flowing southward (Barton & Argote, 1980). Punta Colonet is 68 km north of San Quintín, a conservation priority (Morgan et al., 2005), with abundant fish in volcanic reefs and around Isla San Martín (Albino-Martínez & Rosales-Casián, 2024). Rocky reefs from Punta Colonet are key for reproduction and feeding of resources, as in the Southern California Bight (Pondella et al., 2005). Colonet fish information is null; a study in fishing fields along the temperate Baja California includes this site but does not describe their catches (Rosales-Casián & González-Camacho, 2003). However, considering the habitats of Colonet, it is expected to identify species associated with the *M. pyrifera* beds, demersal fish such as rockfishes and pelagic fish species during warm seasons.

Punta Colonet is pristine, and the first port proposal was suspended (DOF, 2008). In 2022-2023, it was reactivated as an option for the congested ports of Ensenada, Baja California, and California (SEMARNAT, 2022). Fish are an essential component of Punta Colonet, and integrating different methods for catching fish ensures a greater number of species, and

in turn shows the methods for monitoring after the possible port's construction. This study identifies fish species in different habitats, from 5 m to 150 m depth, determines their abundances, seasonality, and order of importance, and provides a baseline for evaluating changes due to the potential construction of the new port.

Materials and Methods

Study Area

Punta Colonet, Colonet or Cabo Colnett (Lat. 30°57' 22.5" N, Long. 116°19'21" W), is 240 km south of California, USA, 132 km south of Ensenada, Mexico (Figure 1), and 6 km from the highway. To the south is Punta San Telmo and northwest Punta Colonet, showing a cliff 4.5 km long and 250 m high protecting the bay that has 2,680 hectares (DOF, 2008; Madrigal-Sánchez, 2009; Castillo-Chávez, 2014).

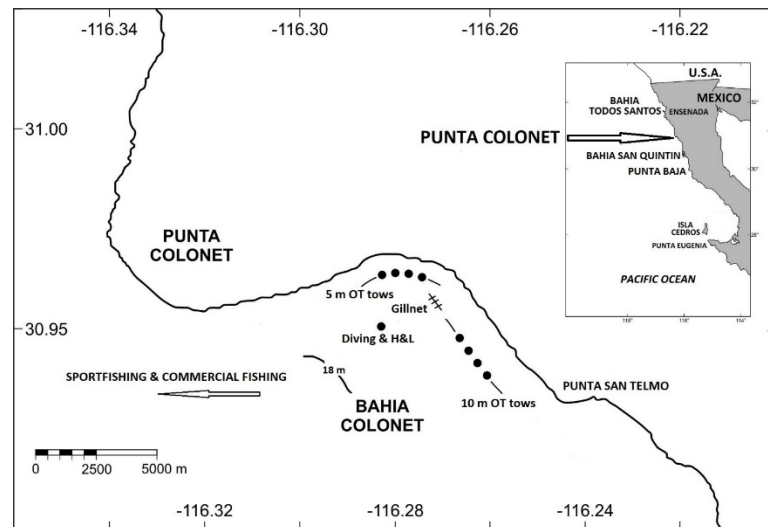


Figure 1. Punta Colonet on the Pacific coast of Baja California (Mexico) and the collection sites. The continuous black dots are repetitions of otter-trawl tows at 5 and 10 m depth. The striped line represents the gillnet collections site at 10 m depth; the separated black dot is the reef (13 m depth) for diving and hook-and-line. The 18 m isobath is shown on the map. The arrow indicates the area for commercial and sport fishing.

Punta Colonet Fish Species

Fish species identification: All fish species identified in monitoring were using the Pacific Northwest keys for the temperate Baja California, Mexico, and California, USA

(Miller & Lea, 1972; Love et al., 2002), and for tropical or subtropical fish species, the key of Humann & DeLoach (2004). In the case of the California sheephead, its scientific name was changed to *Bodianus pulcher* instead of *Semicossyphus pulcher*, according to Santini & Alfaro (2016) and Love & Passarelli (2020).

Diving observations and fishing: In October 1998, I boarded the M/V Horizon (San Diego, California, USA) and visited Colonet on October 29 and November 11. Fish identification and abundances were conducted by three scuba transects (Stephens et al., 2006), spear catch, and fishing with hook-and-line (H&L) at a shallow reef of the bay with 13m depth (latitude 30.91180° N, longitude -116.51791° W).

Otter-trawl and gillnet fish samplings: Before the first Colonet port project (DOF, 2008), sampling was conducted in summer (August 20-21), fall (November 9-10) of 2007, and winter (February 8-9) of 2008. Fish were collected using four repetitions of otter-trawl (7.5m mouth width, 12m length, body 19mm mesh and 5mm at end), both at 5 and 10m depth, for five minutes, 2 knots, and parallel to the coast with a boat (Figure 1); sea surface temperature (SST °C) was recorded at each tow. Also, four gillnet operations (30m length x 2.5m height, and 2.5, 5, and 7.6cm mesh) at 10m depth (Figure 1) and by four hours of soak time were used (Rosales-Casián, 2004a; Rosales-Casián, 2011). Punta Colonet has no restrictions on fish collection since it is not a marine reserve area.

Commercial and sportfishing: Fish species were identified and fish counted in four commercial boats per season, in winter 2008 (February 22), spring (May 23), summer (August 28) and fall (November 28). Sportfishing catches were compiled from Colonet travel reports (2006-2023) on the web (<https://www>) by San Diego boats (241 km to south) and Long Beach, California (411 km to south), and from Ensenada, Ejido Erendira, Colonet and San Quintín, Baja California (bdoutdoors.com; bloodydecks.com; mexfish.com; pacificqueen.net; seaforthlanding.com; sergiosfishing.com; socialsalty.com; sport-fishing.com; wonews.com). The captains or reporters do not provide coordinates from sport or commercial fishing spots due to their secrecy; they only mention some depths and some distances from the coast within the bay or around Punta Colonet.

Colonet fish species sold at seafood market: The Ensenada Seafood Market was monthly monitored (2013-2023), to identify the fish species for sale, determine their abundance and order of importance. This market has 42 stalls, some selling seafood (shrimp, oysters, clams, shellfish, etc.), others selling smoked fish, and others selling whole fish. Up to 20 stalls selling whole fish were monitored per monthly visit,

whose catch came from the Pacific coast of Baja California and the Gulf of California. The catches from Punta Colonet were identified by surveys with the vendor of each stall, who reported the origin of the fish caught.

Preserved fish collection: The Colonet fish species recorded in the Vertebrate Collection of Scripps Institution of Oceanography (SIO), University of California, San Diego (La Jolla, California, USA) were included. Fish collected far from the coast or too deep were not considered, and among the multiple jars with the same species, only the one with the highest number of individuals was included, as in the case of the Northern anchovy, *Engraulis mordax* (<https://scripps.ucsd.edu/marine-vertebrate-collection>).

Fish species importance: To rank the species, the Index of Community Importance (ICI) was selected because it includes only percentages of abundance and frequency of occurrence (Stephens & Zerba, 1981) and covers all data series in the present study. In the first column, the species and relative abundance are in the second column, in the third column, an assigned score (R1); next, frequency of occurrence with scores in the fifth column (R2); the sum of both scores was the ICI, and the lowest values were the most important species. To calculate the frequency of occurrence, the species presence in collections was divided by the total events and expressed as a percentage (Albino-Martínez & Rosales-Casián, 2024). SIO collection species were included as one event in the overall ICI.

Statistical analysis: Fish abundances caught seasonally with nets and from commercial fishing were normally analysed with Levene's test (Zar, 1984). Normality was accepted in abundance with 5m depth otter-trawl tows ($F(2, 9) = 2.925, p = 0.104$); therefore, to determine differences in catch means with respect to time, a parametric ANOVA analysis was used (Zar, 1984). Normality was not accepted for 10 m otter-trawl ($F(2, 9) = 14.847, p = 0.001$) and gill net abundances ($F(2, 9) = 7.672, p = 0.011$), and a Kruskal-Wallis analysis was used to determine catch mean differences with respect to time. Normality of commercial fish catch was accepted (Levene, $F(3, 12) = 1.299, p = 0.319$), and an ANOVA was used to determine differences between the catch per boat means with respect to time. In all statistical analyses and graphics, the Statistica 7.1 program from StatSoft Inc. was used.

Results and Discussion

After Mexican independence, a Swedish colony was established in 1888 and ended the development project ten years later (Heath, 2001-2004). Now, the coast remains pristine with a town 7-8 km away and little development. Punta Colonet forms a semi-protected bay, and with the wind creates

upwelling (Barton & Argote, 1980), resulting in high productivity, abundant fisheries, and is why it is considered a marine priority for conservation (Morgan et al., 2005). Now, Colonet is under a new project as an option to the full ports of Ensenada, Baja California, Mexico and California, USA (DOF, 2008; SEMARNAT, 2022). There are opposing views between conservationists and developers. However, the main idea is to activate its economy, proposing a balance between infrastructure and sustainability with alternative governance for the new port, and maintaining a natural area for conservation (Santes-Álvarez & Riemann-González, 2013).

The SST mean at Punta Colonet was 15.8°C in October-November 1998. Summer 2007 was warmer (20.1°C \pm 0.13 SE) at 5m, and 19.7°C (\pm 0.11) at 10m; in fall, temperatures were 15.2°C and 14.9°C, respectively, with a colder winter 2008 (5m: 13.4°C; 10 m: 13.1°C). Similar SSTs to Punta Baja, a strong upwelling site 120 km south of Colonet, but in spring, it drops to 10°C (Rosales-Casián, 2011). Colonet SSTs are seasonal, with spring upwelling dropping to 12°C (Barton & Argote, 1980).

During the cruise on the M/V Horizon (1998), diving and fishing with H&L accounted for 627 fish (39 species), and diving contributed 69.5% of the total (Table 1). Most abundant species by diving were the Black perch, *Embiotoca jacksoni* (17.7%), the Opaleye, *Girella nigricans* (16.6%), and the Jacksmelt silverside, *Atherinopsis californiensis* (9.6%). The Black perch distribution ranges from Central California, USA, to Baja California, Mexico (Miller & Lea, 1972), where it inhabits kelp and rocks, searching for amphipods as food. Also, their numbers depend on predators such as the Kelp bass (*Paralabrax clathratus*), whose abundance changes with conditions and fishing (Johnson et al., 2019). Those species were registered north in Estero Punta Banda, and south at San Quintín (Rosales-Casián, 1996; 1997). With H&L, the most abundant species were the Starry rockfish (*Sebastes constellatus*, 5.1%), the Vermilion rockfish (*S. miniatus*, 4.1%), and the Greenblotched rockfish (*Sebastes rosemblatti*, 3.8%) (Table 1); rockfishes show 110 species in all oceans, of which 85 are in the temperate-cold zone of the Northeastern Pacific, and 53 species in Mexican waters (Love et al., 2002; Butler et al., 2012). By diving and H&L, 19 fish species accounted for 90.2% of the abundance, and by the ICI, the most important species were *E. jacksoni*, *O. californica*, *H. argenteum*, *G. nigricans*, and the Brown rockfish, *Sebastes auriculatus* (Table 1). Algae and rocky areas provide shelter and food for perches and rockfish species (Love et al., 2002; Miller et al., 2018).

In 2007-2008, 24 otter-trawl and 12 gillnet operations were made, resulting in 1,938 fish from 28 species. Summer 2007

showed the highest number of 832 fish but fewer species (19 species), and winter 2008 had the lowest individuals (383) but a higher number of 24 species, while fall 2007 had intermediate abundances and species (Table 2). The most abundant species were the Dwarf perch (*Micrometrus minimus*, 27.6%), *E. jacksoni* (25%), and the Walleye surfperch, *Hyperprosopon argenteum*, 16.8% (Table 2) several 12 fish species accounting for 91.5% of the abundance.

In summer 2007, abundant species were *M. minimus* (31.3%), *H. argenteum* (30.9%), *E. jacksoni* (13.8%) and the Calico surfperch, *Amphistichus koelzi* (10.2%), which contributed with 86.2% of the total. Most abundant in fall 2007 were *E. jacksoni* (33.1%), *M. minimus* (31.8%), the Shiner perch, *Cymatogaster aggregata* (8.4%), and the Californian needlefish, *Strongylura exilis* (4.6%), which accumulated 77.9% of the total (Table 2); in winter 2008, the most abundant species were *E. jacksoni* (34.2%), *H. argenteum* (13.1%), *M. minimus* (11.5%) and *C. aggregata* (5.0%), which accumulated 63.8% of the total. The most important fish species (ICI) were *E. jacksoni*, *M. minimus*, *H. argenteum*, the Pile perch (*Phanerodon vacca*), and the Giant kelpfish, *Heterostichus rostratus* (Table 2). Above species belong to Embiotocidae except *S. exilis*, a tropical species collected in Southern California Bight, USA, in the 1982-1983 El Niño event (Allen et al., 2006), and in Bahía Todos Santos, Estero Punta Banda, Bahía San Quintín, and Punta Baja, Baja California, Mexico (Hammann & Rosales-Casián, 1990; Rosales-Casián, 2004a; Rosales-Casián, 2011). *H. rostratus* lives in algae and rocky reefs, and shows red, brown and green colours, depending on the surrounding plants (Stepien, 1986).

Highest catches were in 5m otter-trawl tows (801 fish, 47.7% of total), versus 10m and gillnet catches (Table 3). The highest catch in a tow (n = 225 fish), the highest seasonal sum of fish (n = 382), and the highest mean catch (95.5 \pm 45.3 SE fish/tow) occurred with 5m otter-trawl tows in summer 2007; conversely, they were lower at 5 m trawls in winter 2008 (Table 3). Mean catches by 5m trawl tow did not differ (ANOVA, F = 1.571, df = 9, p = 0.259), but, mean catches at 10m trawls (K-W, H(2, 12) = 8.221, p = 0.016) as well as gillnet catches (K-W, H(2, 12) = 9.846, p = 0.007) were different. Compared to other sites, it is difficult due to the soft bottoms, and the Colonet site showed kelp and rocky bottom, which influenced the ichthyofauna and caused net damage between tows. However, in Colonet, the overall mean catch with a 5m otter-trawl was 66.8 fish per trawl (\pm 18.8 SE), while at the San Quintín coast, the mean catch at 5m was 11.2 (\pm 2) fish per trawl (Rosales-Casián, 1997), highlighting the algal habitat in the harbour with high fish numbers.

Artisanal commercial fishing in 2008 showed 1,082 fish individuals belonging to 22 species (Table 4). Annually, the most abundant species were *C. princeps* (42.2%) of the total, followed by *S. miniatus* (20.6%) and *P. nebulifer* (13.5%); the order of the species by the frequency of occurrence was *C. princeps* (93.8%), *S. miniatus* (97.5%) and *S. auriculatus*, 75% (Table 4). The most abundant fish species in winter were *C. princeps* (44.4%), *S. miniatus* (24.2%) and *S. paucispinis* (6.6%). In spring, the most abundant species were *S. miniatus* (28.9%), followed by *C. princeps* (26.5%), and the Barred sand bass (*Paralabrax nebulifer*, 20.1%). In summer, *C. princeps* (46.6%), *P. nebulifer* (25.3%) and *S. miniatus* (10.8%), and during the fall season, *C. princeps* (53.1%), *S. miniatus* (20.6%) and *P. californicus*, 14.5% (Table 4). The order of the most important fish species (ICI) in the artisanal fishing was *C. princeps*, *S. miniatus*, *S. auriculatus*, *P. nebulifer* and three tied, *S. constellatus*, *B. pulcher*, and *P. californicus* (Table 4). Commercial catch showed an overall mean of 67.6 (± 17.3 SE) fish per boat, with the highest in summer (81

± 4.6) fish per boat and the lowest (49.5 ± 4.2 fish per boat) in winter (Figure 2). Mean commercial catches were different over time (ANOVA, $F = 3.996$, $df = 12$, $p = 0.034$). There is no information on Colonet commercial fishing, a study from eight fishing grounds in the temperate Pacific of Baja California, which included Colonet, but did not describe its catches (Rosales-Casián & González-Camacho, 2003). However, San Quintín showed an annual mean catch of $51.8 (\pm 5.2$ SE) fish per boat, and rockfish (*Sebastes* sp.) was the most important (ICI), followed by *P. clathratus* and *C. princeps*; as an inference because it was not part of the present study, differences in the species order and catch per boat maybe due to the environmental conditions, San Quintin (1994-1995) was under Neutral-El Niño conditions (Rosales-Casián & González-Camacho, 2003), and in Colonet (2008) was under La Niña-Neutral conditions (NOAA, 2025); this last condition benefits seasonal upwelling in the California Current (Wang et al., 2022).

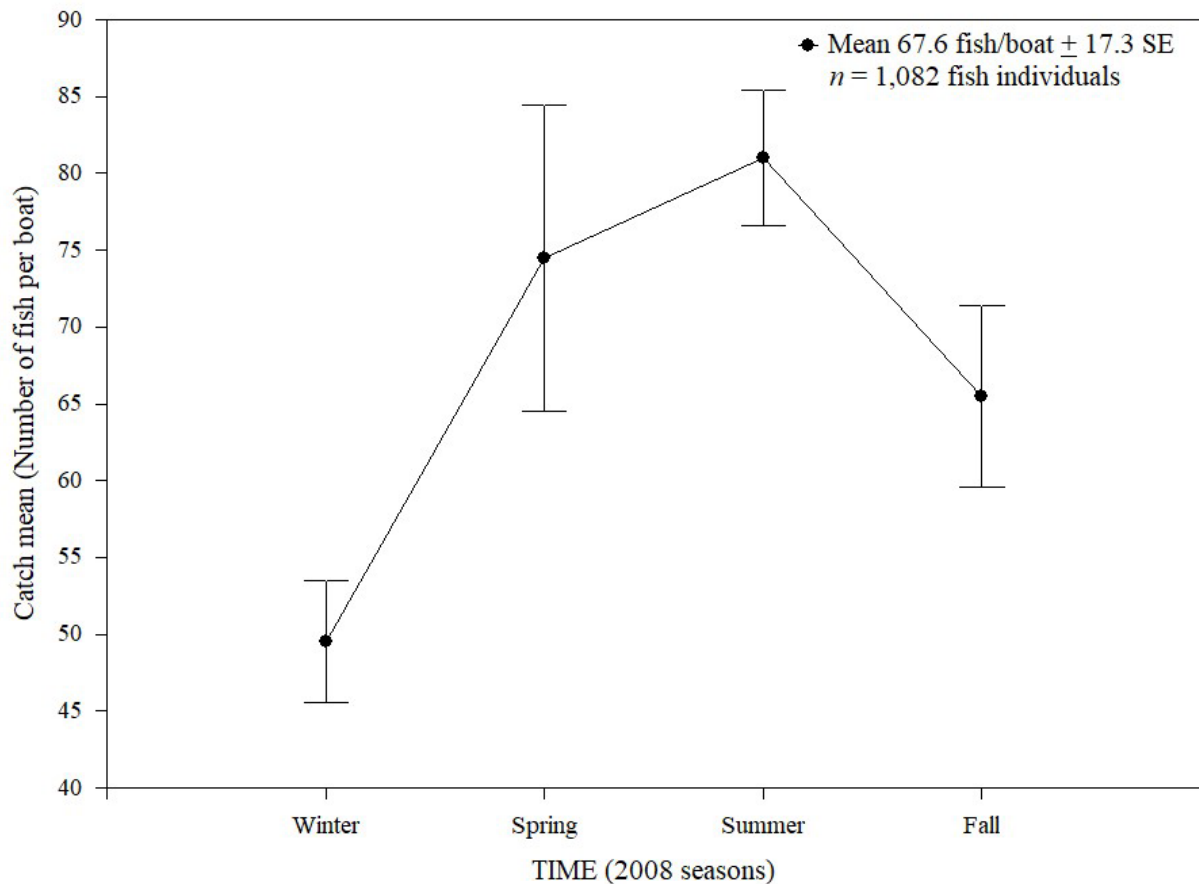


Figure 2. Mean commercial catch of coastal fish (fish per boat \pm SE, standard error) at Punta Colonet, Baja California, Mexico during the 2008 seasons

Table 1. Fish species and abundances by hook-and-line (H&L), spear, and diving in Punta Colonet, Baja California, Mexico (October and November 1998). Rel%: relative abundance %; Cum%: cumulative abundance %

Species	H&L	Spear	Dive1	Dive2	Sum	Rel%	Cum%	R1	FO %	R2	ICI
<i>Embiotoca jacksoni</i>		2	92	14	108	17.2	17.2	1	75	3	4
<i>Oxyjulis californica</i>		2	16	10	28	4.5	53.0	5	75	3	8
<i>Hyperprosopon argenteum</i>		3	3	17	23	3.7	68.3	8.5	75	3	11.5
<i>Girella nigricans</i>		1	103		104	16.6	33.8	2	50	10	12
<i>Sebastes auriculatus</i>		8	6	1	15	2.4	78.9	12.5	75	3	15.5
<i>Paralabrax nebulifer</i>		2	7	3	12	1.9	87.1	16.5	75	3	19.5
<i>Phanerodon furcatus</i>		6	13		19	3.0	71.3	10	50	10	20
<i>Phanerodon vacca</i>			15	3	18	2.9	74.2	11	50	10	21
<i>Paralabrax clathratus</i>			3	8	11	1.8	88.8	18	50	10	28
<i>Bodianus pulcher</i>		1	7		8	1.3	90.1	20	50	10	30
<i>Atherinopsis californiensis</i>			60		60	9.6	43.4	3	25	27	30
<i>Sebastes constellatus</i>	32				32	5.1	48.5	4	25	27	31
<i>Sebastes miniatus</i>	26				26	4.1	57.1	6	25	27	33
<i>Sebastes rosenblatti</i>	24				24	3.8	60.9	7	25	27	34
<i>Embiotoca lateralis</i>			4	1	5	0.8	96.2	25	50	10	35
<i>Micrometrus minimus</i>			4	1	5	0.8	97.0	25	50	10	35
<i>Sebastes paucispinis</i>	23				23	3.7	64.6	8.5	25	27	35.5
<i>Oxylebius pictus</i>		1	1		2	0.3	98.1	29.5	50	10	39.5
<i>Halichoeres semicinctus</i>			1	1	2	0.3	98.7	29.5	50	10	39.5
<i>Caulolatilus princeps</i>	15				15	2.4	76.6	12.5	25	27	39.5
<i>Sebastes chlorostictus</i>	14				14	2.2	81.2	14	25	27	41
<i>Sebastes rosaceus</i>	13				13	2.1	83.3	15	25	27	42
<i>Sebastes lentiginosus</i>	12				12	1.9	85.2	16.5	25	27	43.5
<i>Sebastes hopkinsi</i>	8				8	1.3	91.4	20	25	27	47
<i>Sphyræna argentea</i>	8				8	1.3	92.7	20	25	27	47
<i>Scomber japonicus</i>	6				6	1.0	93.6	22.5	25	27	49.5
<i>Rhacochilus toxotes</i>			6		6	1.0	94.6	22.5	25	27	49.5
<i>Sebastes umbrosus</i>	5				5	0.8	95.4	25	25	27	52
<i>Hypsypops rubicundus</i>			3		3	0.5	97.4	27	25	27	54
<i>Sebastes ovalis</i>	2			3	2	0.3	97.8	29.5	25	27	56.5
<i>Medialuna californiensis</i>			2		2	0.3	98.4	29.5	25	27	56.5
<i>Ophiodon elongatus</i>	1				1	0.2	98.9	35.5	25	27	62.5
<i>Sebastes levis</i>	1				1	0.2	99.0	35.5	25	27	62.5
<i>Sebastes elongatus</i>	1				1	0.2	99.2	35.5	25	27	62.5
<i>Sebastes atrovirens</i>			1		1	0.2	99.4	35.5	25	27	62.5
<i>Embiotoca caryi</i>				1	1	0.2	99.5	35.5	25	27	62.5
<i>Heterostichus rostratus</i>				1	1	0.2	99.7	35.5	25	27	62.5
<i>Haemulon californiensis</i>				1	1	0.2	99.8	35.5	25	27	62.5
<i>Sebastes rastrelliger</i>		1			1	0.2	100.0	35.5	25	27	62.5
Total	191	27	347	62	627	100.0					

Table 2. Seasonal fish species, abundance and importance by otter-trawl (OT) at 5 and 10m, and gillnet (GN) in Punta Colonet, Baja California, Mexico (2007-2008). Rel%: relative abundance %; FO%: frequency of occurrence %; R1: Rel% order; R2: FO% order; ICI: Index of Community Importance

Species	Summer 2007				Fall 2007				Winter 2008				Total	Rel%	R1	FO%	R2	ICI	
	C5	C10	GN	%	C5	C10	GN	%	C5	C10	GN	%							
<i>Embiotoca jacksoni</i>	36	45	34	13.8	113	92	34	33.1	17	84	30	34	485	25.0	2	94.4	1	3	
<i>Micrometrus minimus</i>	217	26	17	31.3	162	68		31.8	24	13	7	12	534	27.6	1	66.7	2	3	
<i>Hyperprosopon argenteum</i>	101	30	126	30.9		7	11	2.5	4	9	37	13	325	16.8	3	63.9	3	6	
<i>Phanerodon vacca</i>	8	9	9	3.1		7	3	1.4		2	4	1.6	42	2.2	7	41.7	5.5	12.5	
<i>Heterostichus rostratus</i>	5	3	2	1.2	10	4	2	2.2	6	3	2	2.9	37	1.9	9.5	55.6	4	13.5	
<i>Phanerodon furcatus</i>		9		1.1	4	25		4.0	8	5	3	4.2	54	2.8	6	33.3	7.5	13.5	
<i>Cymatogaster aggregata</i>		12		1.4		61		8.4	4	11	4	5.0	92	4.7	4	22.2	10	14	
<i>Oxijulis californica</i>	2	2	1	0.6		11	2	1.8		5	5	2.6	28	1.4	12	33.3	7.5	19.5	
<i>Paralichthys californicus</i>	1	2		0.4	1			0.1	7	5	6	4.7	22	1.1	14	41.7	5.5	19.5	
<i>Sebastes auriculatus</i>								16	2.2	1	2	6	2.3	25	1.3	13	25	9	22
<i>Amphistichus koelzi</i>		1	84	10.2						2	1	0.8	88	4.5	5	10.4	20	25	
<i>Genyonemus lineatus</i>								26	3.6			6	1.6	32	1.7	11	16.7	14	25
<i>Atherinopsis californiensis</i>			24	2.9							17	4.4	41	2.1	8	11.1	18	26	
<i>Embiotoca lateralis</i>		2	1	0.4	2	12		1.9		2	1	0.8	20	1.0	15	19.4	12	27	
<i>Strongylura exilis</i>	2			0.2	33			4.6	2			0.5	37	1.9	9.5	11.1	18	27.5	
<i>Syngnathus leptorhynchus</i>	4	1		0.6	2			0.3	11			2.9	18	0.9	16	19.4	12	28	
<i>Sebastes rastrelliger</i>	5		3	1.0						1	3	1	12	0.6	18	19.4	12	29.5	
<i>Paralabrax nebulifer</i>									2	2	2	1.6	6	0.3	20	13.9	16	35.5	
<i>Pleuronichtys guttulatus</i>						1		0.1	2	1	1	1	5	0.3	21	13.9	16	36	
<i>Amphistichus argenteus</i>							11	1.5					11	0.6	18	8.3	22	39.5	
<i>Citharichthys stigmaeus</i>		1		0.1	1	1	1	0.4					4	0.2	24	11.1	18	41.5	
<i>Paralabrax clathratus</i>										3	3	1.6	6	0.3	21	8.3	22	42.5	
<i>Symphurus atricauda</i>									3	1		1	4	0.2	24	8.3	22	45.5	
<i>Seriphus politus</i>			5	0.6									5	0.3	21	2.8	26	46.5	
<i>Scorpaena guttata</i>										2	0.5		2	0.1	27	2.8	26	52.5	
<i>Scorpaenichthys marmoratus</i>	1			0.1									1	0.1	27	2.8	26	52.5	
<i>Medialuna californiensis</i>			1	0.1									1	0.1	27	2.8	26	52.5	
<i>Trachurus symmetricus</i>											1	0.3	1	0.1	27	2.8	26	52.5	
Total	382	143	307		328	289	106		91	151	141		1938	100					
Total by season	Summer, n = 832				Fall, n = 723				Winter, n = 383										

Table 3. Seasonal fish catch, minimum-maximum and mean (\pm SE: Standard error) by otter-trawl tow (OT) at 5 and 10m, and gillnet (GN) in Punta Colonet, Baja California, Mexico (2007-2008)

Season	Otter-trawl 5m catch		Otter-trawl 10m catch		Gillnet catch		Total catch
	Sum (min-max)	Mean (\pm SE)	Sum (min-max)	Mean (\pm SE)	Sum (min-max)	Mean (\pm SE)	
Summer 2007	382 (17-225)	95.5 (\pm 45.3)	143 (20-45)	35.8 (\pm 5.4)	307 (51-142)	76.8 (\pm 21.8)	832
Fall 2007	328 (38-164)	82.0 (\pm 28.6)	289 (44-94)	72.3 (\pm 28.8)	106 (24-30)	26.5 (\pm 1.3)	723
Winter 2008	91 (16-28)	22.8 (\pm 2.6)	151 (20-76)	37.8 (\pm 12.9)	141 (26-40)	35.3 (\pm 3.2)	383
Total catch	801	66.8 (\pm 18.8)	583	48.6 (\pm 7.6)	554	46.2 (\pm 9.4)	1938

Table 4. Seasonal fish species from the commercial catch, abundance and order of importance in Punta Colonet, Baja California, Mexico (2008). Rel%: relative abundance %; FO%: frequency of occurrence %; R1: Rel% order; R2: FO% order; ICI: Index of Community Importance

Fish species	Winter	%	Spring	%	Summer	%	Fall	%	Total	Rel%	R1	FO%	R2	ICI
<i>Caulolatilus princeps</i>	88	44.4	79	26.5	151	46.6	139	53.1	457	42.2	1.0	93.8	1.0	2.0
<i>Sebastes miniatus</i>	48	24.2	86	28.9	35	10.8	54	20.6	223	20.6	2.0	87.5	2.0	4.0
<i>Sebastes auriculatus</i>	8	4.0	19	6.4	16	4.9	1	0.4	44	4.1	4.5	75.0	3.0	7.5
<i>Paralabrax nebulifer</i>	4	2.0	60	20.1	82	25.3			146	13.5	3.0	37.5	6.5	9.5
<i>Sebastes constellatus</i>	7	3.5	14	4.7	2	0.6	3	1.1	26	2.4	7.0	50.0	4.0	11.0
<i>Bodianus pulcher</i>	4	2.0	3	1.0	9	2.8	17	6.5	33	3.0	6.0	43.8	5.0	11.0
<i>Paralichthys californicus</i>	3	1.5			3	0.9	38	14.5	44	4.1	4.5	37.5	6.5	11.0
<i>Sebastes paucispinis</i>	13	6.6					7	2.7	20	1.8	9.0	31.3	8.0	17.0
<i>Seriola lalandi</i>			11	3.7	10	3.1			21	1.9	8.0	18.8	13.5	21.5
<i>Sebastes chlorostictus</i>	3	1.5	2	0.7	3	0.9			8	0.7	13.5	25.0	10.0	23.5
<i>Ophiodon elongatus</i>	2	1.9	3	1.0	2	0.6			7	0.6	15.0	25.0	10.0	25.0
<i>Sebastes umbrosus</i>	4	2.0	5	1.7					9	0.8	11.5	18.8	13.5	25.0
<i>Sebastes babcocki</i>	2	1.0	1	0.3	1	0.3	1	0.4	5	0.5	16.0	25.0	10.0	26.0
<i>Sphyaena argentea</i>			8	2.7					8	0.7	13.5	18.8	13.5	27.0
<i>Atractoscion nobilis</i>	8	4.0	4	1.3					12	1.1	10.0	12.5	17.5	27.5
<i>Scorpaena guttata</i>	2	1.0			7	2.2			9	0.8	11.5	12.5	17.5	29.0
<i>Sebastes atrovirens</i>	2	1.0	2	0.7					4	0.4	17.0	18.8	13.5	30.5
<i>Girella nigricans</i>			1	0.3	1	0.3			2	0.2	18.0	12.5	17.5	35.5
<i>Sebastes caurinus</i>							1	0.4	1	0.1	21	12.5	17.5	38.0
<i>Sebastes flavidus</i>					1	0.3			1	0.1	21	6.3	21.0	41.5
<i>Coryphaena hippurus</i>					1	0.3			1	0.1	21	6.3	21.0	41.5
<i>Stereolepis gigas</i>							1	0.4	1	0.1	21	6.3	21.0	41.5
Total	198		298		324		262		1082	100				

Sportfishing data came from 20 boats that made trips to Punta Colonet and reported their catches online (2006-2023); 14 vessels travelled south from San Diego, CA (USA), and one boat from Long Beach, CA. Furthermore, three trips from Ensenada, Baja California (120 km south), and individual

trips from Erendira (37 km south), San Quintín (60 km north), and one local boat from Colonet. The boats carried between two and 26 anglers and visited rocky spots within and around the bay and occasionally up to 6 km offshore in search of tuna species, and depths of 38–150 m, with a preference for 90–

116 m for Yellowtail and demersal fish. The total caught was 1,193 fish belonging to 33 species, and 18 of those from the *Sebastes* sp. (Table 5). Anglers caught the Yellowtail (*Seriola lalandi*, 32%) by 14 boats, followed by *S. miniatus* (19.1%) in 16 trips, and the Lingcod (*Ophiodon elongatus*, 10.1%) in 12 boats. Most important fish species (ICI), both *S. lalandi* and *S. miniatus* ranked in first place; *S. lalandi* by their highest abundance and *S. miniatus* by the highest frequency of occurrence (80%), followed by *O. elongatus*, with low abundance but high frequency of occurrence of 60% (Table 5). About demersal fish, *S. miniatus* was most abundant by the San Quintín sportfishing in 2005 and also during 2009, changing the first place from *C. princeps* (winter-fall) seasonally, to *S. miniatus* in spring-summer and with the Yellowtail in seventh place (Rodríguez-Santiago & Rosales-Casián, 2008; Rosales-Casián & Delgadillo-Hernández, 2010). San Quintín sportfishing for pelagic fishes during Neutral conditions 2008-2009, El Niño 2009-2010, and La Niña 2010-2011 captured 787 individuals belonging to 12 species, with abundant Yellowtail during Neutral and El Niño years, and a drastic reduction during La Niña (Ibarra-Gonzalez, 2013). In California, the main catch is groundfish; recreational anglers in 2004 landed 212 metric tons of *S. miniatus*, which ranked seventh in abundance; in 2005, in south-central California, anglers caught 2,751 Blue rockfish (*Sebastes mystinus*), with 1,218 individuals of *S. miniatus* ranked second (CDFG, 2005; Stephens et al., 2006; Wang et al., 2022).

At the Ensenada Seafood Market, only 19 stalls monitored over different months and years offered fish caught in Colonet, according to vendor responses, with 1,626 fish belonging to four fish species. *B. pulcher* was first in abundance with 1,080 individuals (66.4%), followed by *C. princeps* (18.7%), *P. nebulifer* (14.7%) and three individuals of the Giant black seabass (*Stereolepis gigas*, 0.2%). These species showed the same importance (ICI) order, *B. pulcher* also with the highest (73.7%) frequency of occurrence (Table 6). A 2013-2014 study at the Seafood Market counted 6,830 individuals from 75 species, with 20 species of the Scorpaenidae family, Sciaenidae (nine species), Embiotocidae (seven species), and Serranidae (six species); the most abundant were *P. nebulifer* (25.7%), *C. princeps* (8.5%), *S. auriculatus* (6.2%), *S. miniatus* (4.8%), and the Gulf corvina (*Cynoscion othonopterus*) with 4.7% (Adame-Fraire, 2015). The last species is endemic to the Gulf of California and is caught during the spawning migration to the Upper Gulf and the Colorado River (Enciso-Enciso et al., 2025).

The SIO Vertebrate Collection (UCSD) showed 544 fish belonging to 47 species from Colonet; the Northern anchovy (*Engraulis mordax*) showed the highest number of individuals ($n = 286$), followed by the White croaker (*Genyonemus*

lineatus), the Spotted kelpfish (*Gibbonsia elegans*), the Longfin sanddab (*Citharichthys xanthostigma*), the Pacific sanddab (*Citharichthys sordidus*), and the Blacksmith, *Chromis punctipinnis* (Table 7). Those species have been collected in Bahía Todos Santos, Bahía and the coast of San Quintín (Hammann & Rosales-Casián, 1990; Rosales-Casián, 1997; Albino-Martínez & Rosales-Casián, 2024), as well as on the California coast (Allen et al., 2006).

All data series of fish showed a total of 7,010 individuals belonging to 106 species; the most abundant species was the California sheephead (*B. pulcher*) with the highest percentage (16%), 1,122 individuals (Table 7) derived from Colonet commercial fishing and sold at the Ensenada Seafood Market. Second was the *C. princeps* (11.7%, 820 individuals) caught with H&L, commercial and sportfishing and sold at the seafood market. In third place, the Black perch (*E. jacksoni*, 8.5%) was collected with otter-trawl tows, gillnet and observed by diving, in fourth place and with the same methods, the Dwarf surfperch (*M. minimus*, 7.7%), and in fifth place *S. miniatus* (6.7%) caught with H&L, by the commercial and sportfishing (Table 7). Based on the fish species identified in the 96 monitoring events at Punta Colonet and the final order of the ICI, the most important species was the Black perch, *Embiotoca jacksoni*, by their highest frequency of occurrence (41.1%), and 8.5% of the abundance (Table 70). This species is associated with kelp beds and shallow rocky reefs, essential habitats for Embiotocidae family members (Froeschke et al., 2007), followed by the Ocean whitefish (*C. princeps*), Vermilion rockfish (*S. miniatus*), California sheephead (*B. pulcher*) and the Dwarf perch, *M. minimus* (Table 8). This study shows the importance of using different methods to represent the fish community, with rocky bottoms and kelp forests for shelter, feeding, reproduction, and migration of species. The 106 species from Punta Colonet are greater than 69 species from the San Quintín and coast system (Rosales-Casián, 1996), although it does not include commercial, sportfishing, or SIO Collection species. However, it is similar to the Bahía Todos Santos-Estero Punta Banda system (120 fish species), using net samplings, ichthyoplankton surveys and diving (Hammann & Rosales-Casián, 1990). Colonet is an essential fish habitat that can be perturbed, such as dredging and filling in California (USA), which reduced fish habitat up to 90% (Kramer, 1990). Based on the abundance of species of the Embiotocidae family in the present study, it is possible to consider them as indicators during the development of the possible port, after its construction in shallow depths (<10m), and using gillnets to avoid active collection methods. In turn, it is possible to select the species of the Scorpaenidae family at greater depths (<20m) using hook-and-line.

Table 5. Fish species from the sportfishing catch, abundance and order of importance in Punta Colonet, Baja California, Mexico (2006-2023). Rel%: relative abundance %; FO%: frequency of occurrence %; R1: Rel% order; R2: FO% order; ICI: Index of Community Importance. Port of boat: COL: Colonet; ENS: Ensenada; ERE: Erendira; LB: Long Beach; SD: San Diego; SQ: San Quintin. NP: Not provided.

YEAR	2006			2007			2008			2009			2010			2014			2016			2017			2019			2020			2021			2022			2023		
MONTH	Jun	Aug	Feb	Oct	Nov	Jan	Feb	Sep	Jul	Jan	Aug	Jan	Jul	Jan	Feb	Mar	Jan	Mar	Apr	Jul	Jan	Feb	Mar	Jan	Mar	Apr	Jul	Jan	Feb	Mar	Jan	Mar	Apr	Jul					
DEPTH (meters)	38-42	NP	21-38	NP	NP	15-45	NP	70-110	NP	NP	60	60-120	60	150	55-150	NP	150	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP							
PORT	ERE	SQ	SD	ENS	ENS	SD	SD	SD	SD	SD	LB	SD	SD	SD	SD	SD	SD	COL	SD	ENS	ERE	SQ	SD	ENS	ENS	SD	SD	SD	COL	SD	ENS								
Fish species/No. boats	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	Rel %	R1	FO %	R2	ICI													
<i>Seriola lalandi</i>	3	6	1	20		6		43	6	118		90		10		8		36	14	23	384	32.2	1.0	70	2.0	3.0													
<i>Sebastes miniatus</i>	12		11		15	26	10		7	18	10	37	6	5	50	6	12		2	1	228	19.1	2.0	80	1.0	3.0													
<i>Ophiodon elongatus</i>			10		12	25			8	26	5	2	8		11		4		7	2	120	10.1	3.0	60	3.0	6.0													
<i>Sebastes paucispinis</i>			10				2		2	10		22	8		8		5		12	8	87	7.3	4.0	50	4.0	8.0													
<i>Sebastes constellatus</i>					2	2			3	8		11	5		10		6				47	3.9	5.0	40	6.0	11.0													
<i>Sarda chiliensis</i>	2			1	6	6						10		2		4	2		1		34	2.8	7.0	45	5.0	12.0													
<i>Sebastes caurinus</i>						10			1	1		6	1								19	1.6	11.5	25	7.0	18.5													
<i>Thunnus albacares</i>				8				25													33	2.8	8.0	20	10.5	18.5													
<i>Sebastes chlorostictus</i>									4	4		8					7				23	1.9	10.0	20	10.5	20.5													
<i>Caulolatilus princeps</i>			10			30											4				44	3.7	6.0	15	15.5	21.5													
<i>Sebastes umbrosus</i>						1				2							10		6		19	1.6	11.5	20	10.5	22.0													
<i>Paralabrax clathratus</i>	4	4						5													13	1.1	14.0	20	10.5	24.5													
<i>Sebastes rastrelliger</i>										2		7	3								12	1.0	14.0	20	10.5	24.5													
<i>Sebastes melanostomus</i>					9		1														10	0.8	17.0	20	10.5	27.5													
<i>Sebastes ovalis</i>						1						6	1				4				12	1.0	14.0	15	15.5	29.5													
<i>Sebastes auriculatus</i>									2	9		13					4				28	2.3	9.0	10	22.0	31.0													
<i>Sebastes babcocki</i>										2		1					5		2		10	0.8	19.0	15	15.5	34.5													
<i>Sebastes serriceps</i>										2		5	1								8	0.7	20.5	15	15.5	36.0													
<i>Sebastes atrovirens</i>										2		3	1								6	0.5	23.0	15	15.5	38.5													
<i>Sebastes rosaceus</i>																	10				10	0.8	17.0	10	22.0	39.0													
<i>Thunnus orientalis</i>														1		1	1				3	0.3	25.5	15	15.5	41.0													
<i>Sebastes serranoides</i>												3					5				8	0.7	20.5	10	22.0	42.5													
<i>Scorpaena guttata</i>			3			4															7	0.6	22.0	10	22.0	44.0													
<i>Sphyrna argentea</i>	3																		1		4	0.3	24.0	10	22.0	46.0													
<i>Paralichthys californicus</i>	1							2	4		5										12	1.0	17.0	5	29.0	46.0													
<i>Sebastes goodei</i>																	3				3	0.3	25.5	5	29.0	54.5													
<i>Scomber japonicus</i>					2																2	0.2	27.5	5	29.0	56.5													
<i>Katsuwonus pelamis</i>				2																	2	0.2	27.5	5	29.0	56.5													
<i>Semicossyphus pulcher</i>													1								1	0.1	31.0	5	29.0	60.0													
<i>Atractoscion nobilis</i>			1																		1	0.1	31.0	5	29.0	60.0													
<i>Sebastes levis</i>					1																1	0.1	31.0	5	29.0	60.0													
<i>Scorpaenichthys marmoratus</i>			1																		1	0.1	31.0	5	29.0	60.0													
<i>Sebastes helvomaculatus</i>							1														1	0.1	31.0	5	29.0	60.0													
Total																					1193																		

Table 6. Fish species offered for sale at the Ensenada Seafood Market stalls from the commercial catch in Punta Colonet, Baja California, Mexico (2013-2023). Rel%: relative abundance %; FO%: frequency of occurrence %; R1: Rel% order; R2: FO% order; ICI: Index of Community Importance.

Fish species	Fish stalls	Total	Rel%	R1	FO%	R2	ICI
<i>Bodianus pulcher</i>	14	1080	66.4	1	73.7	1	2
<i>Caulolatilus princeps</i>	11	304	18.7	2	57.9	2	4
<i>Paralabrax nebulifer</i>	8	239	14.7	3	42.2	3	6
<i>Stereolepis gigas</i>	1	3	0.2	4	10.5	4	8
Total		1626	100				

Table 7. Fish species and numbers of individuals from Punta Colonet, Baja California, Mexico, preserved in the Vertebrate Collection at Scripps Institution of Oceanography (SIO), University of California, San Diego (UCSD). Rel%: relative abundance %; UR: unregistered depth

Fish species	Total	Rel%	Depth m	Fish species	Total	Rel%	Depth m
<i>Engraulis mordax</i>	286	52.5	0-16	<i>Sebastes ensifer</i>	2	0.4	101
<i>Genyonemus lineatus</i>	73	13.4	0-12.8	<i>Sebastes rubrivinctus</i>	2	0.4	27
<i>Gibbonsia elegans</i>	20	3.7	0.25	<i>Icelinus quadriseriatus</i>	2	0.4	38-40
<i>Citharichthys xanthostigma</i>	20	3.7	38-40	<i>Syngnathus californiensis</i>	2	0.4	0-16.5
<i>Citharichthys sordidus</i>	18	3.3	38-40	<i>Hypsoblennius jenkinsi</i>	2	0.4	0-16.5
<i>Chromis punctipinis</i>	13	2.4	16.5	<i>Citharichthys fragilis</i>	2	0.4	38-40
<i>Citharichthys stigmaeus</i>	9	1.7	38-40	<i>Pleuronichthys verticalis</i>	2	0.4	38-40
<i>Anaploma fimbria</i>	8	1.5	0-220	<i>Sebastes paucispinis</i>	1	0.2	UR
<i>Sebastes auriculatus</i>	7	1.3	42-90	<i>Sebastes umbrosus</i>	1	0.2	27
<i>Sebastes macdonaldi</i>	7	1.3	16-220	<i>Heterodontus francisci</i>	1	0.2	20-22
<i>Oligocottus snyderi</i>	7	1.3	Surface	<i>Raja binoculata</i>	1	0.2	14.6-22
<i>Sebastes miniatus</i>	6	1.1	220	<i>Paralepis brevis</i>	1	0.2	UR
<i>Sebastes chlorostictus</i>	6	1.1	91	<i>Zalemnius rosaceus</i>	1	0.2	38-40
<i>Clinocottus analis</i>	5	0.9	0.25	<i>Scorpaena mystes</i>	1	0.2	38-40
<i>Sebastes vexillaris</i>	4	0.7	27	<i>Sebastes chrysomelas</i>	1	0.2	27
<i>Chitonotus pugetensis</i>	4	0.7	38-40	<i>Zaniolepis latipinnis</i>	1	0.2	38-40
<i>Hypsoblennius gilberti</i>	4	0.7	0-18	<i>Syngnathus exilis</i>	1	0.2	UR
<i>Symphurus atricaudus</i>	4	0.7	38-40	<i>Rimicola eigenmanni</i>	1	0.2	0.25
<i>Synodus lucioceps</i>	3	0.6	Surface	<i>Chilara taylori</i>	1	0.2	141
<i>Porichthys notatus</i>	3	0.6	38-40	<i>Ophidion scrippsae</i>	1	0.2	141
<i>Gibbonsia metzi</i>	3	0.6	0.25	<i>Apodichthys fucorum</i>	1	0.2	0.25
<i>Sebastes constellatus</i>	2	0.4	27	<i>Hippoglossus stenolepis</i>	1	0.2	UR
<i>Sebastes rosenblatti</i>	2	0.4	192-220	<i>Parophrys vetulus</i>	1	0.2	38-39.5
				Total	544	100.0	

Table 8. Fish species by importance order from the data series of Punta Colonet, Baja California, Mexico. Cruise (Horizon) 1998; Nets: Otter-trawl and gillnet catch 2007–2008; CF: Seasonal commercial catch 2008; SF: Sportfishing catch 2006–2023; SFM: Ensenada Seafood Market 2003–2023; SIO Coll: Preserved fish species at the Vertebrate Collection of the Scripps Institution of Oceanography, University of California at San Diego, California, USA. Rel%: relative abundance %; FO%: frequency of occurrence %; R1: Rel% order; R2: FO% order; ICI: Index of Community Importance

Species	Cruise	Nets	CF	SF	SFM	SIO	Total	Rel%	R1	FO%	R2	ICI
	1998	2007-08	2008	2006-23	2003-23	Coll						
	Sum	Sum	Sum	Sum	Sum	Sum						
<i>Embiotoca jacksoni</i>	108	485					593	8.46	3.0	41.1	1	4
<i>Caulolatilus princeps</i>	15		457	44	304		820	11.70	2.0	33.3	3.5	5.5
<i>Sebastes miniatus</i>	26		223	228		6	483	6.89	4.0	35.6	2	6
<i>Bodianus pulcher</i>	8		33	1	1080		1122	16.01	1.0	26.7	8	9
<i>Micrometrus minimus</i>	5	534					539	7.69	7.0	28.9	5.5	12.5
<i>Hyperprosopon argenteum</i>	23	325					348	4.96	8.0	28.9	5.5	13.5
<i>Paralabrax nebulifer</i>	12	6	146		239		403	5.75	6.0	24.4	9	15
<i>Sebastes auriculatus</i>	15	26	44	28		7	120	1.71	12.0	33.3	3.5	15.5
<i>Seriola lalandi</i>			21	384			405	5.78	5.0	18.9	13.5	18.5
<i>Sebastes paucispinis</i>	23		20	87		1	131	1.87	10.0	18.9	13.5	23.5
<i>Sebastes constellatus</i>	32		26	47		2	107	1.53	13.0	20.0	11	24
<i>Ophiodon elongatus</i>	1		7	120			128	1.83	11.0	18.9	13.5	24.5
<i>Paralichthys californicus</i>		23	44	12			79	1.13	19.0	27.8	7	26
<i>Heterostichus rostratus</i>	1	37					38	0.54	24.0	23.3	10	34
<i>Phanerodon vacca</i>	18	42					60	0.86	21.0	18.9	13.5	34.5
<i>Phanerodon furcatus</i>	19	54					73	1.04	20.0	15.6	17	37
<i>Oxyjulis californica</i>	28	28					56	0.80	22.0	16.7	16	38
<i>Cymatogaster aggregata</i>		92					92	1.31	17.0	8.9	24	41
<i>Sebastes chlorostictus</i>	14		8	23		6	51	0.73	23.0	11.1	18.5	41.5
<i>Genyonemus lineatus</i>		31				73	104	1.48	15.0	7.8	27.5	42.5
<i>Amphistichus koelzi</i>		88					88	1.26	18.0	7.8	27.5	45.5
<i>Sebastes umbrosus</i>	5		9	19		1	34	0.49	26.5	10.0	21	47.5
<i>Sarda chiliensis</i>				34			34	0.49	26.5	10.0	21	47.5
<i>Atherinopsis californiensis</i>	60	41					101	1.44	16.0	5.6	33.5	49.5
<i>Sebastes rastrelliger</i>	1	12		12			25	0.36	31.5	11.1	18.5	50
<i>Girella nigricans</i>	104		2				106	1.51	14.0	4.4	37	51
<i>Embiotoca lateralis</i>	5	20					25	0.36	31.5	10.0	21	52.5
<i>Paralabrax clathratus</i>	11	6		13			30	0.43	29.0	8.9	24	53
<i>Strongylura exilis</i>		37					37	0.53	25.0	4.4	37	62
<i>Sebastes babcocki</i>			5	10			15	0.21	41.0	8.9	24	65
<i>Sebastes caurinus</i>			1	19			20	0.29	35.5	6.7	30.5	66
<i>Sphyaena argentea</i>	8		8	4			20	0.29	35.5	6.7	31	66
<i>Syngnathus leptorhynchus</i>		18					18	0.26	39.0	7.8	27.5	66.5
<i>Sebastes atrovirens</i>	1		4	6			11	0.16	47.5	7.8	27.5	75
<i>Sebastes ovalis</i>	2			12			14	0.20	42.0	5.6	33.5	75.5
<i>Thunnus albacares</i>				33			33	0.47	28.0	2.2	48	76
<i>Sebastes rosenblatti</i>	24					2	26	0.37	30.0	2.2	48	78

Table 8. Cont.

<i>Citharichthys stigmaeus</i>	3			9	12	0.17	45.0	5.6	33.5	78.5	
<i>Scorpaena guttata</i>	2	9	7		18	0.26	39.0	3.3	40.5	79.5	
<i>Sebastes rosaceus</i>	13		10		23	0.33	33.0	2.2	48	81	
<i>Amphistichus argenteus</i>	11				11	0.16	47.5	3.3	40.5	88	
<i>Engraulis mordax</i>				286	286	4.08	9.0	1.1	80	89	
<i>Symphurus atricaudus</i>	4			4	8	0.11	52.5	4.4	37	89.5	
<i>Sebastes serriceps</i>			8		8	0.11	52.5	3.3	40.5	93	
<i>Atractoscion nobilis</i>		12			12	0.17	45.0	2.2	48	93	
<i>Pleuronichthys guttulatus</i>	5				5	0.07	60.0	5.6	33.5	93.5	
<i>Sebastes melanostomus</i>			10		10	0.14	49.0	2.2	48	97	
<i>Sebastes serranoides</i>			8		8	0.11	52.5	2.2	48	100	
<i>Scomber japonicus</i>	6		2		8	0.11	52.5	2.2	48	101	
<i>Thunnus orientalis</i>			3		3	0.04	69.0	3.3	40.5	110	
<i>Stereolepis gigas</i>		1		3	4	0.06	63.5	2.2	48	111	
<i>Gibbonsia elegans</i>					20	20	0.29	35.5	1.1	80	115
<i>Citharichthys xanthostigma</i>					20	20	0.29	35.5	1.1	80	115
<i>Citharichthys sordidus</i>					18	18	0.26	39.0	1.1	80	119
<i>Chromis punctipinis</i>					13	13	0.19	43.0	1.1	80	123
<i>Sebastes lentiginosus</i>	12				12	0.17	45.0	1.1	80	125	
<i>Oxylebius pictus</i>	2				2	0.03	78.5	2.2	48	126	
<i>Halichoeres semicinctus</i>	2				2	0.03	78.5	2.2	48	126	
<i>Sebastes levis</i>	1		1		2	0.03	78.5	2.2	48	126	
<i>Sebastes hopkinsi</i>	8				8	0.11	52.5	1.1	80	132	
<i>Anaploma fimbria</i>					8	8	0.11	52.5	1.1	80	132
<i>Sebastes macdonaldi</i>					7	7	0.10	56.5	1.1	80	136
<i>Oligocottus snyderi</i>					7	7	0.10	56.5	1.1	80	136
<i>Rhacochilus toxotes</i>	6				6	0.09	58.0	1.1	80	138	
<i>Seriphus politus</i>		5			5	0.07	60.0	1.1	80	140	
<i>Clinocotus analis</i>					5	5	0.07	60.0	1.1	80	140
<i>Sebastes vexillaris</i>					4	4	0.06	63.5	1.1	80	144
<i>Chitonotus pugetensis</i>					4	4	0.06	63.5	1.1	80	143
<i>Hypsoblennius gilberti</i>					4	4	0.06	63.5	1.1	80	143
<i>Hypsypops rubicundus</i>	3				3	0.04	69.0	1.1	80	149	
<i>Medialuna californiensis</i>	2	1			3	0.04	69.0	1.1	80	149	
<i>Sebastes goodei</i>			3		3	0.04	69.0	1.1	80	149	
<i>Synodus lucioceps</i>					3	3	0.04	69.0	1.1	80	149
<i>Porichthys notatus</i>					3	3	0.04	69.0	1.1	80	149
<i>Gibbonsia metzi</i>					3	3	0.04	69.0	1.1	80	149
<i>Scorpaenichthys marmoratus</i>	1		1		2	0.03	78.5	1.1	80	158	
<i>Katsuwonus pelamis</i>			2		2	0.03	78.5	1.1	80	158	
<i>Sebastes ensifer</i>					2	2	0.03	78.5	1.1	80	158

Table 8. Cont.

<i>Sebastes rubrivinctus</i>						2	2	0.03	78.5	1.1	80	158.5
<i>Icelinus quadriseriatus</i>						2	2	0.03	78.5	1.1	80	158.5
<i>Syngnathus californiensis</i>						2	2	0.03	78.5	1.1	80	158.5
<i>Hypsoblennius jenkinsi</i>						2	2	0.03	78.5	1.1	80	158.5
<i>Citharichthys fragilis</i>						2	2	0.03	78.5	1.1	80	158.5
<i>Pleuronichthys verticalis</i>						2	2	0.03	78.5	1.1	80	158.5
<i>Haemulon californiensis</i>	1						1	0.01	95.5	1.1	80	175.5
<i>Sebastes elongatus</i>	1						1	0.01	95.5	1.1	80	175.5
<i>Embiotoca caryi</i>	1						1	0.01	95.5	1.1	80	175.5
<i>Trachurus symmetricus</i>		1					1	0.01	95.5	1.1	80	175.5
<i>Sebastes flavidus</i>			1				1	0.01	95.5	1.1	80	175.5
<i>Coryphaena hippurus</i>			1				1	0.01	95.5	1.1	80	175.5
<i>Atractoscion nobilis</i>				1			1	0.01	95.5	1.1	80	175.5
<i>Sebastes helvomaculatus</i>				1			1	0.01	95.5	1.1	80	175.5
<i>Heterodontus francisci</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Raja binoculata</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Paralepis brevis</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Zalembeus rosaceus</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Scorpaena mystes</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Sebastes chrysomelas</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Zaniolepis latipinnis</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Syngnathus exilis</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Rimicola eigenmanni</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Chilara taylori</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Ophidion scrippsae</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Apodichthys fucorum</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Hippoglossus stenolepis</i>						1	1	0.01	95.5	1.1	80	175.5
<i>Parophrys vetulus</i>						1	1	0.01	95.5	1.1	80	175.5
Total		627	1938	1082	1193	1626	544	7010	100			

Conclusion

This study highlights Punta Colonet, Baja California, Mexico, for fish species diversity, with commercial and recreational fishing. This study identified 106 coastal fish species using various methods, including their abundances, frequencies of occurrence, and species importance. These findings, along with other studies, will support decision-makers prior

to port construction. Future studies on fish and invertebrates and their relationship to changing conditions during El Niño and La Niña will be necessary for understanding the dynamics of the bay of Punta Colonet.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures with no animal experiments.

Data availability: The data will be made available upon request from the author.

Funding disclosure: The Oceanology Division of Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE) funded this study through different projects headed by Jorge A. Rosales-Casián in the Marine Ecology Department.

Acknowledgements: Thanks to Milton Love, University of California, Santa Barbara, for his Horizon cruise and Cesar Almeda (CICESE) for help. Thanks to students Rubí Ruz, Anelena Campuzano, and Alejandro Rodríguez for fish sampling, and boat operators Luis Arce and Iván Castro. Humberto Delgadillo in seafood market monitoring, Jorge I. Rosales-Vásquez in sampling and by the Colonet bay map.

Disclosure: -

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Effects of environmental variables on Oligochaeta (Annelida) assemblages in Çardak Lagoon (Turkish Straits System)

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Cite this article as:

Ateş, A.S., Dağlı, E., Acar, S. (2026). Effects of Environmental variables on Oligochaeta (Annelida) assemblages in Çardak Lagoon (Turkish Straits System). *Aquatic Research*, 9(2), 107-117. <https://doi.org/10.3153/AR26010>

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ABSTRACT

This study aimed to investigate the relationships between the abundance of the Oligochaeta assemblages in Çardak Lagoon, an area impacted by domestic pollutants. A total of 3 oligochaeta species [(*Thalassodrilides gurwitschi* (Hrabě, 1971), *Thalassodrilides* sp., and *Oligochaeta* sp.)] were recorded. The peak abundance periods for these communities were determined to be spring and summer. Analysis of the environmental variables across the sampling seasons revealed that the anionic detergent level in the water and the percentage of gravel in the sediment exhibited the highest correlation with overall oligochaete abundance. At the sampling points, the maximum correlation value was recorded specifically between the percentage of organic matter (OM%) in the sediment and the abundance of *Thalassodrilides gurwitschi*.

Keywords: Oligochaeta, Sewage pollution, Çardak lagoon, Turkish straits system

Submitted: 12.11.2025

Revision requested: 26.12.2025

Last revision received: 15.01.2026

Accepted: 02.02.2026

Published online: 27.03.2026

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Available online at

<http://aquatres.scientificwebjournals.com>

Introduction

Oligochaetes constitute one of the major macrozoobenthos groups and play a significant ecological role in aquatic ecosystems. Although marine oligochaetes are frequently encountered in shallow waters, studies investigating the environmental factors influencing these populations remain relatively scarce (Pfannkuche, 1978; Thompson & Shin, 1983; Diaz et al., 1987; Diaz & Erseus, 1994; Gierre, 2006; Lafont & Vivier, 2006; Gamito, 2008; Coelho et al., 2015; Guimarães et al., 2024). The structure of oligochaete communities, which is indicative of aquatic ecosystem health, can be significantly influenced by environmental factors across diverse water bodies, including marine, freshwater, and brackish environments (Guimarães et al., 2024). These ecological variables encompass the availability and quality of food resources, contaminants in the water and sediment, and physicochemical characteristics (Behrend et al., 2012).

Most Oligochaeta species typically inhabit areas where the sediment is rich in organic material. Furthermore, they often displace other macrozoobenthos communities that exhibit a lower tolerance for elevated organic content (Rosa et al., 2014).

Aquatic oligochaetes fulfil a significant ecological function within the food web and in sediment mixing. They also serve as a critical food source for various fish and crustacean species (Guimarães et al., 2024). The particle composition and the level of organic matter in the sediment are primary factors influencing the structure of marine ecosystems. Crucially, the burrowing activity of oligochaetes facilitates the release of accumulated biogenic compounds and pollutants from the sediment into the surrounding water column.

The distribution of marine oligochaetes is significantly influenced by key water quality parameters, including oxygen and salinity concentrations, as well as the presence of hydrogen sulfide (Giere, 2006). Oligochaetes can thrive in sediments rich in organic matter, provided there is sufficient oxygen availability. However, these assemblages are particularly vulnerable to heavy metal contamination (Rodríguez & Reynoldson, 2011). Species recognised as indicators of nutrient-rich environments often exhibit strong tolerance to silty substrates heavily loaded with organic material. Consequently, analysing current oligochaete populations plays a crucial role in assessing the trophic status of water bodies (Chapman et al., 1982).

Anthropogenic pollution has significantly escalated worldwide in marine environments, leading to severe detrimental effects on benthic communities, encompassing both meiofauna and macrofauna. Oligochaetes, a key macrozoobenthic

group, are widely recognised as effective indicators of pollution (Chapman et al., 1982; Abubakr et al., 2018).

These organisms are frequently encountered in surface sediments and thrive in areas characterised by low oxygen levels (Lafont & Vivier, 2006). Marine oligochaetes, notable for their high numbers and large biomass, particularly in nutrient-rich coastal areas, are primarily regarded as indicators of both water quality and sediment contamination (Pfannkuche, 1978).

Sewage pollution constitutes a major challenge for coastal regions. The sludge from household waste, often containing carbon levels between 50% and 70%, serves as a significant nutrient source for oligochaetes. Furthermore, they play a crucial role in the formation of sea floor sediment and substantially influence the mineralisation process within aquatic sediments (Ratsak & Verkuijlen, 2006).

This study investigates the relationships between oligochaete abundance in a contaminated lagoon and various temporal and spatial environmental variables.

Materials and Methods

Samplings

The sampling area was established across seven distinct points (GPS Coordinates: 40 ° 23 ' 14 " N, 26 ° 43 ' 30 " E) within the Çardak Lagoon, located in the northeastern part of the Çanakkale Strait. The sampled depths ranged from 1 to 1.8 meters (Figure 1).

Faunistic Analysis

Oligochaete samples were collected by a SCUBA diver using a 30x30 cm metal-framed quadrat during October 2018, and subsequently in February, April, and June 2019. The samples were preserved in 5 L plastic containers using a 4% neutralized formaldehyde solution. In the laboratory, the sediment was processed by rinsing with pressurised water and passing it through a three-tiered sieve system with mesh sizes of 0.5, 1, and 2 mm. Individuals retained on the sieves were collected (both macro- and micro-levels) and preserved in 70% ethanol in 50 cc glass tubes. All identified oligochaetes were examined and counted under a trinocular stereomicroscope based on classifications from previous studies. Identification relied specifically on the definitions provided by Brinkhurst (1971, 1980, 1982). The relationships between environmental variables and oligochaete populations across the seven distinct sampling sites and four seasonal periods were statistically evaluated using the Pearson correlation coefficient (r) via the PAST software.

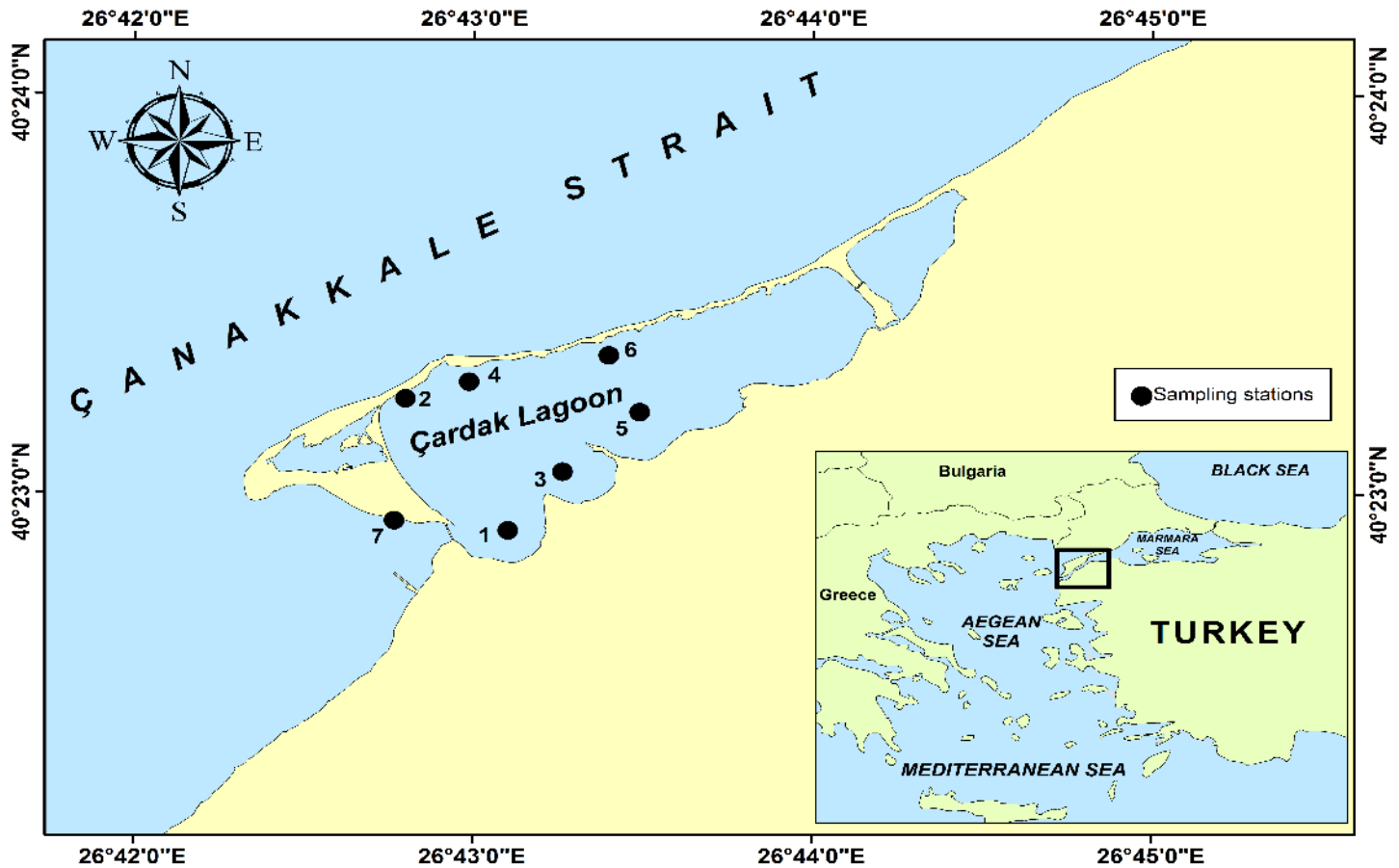


Figure 1. Map of the study area showing the sampling points

Water and Sediment Analyses

Water quality variables of the lagoon water were measured in situ using a YSI 650 MDS multi-parameter device. Nutrient levels (NO_2^- , NO_3^- , NH_4^+ , $\text{PO}_4\text{-P}$, SiO_2) and total suspended solids (TSS) in the lagoon water were determined in the laboratory following the analytical methods established by Strickland and Parsons (1972). These measurements utilised various wavelengths on a Jasco Brand UV spectrophotometer located at the Faculty of Marine Sciences and Technology laboratory. The determination of percentage organic matter and particle content in the sediment was performed at the Central Laboratory of Çanakkale Onsekiz Mart University. Sediment particle size analyses were carried out according to the standards established by Allen (1997).

Results and Discussion

Water and Sediment Variables Data

Environmental conditions in the lagoon varied seasonally. Water temperature ranged from 7.57 to 27.34 °C, peaking at

27.34 °C in June 2019. Surface water salinity fluctuated between 20.17‰ and 24.40‰. The pH levels, spanning 7.78 to 8.56, remained within the typical range for seawater. Dissolved oxygen concentrations in the water varied from 5.90 to 9.51 mg L^{-1} .

Regarding nutrients, ammonium (NH_4^+) concentrations were consistently minimal ($<0.01 \text{ mg L}^{-1}$) across all sampling sites throughout the study period. Phosphate ($\text{PO}_4\text{-P}$) levels ranged from 0.01 to 0.03 mg L^{-1} . Furthermore, total phosphate concentrations varied between 0.02 and 0.17 mg L^{-1} , with the maximum total nitrogen measurement recorded at 0.99 mg L^{-1} .

The maximum $\text{NO}_2^- + \text{NO}_3^-$ concentration (0.195 mg L^{-1}) was recorded during the autumn of 2018. The peak chlorophyll-*a* (Chl-*a*) level observed in the lagoon water (12.85 $\mu\text{g L}^{-1}$) occurred in the summer of 2019, while the highest total suspended solids (TSS level (71.33 mg L^{-1}) was recorded in the winter of 2019. A significant negative linear relationship ($p < 0.001$) was found between Chl-*a* and TSS values. This finding suggests that the suspended load in the lagoon was predominantly of terrestrial rather than phytoplanktonic origin.

Chemical Oxygen Demand (COD) values were higher in autumn and winter compared to other seasons. Conversely, the

concentrations of anionic detergents were lower during autumn and winter. Table 1 presents the average values for all environmental variables throughout the sampling periods.

Table 1. Spatiotemporal variability in water chemistry, nutrients, chlorophyll-*a* (chl-*a*), total suspended solids (TSS), chemical oxygen demand (COD), and anionic surfactant (AS) values measured in the study

Variable	Station	Mean±SD	Min.	Max.	Variable	Station	Mean±SD	Min.	Max.
T (°C)	St. 1	15.59±6.77	8.46	24.75	pH	St. 1	7.97±0.19	7.78	8.20
	St. 2	16.74±8.07	7.86	27.34		St. 2	8.29±0.08	8.18	8.37
	St. 3	15.61±7.21	7.69	25.20		St. 3	8.18±0.09	8.05	8.26
	St. 4	15.53±6.61	7.96	24.07		St. 4	8.32±0.09	8.23	8.43
	St. 5	15.89±7.85	7.57	26.31		St. 5	8.25±0.15	8.07	8.40
	St. 6	16.33±7.88	7.68	26.54		St. 6	8.34±0.20	8.14	8.56
	St. 7, ref.	16.19±7.03	8.72	25.46		St. 7, ref.	8.25±0.09	8.12	8.35
S (‰)	St. 1	21.91±1.69	20.29	23.92	O ₂ (mg L ⁻¹)	St. 1	8.18±1.49	6.11	9.51
	St. 2	22.01±1.37	20.78	23.92		St. 2	7.76±1.19	6.85	9.50
	St. 3	21.79±1.20	20.76	23.00		St. 3	7.21±1.08	6.15	8.17
	St. 4	22.04±1.37	20.77	23.69		St. 4	7.07±1.01	5.99	8.27
	St. 5	22.04±0.88	20.80	22.85		St. 5	7.18±1.12	6.16	8.47
	St. 6	21.87±0.84	20.74	22.74		St. 6	7.08±0.81	5.90	7.69
	St. 7, ref.	22.04±1.93	20.17	24.40		St. 7, ref.	7.76±0.37	7.41	8.20
PO ₄ (mg L ⁻¹)	St. 1	0.02±0.012	0.01	0.03	NH ₄ ⁺ (mg L ⁻¹)	St. 1	0.01±0.00	0.01	0.01
	St. 2	0.015±0.006	0.01	0.02		St. 2	0.01±0.00	0.01	0.01
	St. 3	0.013±0.005	0.01	0.02		St. 3	0.01±0.00	0.01	0.01
	St. 4	0.015±0.006	0.01	0.02		St. 4	0.01±0.00	0.01	0.01
	St. 5	0.01±0.00	0.01	0.01		St. 5	0.01±0.00	0.01	0.01
	St. 6	0.015±0.01	0.01	0.03		St. 6	0.01±0.00	0.01	0.01
	St. 7, ref.	0.01±0.00	0.01	0.01		St. 7, ref.	0.01±0.00	0.01	0.01
TP (mg L ⁻¹)	St. 1	0.048±0.026	0.02	0.07	TN (mg L ⁻¹)	St. 1	0.480±0.206	0.30	0.74
	St. 2	0.029±0.013	0.02	0.04		St. 2	0.230±0.126	0.10	0.37
	St. 3	0.026±0.009	0.02	0.04		St. 3	0.427±0.170	0.25	0.66
	St. 4	0.035±0.018	0.02	0.05		St. 4	0.162±0.047	0.10	0.20
	St. 5	0.051±0.066	0.02	0.15		St. 5	0.498±0.379	0.20	0.99
	St. 6	0.065±0.073	0.02	0.17		St. 6	0.498±0.379	0.20	0.99
	St. 7, ref.	0.052±0.072	0.02	0.16		St. 7, ref.	0.262±0.075	0.20	0.35
NO ₂ +NO ₃ (mg L ⁻¹)	St. 1	0.083±0.04	0.04	0.13	SiO ₂ (mg L ⁻¹)	St. 1	0.367±0.241	0.15	0.60
	St. 2	0.071±0.059	0.04	0.13		St. 2	0.563±0.442	0.20	1.20
	St. 3	0.071±0.059	0.02	0.13		St. 3	0.292±0.225	0.05	0.55
	St. 4	0.036±0.02	0.01	0.06		St. 4	0.575±0.561	0.20	1.40
	St. 5	0.088±0.074	0.03	0.20		St. 5	0.555±0.632	0.17	1.50
	St. 6	0.088±0.063	0.03	0.18		St. 6	0.186±0.156	0.02	0.40
	St. 7, ref.	0.094±0.041	0.05	0.14		St. 7, ref.	0.487±0.477	0.20	1.20
Chl- <i>a</i> (µg L ⁻¹)	St. 1	3.64±3.84	1.61	9.39	TSS (mg L ⁻¹)	St. 1	11.00±4.62	6.80	17.60
	St. 2	2.64±1.52	1.06	3.96		St. 2	8.07±2.74	4.30	10.20
	St. 3	3.39±2.27	1.09	5.75		St. 3	22.1±22.5	3.80	54.40
	St. 4	2.96±2.26	0.97	6.19		St. 4	9.35±4.85	4.00	15.60
	St. 5	2.59±2.13	1.31	5.76		St. 5	19.1±24.8	3.20	56.00
	St. 6	4.47±5.61	1.07	12.85		St. 6	28.4±29.7	7.20	71.30
	St. 7, ref.	1.49±0.44	1.05	1.98		St. 7, ref.	15.98±8.36	7.70	24.40
COD (mg L ⁻¹)	St. 1	127.9±52	76.00	198	AS (mg L ⁻¹)	St. 1	0.045±0.022	0.02	0.07
	St. 2	152.1±102.8	76.00	295		St. 2	0.027±0.016	0.02	0.05
	St. 3	105.0±55.2	40.00	158		St. 3	0.032±0.014	0.02	0.05
	St. 4	87.5±64.1	40.00	181		St. 4	0.040±0.014	0.02	0.05
	St. 5	120.5±111.8	40.00	277		St. 5	0.034±0.011	0.02	0.05
	St. 7, ref.	101.5±79.6	40.00	207		St. 7, ref.	0.032±0.016	0.02	0.05

Sediment analysis in the sampling area revealed a composition dominated by sand (71.59%), followed by gravel and shell content (20.16%), and mud (encompassing both clay and silt) at 8.19%. The highest proportion of sand (92%) was recorded at Station 6. Conversely, Station 5 exhibited the maximum levels of mud (16.71%) and gravel/shell content (25.64%) (Table 2).

Regarding the organic matter content in the sediment, the peak measurement (16.88%) occurred at Station 3 during both the spring and summer sampling periods. The reference station consistently showed the lowest average organic matter content, recording 1.79% (Table 3). Seasonal variations were also observed in the water column: the maximum organic matter concentration in water (14.6 mg L⁻¹) was recorded in winter at Station 2. Conversely, the minimum concentration (9.6 mg L⁻¹) was noted in spring at Stations 2, 5, and 7. (Seasonal variations in sediment organic matter are further detailed in Table 3).

Faunistic Data

A total of 937 oligochaete specimens, encompassing three species (*Thalassodrilides gurwitschi* (Hrabě, 1971), *Thalassodrilides* sp., and *Oligochaeta* sp.), were recorded in Çardak Lagoon between autumn 2018 and summer 2019. *Oligochaeta* sp. was identified as the most dominant species in the study area, exhibiting a density of 886 ind. 0.09 m⁻². This was followed by *Thalassodrilides* sp., from which 42 specimens were recorded (Table 4). The highest abundance of *Oligochaeta* sp. (616 ind. 0.09 m⁻²) was recorded at the reference site, which was characterised by the lowest sediment organic matter content (1.73%). Temporally, spring exhibited the maximum number of *Oligochaeta* sp. individuals (448 ind. 0.09 m⁻²). Conversely, the marine oligochaete species *T. gurwitschi* was the least frequently encountered throughout the entire study, with only 9 individuals sampled across all periods.

Table 2. Mean seasonal percent granulometry ratios recorded at stations

Factor	Clay+silt	Particle type %						
		Very fine sand	Fine sand	Medium sand	Coarse sand	Very coarse sand	Gravel and shell	Coarse gravel and shell
Stn, 1	14.16	13.89	18.04	9.43	8.91	11.46	14.74	9.3
Stn, 2	3.17	10.96	15.93	19.57	16.49	15.72	10.47	7.65
Stn, 3	13.24	7.25	7.92	9.85	12.82	17.3	19.72	11.83
Stn, 4	5.27	20.08	43.68	7.1	8.91	6.42	4.12	4.36
Stn, 5	16.71	23.66	9.27	7.17	7.75	9.76	11.85	13.79
Stn, 6	3.29	13.67	43.94	25.06	6.16	3.91	2.59	1.38
Stn, 7, ref,	1.49	3.1	15.26	17.55	15.88	17.3	16.11	13.25
Mean	8.19±6.27	13.23±7.06	22.00±15.32	13.67±4.04	10.98±4.08	11.69±5.34	11.37±6.24	8.79±4.65

Table 3. Seasonal values of organic matter content in water and sediment. WOM: Organic matter in water, SOM: Organic matter in sediment

Sampling point	Sampling period							
	Autumn 18		Winter 19		Spring 19		Summer 19	
	WOM (mg L ⁻¹)	SOM (%)	WOM (mg L ⁻¹)	SOM (%)	WOM (mg L ⁻¹)	SOM (%)	WOM (mg L ⁻¹)	SOM (%)
St. 1	11	11,43	11,6	8,9	10,6	10,14	11,2	12,14
St. 2	10,8	2,73	14,6	3,99	9,6	3,6	11,6	1,82
St. 3	11,1	14,21	12,8	14,13	11,4	16,88	11,2	16,88
St. 4	11,2	4,38	10,6	2,97	10	4,08	11,6	2,56
St. 5	11	7,76	12,2	7,53	9,6	6,76	12	9,6
St. 6	10,9	2,69	12,4	2,94	10	2,73	11,4	2,64
St. Ref.	11,2	1,76	11,2	1,4	9,6	2,1	11,4	1,69

Table 4. The number of individuals of oligochaeta species by sampling periods and stations. Σ : Total abundance, Di%: Dominance.

OLIGOCHAETA	St. 1	St.2	St.3	St. 4	St. 5	St. 6	Ref.	Aut. 18	Win. 19	Spr. 19	Sum. 19	Σ	Di%
<i>Thalassodrilides gurwitschi</i>	0	0	8	0	0	1	0	0	0	9	0	9	0,96
<i>Thalassodrilides</i> sp.	17	0	0	0	0	15	10	13	5	24	0	42	4,48
<i>Oligochaeta</i> sp.	0	171	39	15	6	39	616	41	151	448	246	886	94,55
Total	17	171	47	15	6	55	626	54	156	481	246	937	

Temporal and Spatial Correlations Between Environmental Factors and Oligochaeta Abundance in the Study Area

Correlation analysis revealed several strong and statistically significant relationships between oligochaeta abundance and environmental parameters. *Thalassodrilides gurwitschi* abundance exhibited the most robust seasonal positive correlation ($r=0.98$; $p < 0.05$) among all variables examined, specifically with the anionic detergent level in the water. Across all sampling periods, its abundance also demonstrated a significant positive correlation ($r=0.75$; $p < 0.05$) with the percentage of organic matter in the sediment. Similarly, the abundance of *Oligochaeta* sp., the most dominant species in the study area, showed strong and statistically significant positive correlations with both the anionic detergent concentration in water ($r=0.95$; $p < 0.05$) and the percentage of gravel in the sediment ($r=0.96$; $p < 0.05$) (Fig. 2, 3).

Correlations With Environmental Variables for Abundance

The Principal Component Analysis (PCA) of the oligochaeta data visualises stations as blue dots and environmental and biological variables as red arrows. Together, PC1 and PC2 account for 50.5% of the total variance. The *Oligochaeta* sp. vector extends towards high organic matter (OM%, WOM) and fine-grained sediment (Mud%), reflecting its tolerance to organic pollution; conversely, *Thalassodrilides gurwitschi* and *Thalassodrilides* sp. exhibit sensitivity to more oxygenated habitats with lower organic content. PC1 (28.3%) represents the sediment and trophic conditions, while PC2 (22.2%) reflects temperature and oxygen dynamics. The distribution of the stations along these two axes indicates the segregation of species according to the environmental stress gradient (Figure 4).

This table clearly demonstrates that the species occupy distinct ecological niches within the lagoon environment; *Thalassodrilides gurwitschi*: Indicator of organically rich but low-stress habitats, *Thalassodrilides* sp.: Adapted to nutrient-rich and well-oxygenated environments, *Oligochaeta* sp.: Tolerant species, resilient to increased organic load and salinity. These relationships quantitatively support the species–envi-

ronment segregation observed in the PCA analysis and confirm that gradients of organic matter, salinity, and nutrients are the primary drivers shaping the benthic community composition within the lagoon system.

Considering the sampling stations, *T. gurwitschi* preferentially inhabits sediments rich in organic matter but moderately oxygenated. *Thalassodrilides* sp. thrives in areas characterised by high nutrient enrichment (verging on eutrophication). Conversely, *Oligochaeta* sp. is capable of tolerating very high levels of organic matter, yet its optimum density is observed under intermediate organic conditions. As for the sampling seasons, *T. gurwitschi* preferentially inhabits coarse-grained, oxygenated, and detritus-rich sediments, whereas *Thalassodrilides* sp. is tolerant of habitats that are organically rich, highly nutrient-loaded, and characterised by temperate temperatures. Conversely, *Oligochaeta* sp. is adapted to coarse-grained, low-oxygen, and organic matter-rich environments.

The most recent literature concerning the ecology and biology of marine oligochaetes was extensively analysed by Giere (2006). In lagoon and estuarine environments subjected to pollution, the increase in oligochaeta abundance coupled with the decline in pollution-sensitive polychaete species has become increasingly pronounced (Díaz, 1980). However, oligochaetes are not universal indicators; therefore, other taxa must be concurrently considered to obtain comprehensive information on the ecological quality of aquatic environments (Lafont & Vivier, 2006). Oligochaetes significantly influence sediment properties—biologically, chemically, and physically—through bioturbation, which facilitates mineralisation and organic matter decomposition (Ito et al., 2016).

Although the organic matter content in the sediment is a crucial environmental variable, it is important to note that oligochaetes exhibit a stronger correlation with the organic matter in polluted sediments. Elevated organic matter in aquatic environments often leads to anoxic conditions. This restriction inhibits the growth of filter-feeding species, such as mussels, resulting in a shift in species composition where bivalves are

typically replaced by polychaetes and oligochaetes (Coelho et al., 2015).

Considering the aquatic oxygen level, oligochaete species are highly tolerant to low dissolved oxygen levels, enabling them to inhabit polluted sediments where they can constitute nearly 100% of the macrozoobenthos in terms of both number and biomass (Sowa & Krodkiewska, 2020). While most aquatic oligochaetes are highly sensitive to oxygen depletion and

consequently inhabit cleaner and/or colder water bodies, certain observations indicate their presence in deeper, anoxic sections of muddy bottoms, where they engage in feeding and predator avoidance. The distribution of oligochaetes is therefore primarily dictated by the availability of dissolved oxygen in and on the sediment. Consistent with this tolerance, our study also revealed weak and negative relationships between water oxygen levels and overall oligochaete abundance.

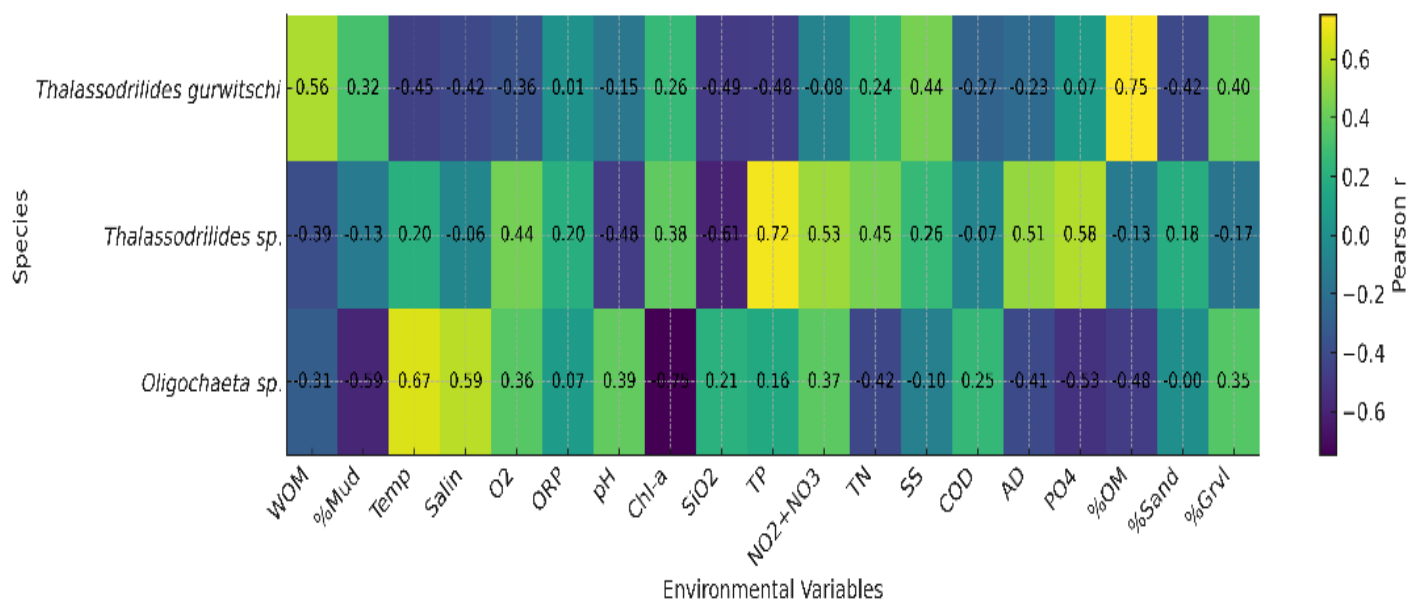
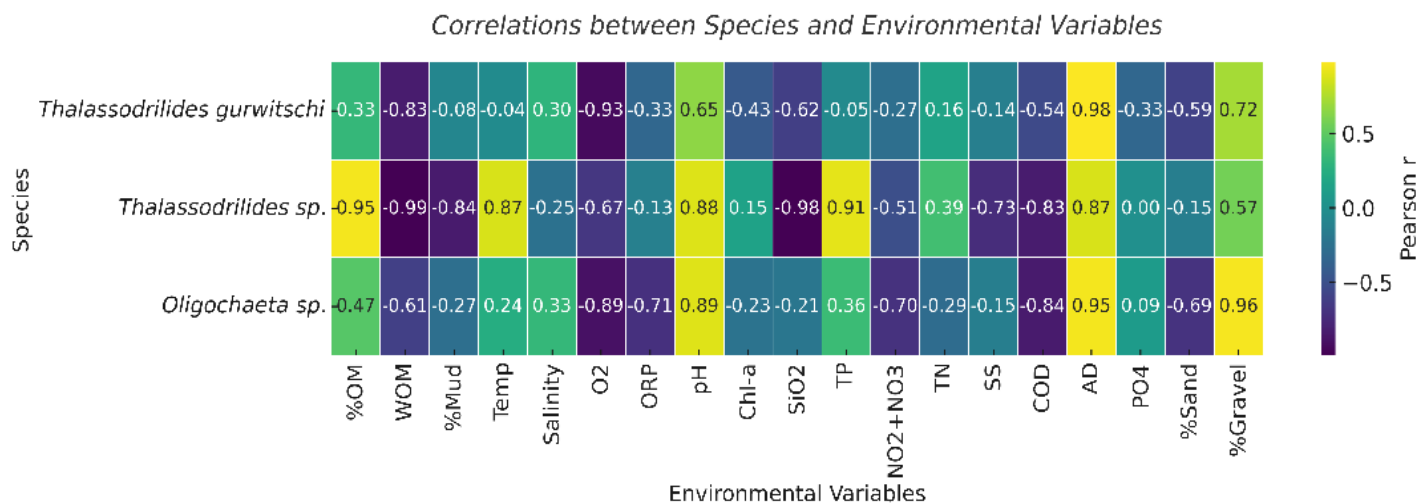


Figure 2. Diagram of heat correlation between oligochaeta abundance and environmental variables based on sampling points



WOM: Organic matter in water, OM: Organic matter in sediment, O₂: Oxygen, ORP: Oxygen reduction potential, Chl-*a*: Chlorophyll-*a*, TP: total phosphate, TN: Total nitrogen, SS: Suspend solids, SiO₂: Silicate, COD: Chemical oxygen demand, AD: Anionic detergent, PO₄: Fosfat, Grvl%: Gravel.

Figure 3. Diagram of heat correlation between oligochaeta abundance and environmental variables based on sampling seasons

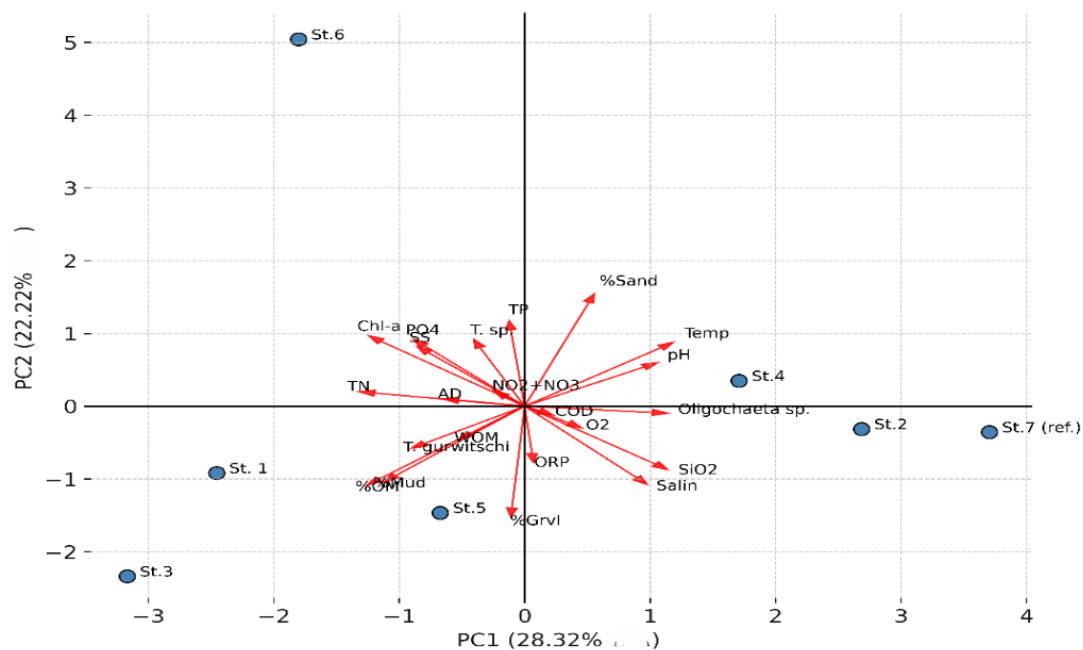


Figure 4. PCA ordination diagram for spatial mean environmental variables and abundance

The average abundance of several freshwater oligochaetes is positively correlated with the concentrations of various pollutants in the water column, including dissolved organic matter, ammonium, and phosphate (Rashid & Pandit, 2014). Specifically, freshwater *Limnodrilus* species are recognised as the most tolerant oligochaetes to organic pollution (Abubakar et al., 2018). Among these, *Limnodrilus hoffmeisteri* is known as an indicator of severe organic matter pollution in freshwater environments (Brinkhurst, 1980; Uzunov et al., 1988).

In this study, strong correlations were observed between the abundance of the marine oligochaete, *Thalassodrilides* species and sedimentary organic matter levels, which varied with station and season. Conversely, the abundance of *Oligochaeta* sp., which was the most abundant species recorded, demonstrated weak and negative correlations with the organic matter content. Based on these findings regarding organic matter, the *Thalassodrilides* species can be proposed as an effective indicator of organic matter pollution in this study area. In our study, the marine oligochaete *Thalassodrilides gurwitschi* was recorded in low numbers, which is consistent with its generally low prevalence in marine and brackish water environments (Torii et al., 2016). However, *T. gurwitschi* was previously identified by Thompson and Shin (1983) as one of the most dominant opportunistic species in Victoria Harbour (Hong Kong), particularly in bottoms where silt content exceeded 70%. Similarly, in the study area, the maximum

number of *T. gurwitschi* individuals was encountered in bottom sediments characterised by a high silt and clay content (13.24%). Stoner and Acevedo (1990) also documented *T. gurwitschi* as the most dominant (2916 ind.m⁻²) species in Joyuda Lagoon (western Puerto Rico).

Many oligochaete species exhibit wide thermal tolerance, surviving both low (around 0 °C) and high (above 20 °C) temperatures (Timm & Martin, 2015). Research on oligochaete population structure, particularly by Japanese scientists, including Kuniyasu et al. (1997) and Inamori et al. (1983), has examined the effects of water temperature, pH, and phosphate concentration on the population growth of freshwater species. Oligochaetes are typically found in environments characterized by elevated nutrient levels and chlorophyll-*a* content (Çelik et al., 2010). Furthermore, oligochaete abundance peaked during the spring and summer periods when the surface water temperature was elevated. In our study, although weak and negative correlations were observed between oligochaete abundance and the water variables of phosphate and temperature, strong seasonal positive correlations were recorded with pH levels.

Regarding oligochaete communities in the Mediterranean Sea, Casellato (1994) conducted a study on the oligochaete fauna, including *T. gurwitschi*, distributed across various lagoons and brackish water areas of the Po Delta (Northern

Adriatic). Furthermore, several studies have investigated oligochaete communities and their relationships with environmental variables in brackish and lagoon areas off the coast of Portugal (Gamito, 2008; Silva et al., 2012; Coelho et al., 2015). Among these studies, Gamito (2008) noted that the most heavily polluted bottoms of the Ria Formosa Lagoon (Southern Portugal, Western Mediterranean) were dominated by oligochaetes, particularly tubificids. In the Arade estuary, another aquatic area in Southern Portugal, Silva et al. (2012) documented high abundances of both *Capitella* spp. and oligochaetes associated with freshwater discharges. Furthermore, in the Salgado Lagoon (Southern Portugal), Coelho et al. (2015) established that oligochaete abundance exhibited a positive correlation with sediment phosphorus content, clay content, and chlorophyll *a* concentration. Conversely, despite the high concentration of chlorophyll-*a* in the water, particularly during the spring, we observed weak and negative correlations between Chl-*a* levels and oligochaete abundance recorded in the lagoon area.

While studies conducted in Türkiye on this subject have primarily focused on freshwater habitats, there remain limited investigations carried out in marine coastal areas such as lagoons and estuaries (Çınar et al., 2011; Aydın et al., 2022). Specifically, Çınar et al. (2011) reported five oligochaete species from depths ranging from 0 to 66 m in the Sea of Marmara; notably, *T. gurwitschi*, which was also recorded in the present study, was found at depths between 0 and 25 m. In another recent study within the Turkish Strait System, Aydın et al. (2022) documented oligochaetes exhibiting a dominance of 19.7% in Küçükçekmece Lagoon. More recently, Çınar et al. (2024) reported a total of 18 oligochaete species from Turkish seas and lagoons, noting that 11 of these species were distributed in the Aegean Sea. The same authors highlighted that no marine oligochaete species had been observed on the Turkish Mediterranean coast to date.

In the Enez Lagoon (northeastern Aegean Sea), which is in proximity to the present study area, Güher et al. (2005) examined the seasonal limnological characteristics. They analysed the faunal components in relation to environmental variables. As a result of their description of the benthic communities in Enez Lagoon, they concluded that the area exhibits a mesotrophic character.

While the organic matter content in the water was highly consistent across the study area, the percentage of organic matter in the sediment varied considerably. Sediment samples collected near domestic discharge points exhibited elevated organic matter levels. Specifically, *Oligochaeta* sp. was the most dominant species at Station 7, the site characterised by

the lowest sediment organic matter and silt/clay content. Similarly, *Oligochaeta* sp. was dominant at Stations 2 and 6, where the combined organic matter and silt/clay content remained low (3%).

In the present study, the primary environmental variables determining oligochaete abundance across all sampling seasons were the anionic detergent level in the water and the percentage of sediment gravel. Furthermore, the percentage of organic matter, a well-established sediment variable influencing oligochaete distribution, was specifically found to affect the abundance of two *Thalassodrilides* species significantly.

Conclusion

The specific distribution and high abundance of *Oligochaeta* sp. in low-organic sediment, contrasted by the sensitivity of *Thalassodrilides* species to organic matter, highlight the need for species-specific analysis when assessing the ecological status of coastal lagoons. Future studies should focus on the chronic effects of anionic detergents and gravel content on benthic fauna in brackish water environments. The results of our study partially corroborate findings reported in various brackish, deltaic, and lagoon systems across the Mediterranean and global geographies concerning oligochaete distribution patterns and the relationships between their abundance and environmental variables. Çardak Lagoon, due to its semi-restricted nature (or slightly closed characteristic), appears to be less severely impacted by anthropogenic effects. However, the lagoon is subjected to multiple environmental stressors that influence the structure of the existing oligochaete community. The community's response to this stress is indicated by low densities, particularly at coastal sampling points where sediment pollution was observed. Therefore, further analysis of oligochaete communities in relation to environmental variables is crucial for gaining detailed insight into the ecosystem functioning of this lagoon area.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures, with no animal experiments.

Data availability: The data will be made available upon request from the author.

Funding disclosure: This study was derived from the results of the COST Action Project 117Y510, supported by TÜBİTAK.

Acknowledgements: -

Disclosure: -

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Impact of *Aloe vera* gel and patchouli essential oil-based edible coatings on the shelf life and quality of trout fillets

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Cite this article as:

Oğuzhan Yıldız, P. (2026). Impact of *Aloe vera* gel and patchouli essential oil-based edible coatings on the shelf life and quality of trout fillets. *Aquatic Research*, 9(2), 118–128. <https://doi.org/10.3153/AR26011>

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Submitted: 17.12.2025

Revision requested: 06.01.2026

Last revision received: 02.02.2026

Accepted: 05.02.2026

Published online: 30.03.2026

ABSTRACT

In this study, the effectiveness of edible coatings containing *Aloe vera* gel (AVG) and patchouli oil (PY) on the quality preservation of trout (*Oncorhynchus mykiss*) fillets during cold storage ($4\pm 1^\circ\text{C}$, 12 days) was investigated. Experimental groups were composed of uncoated control (K0), AVG-coated (K1), AVG+PY-coated (1%; K2) and AVG+PY-coated (2%; K3) samples. Microbiological (total mesophilic bacteria, total psychrotrophic bacteria, yeast and mould), chemical (pH, TVB-N, TBARS), and sensory analyses were performed to evaluate quality changes during storage. The results demonstrated that AVG+PY coatings significantly reduced lipid oxidation, as evidenced by lower TBARS values compared to the control group. TVB-N levels also remained lower in coated samples, indicating delayed spoilage. Overall, the synergistic combination of AVG and PY proved effective in slowing microbial growth and oxidative deterioration, thereby enhancing the storage stability of trout fillets. Stability was maintained up to 12 days under refrigerated storage, ensuring acceptable quality throughout the period. These findings highlight the potential of AVG+PY coatings as a natural, eco-friendly, and functional alternative to synthetic preservatives in aquatic product preservation.

Keywords: *Aloe vera* gel, Patchouli oil, Trout fillet, Edible coating, Shelf life

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Introduction

Fish and fish products constitute an essential component of the human diet owing to their richness in high-quality proteins, omega-3 fatty acids, vitamins, and minerals. Nevertheless, their high water activity, neutral pH, and nutrient-dense composition render them highly vulnerable to microbial proliferation and oxidative spoilage. This susceptibility significantly restricts the shelf life of fish fillets and often results in quality deterioration before reaching consumers. Consequently, there has been a growing interest in natural and safe preservation approaches to extend the shelf life of fish products (Mei et al., 2019; Aberoumand et al., 2024; Liu et al., 2024; Mozzon et al., 2024; Noreen et al., 2025), with edible coatings emerging as a promising strategy to enhance the storage stability of seafood by forming a protective barrier against microbial contamination and oxidative reactions (Usman et al., 2025).

Aloe vera, a member of the Liliaceae family and the most commercially important species of the *Aloe* genus, has attracted considerable attention in this context. Its fleshy leaves contain a viscous mucilaginous gel rich in approximately 75 nutrients and more than 200 bioactive compounds, including carbohydrates, amino acids, saponins, enzymes, vitamins, minerals, lignin, and salicylic acid (Basmatker et al., 2011; Thy et al., 2023; Sharma & Kaur, 2025). This diverse biochemical composition endows *Aloe vera* gel (AVG) with notable antioxidant, antimicrobial, and humectant properties, which have been widely exploited in medical, cosmetic, and food applications. In food preservation, *Aloe vera* gel (AVG) has been extensively investigated as an edible coating material in various products, including fruits, vegetables, poultry, and seafood, due to its ability to reduce water loss, maintain textural integrity, delay oxidative reactions, and inhibit microbial growth (Ali et al., 2021; Sarker & Grift, 2021; Al-Hijazeen & Ibrahim, 2024; Waris & Pilavtepe-Celik, 2025). Despite these multifunctional properties, most studies have focused on AVG alone, while research combining AVG with essential oils remains limited. Compared to other essential oils such as thyme, clove, and peppermint, which have been extensively studied in seafood preservation, patchouli oil remains relatively underexplored in this context. In particular, investigations involving patchouli oil in seafood preservation are lacking, highlighting a gap in the literature and the need to explore potential synergistic effects. Patchouli oil, derived from the leaves of *Pogostemon cablin*, is a natural essential oil traditionally used in medicine, cosmetics, aromatherapy, and food applications. Its chemical profile is dominated by sesquiterpenes such as patchoulol, which confer strong antimicrobial, antifungal, and antioxidant activities. These bioactive properties make patchouli oil a promising candidate for

food preservation, as it can suppress microbial growth and mitigate oxidative deterioration in perishable products (Beşirik & Göger, 2023; Mrisho et al., 2025). Unlike other essential oils widely applied in seafood preservation, patchouli oil offers a distinctive sesquiterpene-rich composition and has been scarcely investigated in food systems. Therefore, the present study introduces patchouli oil as a novel candidate in combination with *Aloe vera* gel, aiming to provide new scientific insights into their synergistic antimicrobial and antioxidant effects, while also offering a practical, environmentally friendly preservation strategy for seafood products.

Accordingly, the present study aims to evaluate the effectiveness of *Aloe vera* gel combined with patchouli oil as an edible coating for rainbow trout fillets. The research focuses on the coating's ability to inhibit microbial growth, reduce oxidative spoilage, and preserve sensory attributes during refrigerated storage. Rainbow trout fillets typically exhibit a short shelf life under cold storage due to their high water activity and susceptibility to microbial and oxidative deterioration. Therefore, extending the shelf life of this perishable product is of great importance for ensuring food safety, reducing economic losses, and meeting consumer demand for fresh and high-quality seafood. By addressing this gap, the study seeks to contribute to the development of natural and environmentally friendly preservation strategies for seafood.

Materials and Methods

Material

Sixty rainbow trout (*Oncorhynchus mykiss*) fillets, averaging 250 ± 10 g in weight and 27.0 ± 1.2 cm in length, were purchased skin-on from the Faculty of Fisheries at Atatürk University (Erzurum, Türkiye) and transported to the laboratory under cold-chain conditions. *Aloe vera* leaves were obtained from three-year-old plants harvested at maturity from a local herbal supplier in Erzurum, Türkiye. On average, 2.5–3.0 kg of leaves were used, each measuring approximately 50 cm in length and 7 cm in width, and the gel was extracted manually. Although *Aloe vera* gel is known to contain polysaccharides, amino acids, vitamins, minerals, and phenolic compounds that confer antioxidant, antimicrobial, and humectant properties (Nicolau-Lapeña et al., 2021), detailed compositional analyses were not performed in this study. Patchouli oil ($\geq 98\%$ purity; Aksuvital, Türkiye), rich in sesquiterpenes such as patchoulol, was obtained from a commercial supplier. It is recognised for its antimicrobial and antioxidant activities (Isnain et al., 2025; Taupik et al., 2025), but its physical, chemical, and functional properties were not analysed within the scope of this work.

Method

Preparation of Aloe Vera Gel and Coating Solutions

Aloe vera gel was extracted following the procedure described by Chin et al. (2017) with slight modifications. Fresh leaves from three-year-old plants were washed, peeled, and the inner gel was collected. The gel was homogenised using a blender, filtered through muslin cloth to remove fibrous material, and stored at 4 °C until use. For coating preparation, *Aloe vera* gel was used at a concentration of 25% (w/v). Glycerol was added at 0.7% (w/v) as a plasticiser, corresponding to a ratio of 25:0.7 (AVG: glycerol). The mixture was stirred for 30 minutes to obtain a homogeneous base solution.

To prepare the coating formulations, patchouli oil (PY) was incorporated into the *Aloe vera* gel base at two concentrations (1% and 2%, v/v) to evaluate its antimicrobial and antioxidant effectiveness. Prior to mixing, the essential oil was emulsified with 0.25% (v/v) Tween 80 to ensure uniform dispersion. The emulsified oil was then blended with the AVG solution using a high-speed homogeniser at 12,000 rpm for 5 minutes to obtain a homogeneous mixture. The final coating formulations, therefore, contained 25% (w/v) *Aloe vera* gel, 0.7% (w/v) glycerol, 0.25% (v/v) Tween 80, and patchouli oil at either 1% or 2% (v/v).

Fillets were obtained from independent fish and randomly assigned to four experimental groups, each containing 15 fillets:

KO: Control (uncoated fillets)

K1: AVG coating

K2: AVG + 1% PY coating

K3: AVG + 2% PY coating

For coating, trout fillets were immersed in the respective solutions for 2 min to ensure uniform coverage, drained for 5 min to remove excess coating, and air-dried at room temperature for about 15 min on each side to allow film formation. The fillets were then placed in polyethylene containers, tightly sealed with lids to minimise exposure to air and microbial contamination, and stored at 4 ± 1 °C for a period of 12 days. Analyses were conducted on days 0, 3, 6, 9, and 12 of storage, with each measurement performed in triplicate (n = 3).

Chemical Analyzes

For pH measurement, 5 g of trout fillet was homogenised with 50 mL of distilled water using an Ultra-Turrax, and values were recorded with a pH meter (Yavuzer, 2018). Lipid oxida-

tion was determined as thiobarbituric acid reactive substances (TBARS) following Malle & Poumeyrol (1989), and results were expressed as µmol MDA/kg. Total volatile basic nitrogen (TVB-N) was analysed according to Malle & Tao (1987) and expressed as mg/100 g.

Microbiological Analysis

For microbiological evaluation, 10 g of trout fillet was aseptically transferred into sterile stomacher bags containing 90 ml of 0.1% buffered peptone water and homogenised for 2 minutes. Serial dilutions were prepared and spread on selective agar media using the spread plate method. Total mesophilic aerobic bacteria (TMAB) were enumerated after incubation at 37 °C for 2 days. Total psychrotrophic bacteria (TPB) were determined by incubation at 7 °C for 10 days. Yeast and mould (TYM) counts were obtained after incubation at 25 °C for 5 days (Halkman, 2005).

Sensory Analysis

A panel of five trained assessors carried out sensory evaluation of trout fillets. Samples were judged using a five-point hedonic scale, where 1 corresponded to “extremely undesirable” and 5 to “extremely desirable,” with texture, colour, odour, and overall acceptability taken into account (adapted from Çoban et al., 2018). In this study, no strict cut-off value was established for sensory acceptability; instead, mean panel scores were used as the basis for interpretation, and values greater than 3 (“moderately desirable”) were considered indicative of acceptable quality during storage. All sensory procedures followed internationally recognised standards, ISO 8586 for panelist selection and training, and ISO 13299 for sensory analysis methodology, ensuring consistency and reproducibility of the evaluation.

Statistical Analysis

All statistical procedures were performed using the SPSS software package (version 27.0, IBM Corp., Armonk, NY, USA). Differences among treatments (KO, K1, K2, K3) and storage periods (0, 3, 6, 9, 12 days), as well as their interaction, were tested for significance at $\alpha = 0.05$ using two-way analysis of variance (ANOVA). When significant differences were detected, Duncan’s multiple range test was applied for post hoc comparisons. Results are expressed as mean ± standard deviation (SD).

Results and Discussion

Microbial Analysis Results

Edible coatings prepared with *Aloe vera* gel and patchouli oil at different concentrations, along with the microbial changes

observed during storage of coated trout fillets, are presented in Table 1. In the control group (KO), TMAB values increased markedly during storage. While the AVG coating (K1) slightly slowed microbial growth, the AVG+1% PY (K2) and, particularly, the AVG+2% PY (K3) coatings provided more effective microbial suppression. At the end of day 5, the TMAB value was 5.26 log cfu/g in the K3 group, compared to 7.11 log cfu/g in the control group. For seafood, the microbiological acceptability limit for total mesophilic aerobic bacteria (TMAB) was considered as 7 log cfu/g, in accordance with the criteria reported by Genç & Kayhan (2023). The control group reached this limit at day 12, whereas all coated groups remained below this threshold throughout the storage period. The findings indicate that the coating containing 2% patchouli oil (K3) significantly suppressed the growth of total mesophilic aerobic bacteria (TMAB) compared to the control group. These results highlight the potential of plant-derived antimicrobial compounds to extend the shelf life of fish products effectively. The film-forming capacity of *Aloe vera* gel reduces oxygen permeability on the food surface, thereby limiting microbial proliferation. In parallel, phenolic compounds and terpenoids present in patchouli oil compromise cell membrane integrity and inhibit bacterial metabolism. This synergistic interaction enhances the overall antimicrobial efficacy of the coatings. Our findings are consistent with previous reports where *Aloe vera*-based edible coatings containing essential oil nanoemulsions significantly reduced microbial counts and extended the shelf life of trout fillets (Jamali et al., 2023). Patchouli oil, rich in sesquiterpenes, has been reported to exhibit strong antimicrobial and antioxidant activities, similar to ginger oil, which has also been extensively documented for its preservative potential (Avci et al., 2020; Beşirik & Göger, 2023; Ayustaningwarno et al., 2024; Mrisho et al., 2025).

Total psychrotrophic bacteria counts increased progressively during cold storage in all groups, but the rate of growth was markedly different depending on the coating. The counts of psychrotrophic bacteria in trout fillets varied significantly depending on treatment type, storage period, and their combined interaction ($p < 0.05$). In the control samples, TPAB reached 7.17 log cfu/g by day 12, indicating rapid spoilage under refrigeration. In contrast, the AVG+patchouli oil coatings significantly suppressed psychrotrophic growth. At the end of the 12th day of storage, the K1 group (AVG) maintained TPAB at 6.37 log cfu/g, while the K2 group (AVG + 1% PY) showed 5.73 log cfu/g, and the K3 group (AVG + 2% PY) exhibited the lowest value of 5.13 log cfu/g. These findings confirm that the incorporation of plant-derived antimicrobials into edible coatings can effectively delay the proliferation of cold-tolerant spoilage bacteria. Both the treatment and the storage duration had statistically significant effects on TPAB

counts ($p < 0.05$). Similar results were reported by Hassanpour et al. (2024) in their study on *Oncorhynchus aguabonita* fillets coated with *Trachyspermum copticum* essential oil nanoemulsions, where psychrotrophic growth was significantly delayed during storage at 4°C. Likewise, Çoban & Ergür (2023) demonstrated that chia mucilage coatings enriched with gojiberry extract effectively extended the shelf life of trout fillets by exerting both antibacterial and antioxidative effects. The observed microbial suppression in these studies was attributed to the synergistic interaction between the biopolymer matrix and the bioactive compounds of the plant-derived additives. In both cases, the coating formulation and storage duration had statistically significant effects on TPAB counts ($p < 0.05$), confirming the potential of natural antimicrobial agents in prolonging the shelf life of refrigerated fish products.

Yeast and mould counts increased progressively during storage. The values of yeast and mould varied markedly depending on treatment group, storage time, and the interaction of these variables ($p < 0.05$). At day 12, the highest yeast and mould count was observed in the control group (3.37 log cfu/g), whereas the coated groups, particularly K2 and K3, maintained significantly lower values (around 2.08–2.18 log cfu/g). Statistical analysis confirmed that treatment, storage period, and the interaction between these factors had a significant effect on yeast and mould counts ($p < 0.05$). *Aloe vera*-based coatings effectively suppressed yeast–mold growth compared to the control, with the K3 samples exhibiting the strongest inhibitory effect. These findings highlight the potential of *Aloe vera* as a natural preservative to extend the shelf life of refrigerated products. The results of this study also confirm the protective role of patchouli essential oil. In line with our findings, Kalkan et al. (2019) reported that chitosan films enriched with peppermint essential oil, when applied to bonito fillets, significantly reduced yeast–mold counts. Likewise, Faraj & Nouri (2024) demonstrated that mucilage coatings containing nanoencapsulated essential oils effectively delayed fungal proliferation in button mushrooms, thereby extending their shelf life.

Chemical Analysis Results

The chemical variations observed during storage are illustrated in Figure 1 (a–c). A progressive increase in TVB-N values was observed in all samples during the storage period. The highest mean values were recorded in the control group, whereas lower TVB-N levels were detected in the coated groups. While the average TVB-N value at the end of storage in the control group reached 26.11 mg/100 g, the coated samples exhibited lower levels, with K3 maintaining 21.09 mg/100 g. Duncan's multiple comparison test revealed significant differences among the samples ($p < 0.05$). This reduction clearly

indicates the effectiveness of the coating in slowing protein degradation and extending the shelf life of the product. Similarly, Waris & Pilavtepe-Celik (2025) reported that the application of *Aloe vera* gel coating markedly lowered TVB-N accumulation in cold-stored sea bass fillets, thereby prolonging their shelf life. In addition, Izadi et al. (2023) demonstrated that tomato seed mucilage coatings enriched with shallot essential oil effectively reduced chemical spoilage and preserved the quality of frozen rainbow trout fillets. These findings are consistent with the present study, further supporting the effectiveness of natural coatings in delaying chemical deterioration and extending the shelf life of fish products.

A marked increase in TBARS values was observed across all groups during storage. By the end of the storage period, the control group reached an average of 6.97 mg MDA/kg, whereas coated samples exhibited lower levels. This highlights the inhibitory effect of the coatings on oxidative deterioration. Statistical analysis revealed that both sample and storage duration had significant effects on TBARS values ($p < 0.05$). In contrast, the sample \times storage interaction was not significant ($p > 0.05$), suggesting that the protective role of the coatings is maintained regardless of storage time. In the study conducted by El-Chaghaby et al. (2024), it was reported that the edible coating prepared from *Aloe vera* gel combined with lemongrass extract significantly reduced lipid oxidation and limited TBARS accumulation in cold-stored Nile tilapia fillets. Similarly, Mohammadian et al. (2025) demonstrated that

innovative chitosan coatings enriched with *Cuminum cyminum* essential oil effectively suppressed oxidative spoilage and extended the shelf life of *Hypophthalmichthys molitrix* fillets. These findings are consistent with the present study.

By the end of the storage period, the highest average pH was recorded in the control group (7.23), whereas initial values were considerably lower. This highlights the progressive effect of storage duration on pH elevation. Statistical analysis revealed that both sample type and storage duration had significant effects on pH values ($p < 0.05$). In contrast, the sample \times storage interaction was not significant ($p > 0.05$), suggesting that the influence of storage time on pH increase is consistent across all samples. Similar trends have been reported in fish preservation studies. Fofandi & Tanna (2020) demonstrated that *Aloe vera* coating effectively slowed quality deterioration in Indo-Pacific King Mackerel chunks during chilled storage, as reflected in lower pH values compared to the control group. This indicates that coated samples maintained a more stable pH profile. In contrast, the control group exhibited a marked increase, underscoring the protective role of *Aloe vera* against biochemical changes during storage. Likewise, in the present study, coated samples consistently maintained lower pH values compared to the control group throughout storage, confirming the inhibitory effect of coatings on pH elevation.

Table 1. Microbial changes (log cfu/g) in trout fillets coated with *Aloe vera* gel and patchouli oil during cold storage

Analyses	Storage Time (days)	K0	K1	K2	K3	Mean (X±SD)
TMAB	0	3.15±0.21	2.95±0.14	2.62±0.21	2.45±0.19	2.79±0.33 ^a
	3	4.13±0.16	3.80±0.23	3.19±0.10	2.99±0.03	3.53±0.50 ^b
	6	5.34±0.11	4.35±0.45	3.99±0.12	3.82±0.10	4.37±0.65 ^c
	9	6.18±0.11	5.34±0.41	4.94±0.09	4.63±0.26	5.27±0.65 ^d
	12	7.11±0.18	6.35±0.26	5.68±0.26	5.13±0.16	6.07±0.81 ^e
	Mean (X±SD)	5.18±1.49 ^D	4.56±1.23 ^C	4.08±1.18 ^B	3.80±1.05 ^A	
TPAB	0	3.35±0.31	3.05±0.07	2.74±0.26	2.54±0.18	2.92±0.37 ^a
	3	4.17±0.23	4.05±0.09	3.10±0.11	3.08±0.10	3.60±0.55 ^b
	6	5.56±0.14	4.50±0.45	4.18±0.08	3.97±0.04	4.55±0.67 ^c
	9	6.15±0.09	5.20±0.28	4.99±0.07	4.54±0.32	5.22±0.65 ^d
	12	7.17±0.22	6.37±0.19	5.73±0.25	5.13±0.11	6.10±0.82 ^e
	Mean (X±SD)	5.28±1.45 ^D	4.64±1.19 ^C	4.15±1.19 ^B	3.85±1.00 ^A	
TYM	0	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00 ^a
	3	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00 ^a
	6	2.24±0.10	2.11±0.08	2.00±0.00	2.00±0.00	2.08±0.11 ^b
	9	2.98±0.05	2.60±0.22	2.02±0.02	2.00±0.00	2.40±0.44 ^c
	12	3.37±0.20	2.96±0.06	2.18±0.04	2.08±0.04	2.65±0.58 ^d
	Mean (X±SD)	2.52±0.59 ^C	2.33±0.41 ^B	2.04±0.07 ^A	2.01±0.03 ^A	

KO: control (uncoated fillets), K1: AVG-coated, K2: AVG+1% PY-coated, K3: AVG+2% PY-coated samples. Values are expressed as mean \pm standard deviation (SD). Significant differences among storage days are indicated by lowercase superscript letters (a–f), while differences among treatment groups within the same day are denoted by uppercase letters (A–D) ($p < 0.05$)

Sensory Analysis Results

The sensory evaluation results are illustrated in Figure 2 (a-d). Sensory analysis results demonstrated that storage time had a significant effect on all parameters. Storage duration created statistically significant differences in texture, colour, odour, and overall acceptability values ($p < 0.05$). Although the sample factor showed some initial differences, the sample \times storage interaction was not statistically significant ($p > 0.05$). At day 0, all samples received high scores for texture (range 4.2–4.8). However, texture scores steadily decreased as storage progressed. By day 9, values had dropped to 1.4–2.0, and by day 12, they reached their lowest levels (1.0–1.4). Similarly, colour scores decreased significantly with storage time. Samples that were initially bright and acceptable (4.0–5.0) declined to their lowest values (1.0–1.4) on day 12. Odour was among the most sensitive attributes to storage. Initial odour scores (4.0–4.8) decreased to 1.2–2.0 on day 9 and further to 1.0–1.4 on day 12. The observed decline in odour is thought to be associated with the characteristic volatile components of patchouli oil. Patchouli oil contains compounds such as patchoulol, α -bulnesene, and seychellene, which are known for their intense, woody, and earthy aromatic properties. Pannellists perceived this distinctive aroma as divergent from the product's natural profile, leading to reduced sensory acceptability. This finding suggests that while natural additives may initially enhance sensory quality, their dominant aromatic profiles can ultimately limit consumer preference. Overall acceptability values also showed a consistent decline across all samples with increasing storage time. Samples that initially scored high reached their lowest values, particularly on days 9 and 12. This indicates that the products were unable to maintain sensory quality throughout their shelf life, and consumer acceptance remained restricted by storage duration. Pannellists noted that acceptability levels, evaluated on a 5-point hedonic scale, were initially higher in the groups treated with additives, but this effect diminished progressively as storage time increased. In this study, mean scores above 3 (“moderately desirable”) were considered indicative of acceptable quality, and values declined below this threshold by days 9 and 12. From day 6 onwards, the K1 group consistently exhibited superior sensory properties compared to the other treatments. Although *Aloe vera*–patchouli coatings effectively delayed microbial and chemical deterioration, the strong aromatic profile of patchouli oil negatively influenced odour acceptability. This trade-off underscores the balance between preservation efficacy and sensory quality, as reduced odour scores directly affect consumer acceptance and practical applicability. Therefore, while the coatings enhanced shelf life, their sensory impact must be carefully considered in future applications, particularly in relation to consumer preferences and product marketability. Similar trade-offs have been reported

in previous studies. Vital et al. (2018) reported that alginate-based coatings containing essential oils applied to Nile tilapia fillets maintained higher sensory acceptability scores during storage compared to controls, confirming the protective role of natural coatings in delaying sensory deterioration. Similarly, Zomorodian et al. (2023) demonstrated that *Zataria multiflora* essential oil incorporated into chitosan coatings significantly improved sensory characteristics in salmon fillets, highlighting that essential oil-based coatings can effectively preserve sensory quality during cold storage. These studies are consistent with the present findings.

Limitations and Opportunities

The present study has several limitations that should be acknowledged. First, although the novelty of combining *Aloe vera* gel with patchouli oil was emphasised, the absence of direct chemical characterisation of the patchouli essential oil (e.g., GC–MS analysis) limits the ability to link the observed preservative effects to specific bioactive compounds. It may affect reproducibility, given the known variability of essential oil composition. Similarly, the physicochemical properties of *Aloe vera* gel and patchouli oil were not experimentally analysed in this work; instead, their functional attributes were discussed based on literature reports. In addition, while statistical analyses were performed using ANOVA and Duncan's test, the study was restricted to the parameters measured. It did not include advanced modelling approaches that could further strengthen the interpretation of results. Finally, the findings are based on rainbow trout fillets under refrigerated storage, and broader validation across different seafood species and storage conditions would be valuable to generalise the applicability of this preservation strategy.

Conclusion

Aloe vera–patchouli coatings effectively suppressed microbial growth, delayed chemical spoilage, and supported sensory quality in trout fillets during refrigerated storage. The coatings maintained acceptable quality for at least 12 days, providing a clear shelf-life extension compared to the control group. The AVG+2% patchouli oil formulation exhibited the strongest protective effect, maintaining lower microbial counts and chemical deterioration indicators compared to the control group. However, the distinctive volatile compounds of patchouli oil influenced odour perception, which limited consumer preference over extended storage. These findings highlight the potential of *Aloe vera*–based coatings enriched with essential oils as natural preservation strategies for aquaculture products. Future studies should focus on optimising additive concentrations, exploring microencapsulation or controlled release systems, and combining essential oils with other natural preservatives to balance functional efficacy with sustained sensory quality and consumer acceptance.

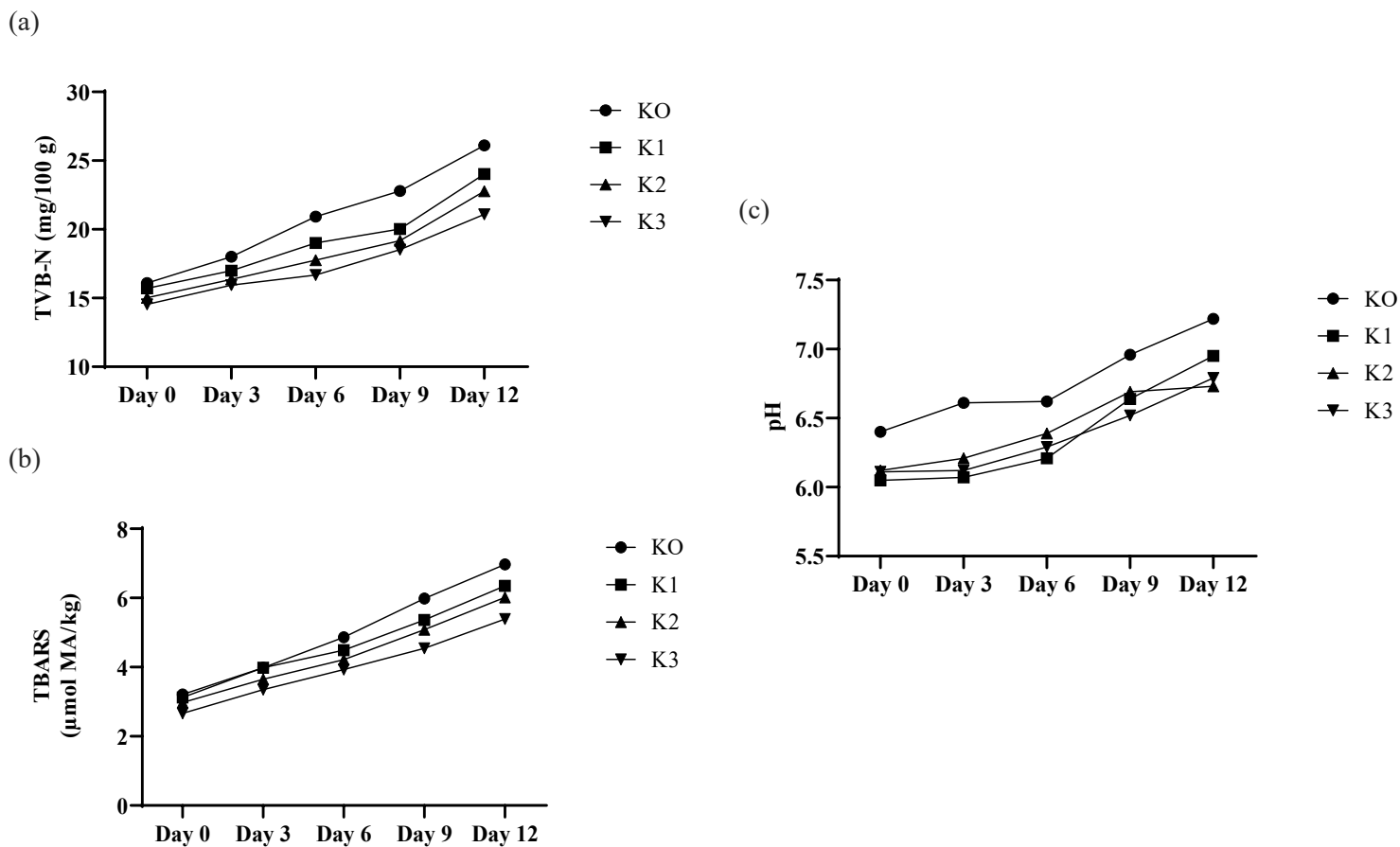
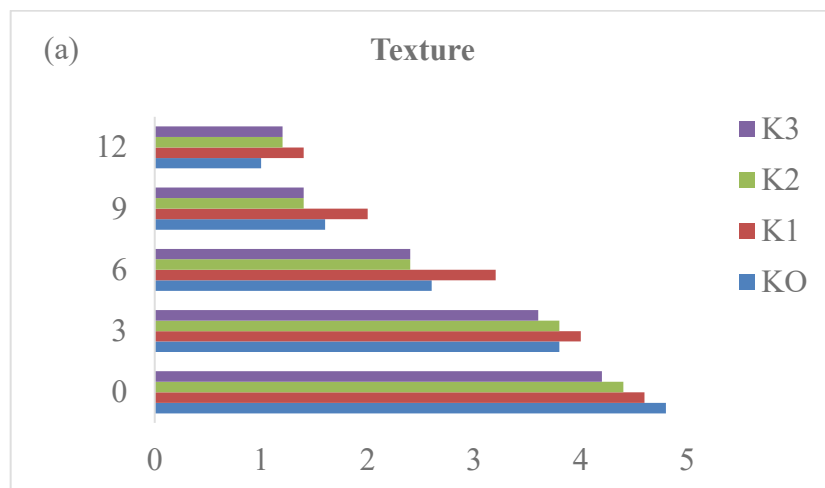


Figure 1. a) TVB-N, b) TBARS and c) pH changes in trout fillets coated with changes in trout fillets coated with *Aloe vera* gel and patchouli oil during cold storage. KO: control (uncoated fillets), K1: AVG-coated, K2: AVG+1% PY-coated, K3: AVG+2% PY-coated samples



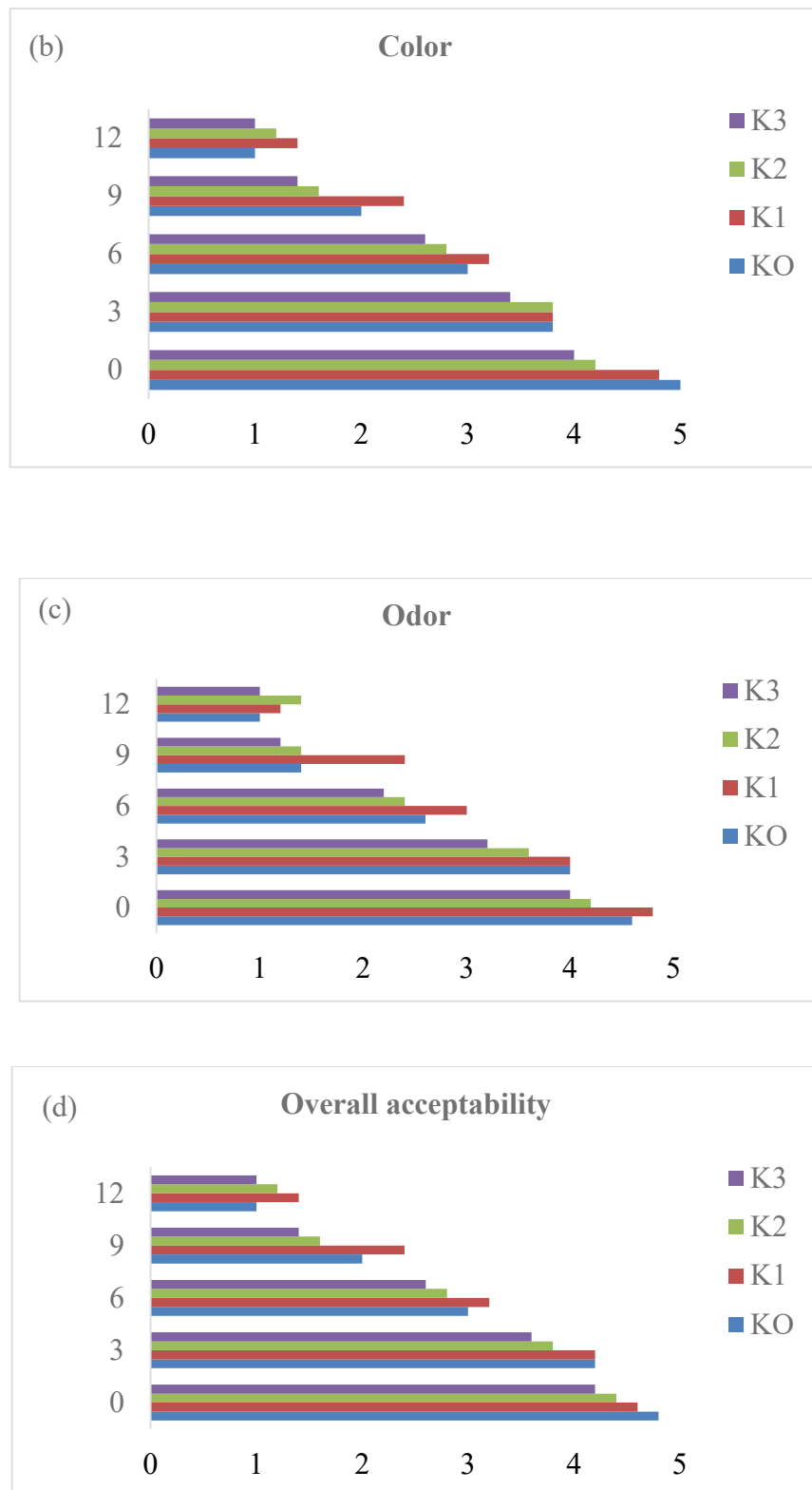


Figure 2. a) Texture, b) Color, c) Odor and d) Overall acceptability changes in trout fillets coated with changes in trout fillets coated with *Aloe vera* gel and patchouli oil during cold storage. KO: control (uncoated fillets), K1: AVG-coated, K2: AVG+1% PY-coated, K3: AVG+2% PY-coated samples

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures, with no animal experiments.

Data availability: The data will be made available upon request from the author.

Funding disclosure: -

Acknowledgements: -

Disclosure: -

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Functional effects of quercetin via the haematology–gut axis: Resistance to *Lactococcus petauri* in *Oncorhynchus mykiss*

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Cite this article as:

Köse, Ö. (2026). Functional effects of quercetin via the haematology–gut axis: Resistance to *Lactococcus petauri* in *Oncorhynchus mykiss*. *Aquatic Research*, 9(2), 129–147. <https://doi.org/10.3153/AR26012>

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Submitted: 26.12.2025

Revision requested: 31.01.2026

Last revision received: 05.02.2026

Accepted: 09.02.2026

Published online: 30.03.2026

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ABSTRACT

This study evaluated the effects of low-fat (LFD), high-fat (HFD), and quercetin-supplemented high-fat (HFD+Q; 0.2 g/kg) diets on the haematological responses, intestinal histomorphology/histopathology, and resistance to *Lactococcus petauri* infection in rainbow trout (*Oncorhynchus mykiss*). A total of 270 fish (initial mean weight: 38.37 g) were randomly distributed into three dietary groups with three replicates per treatment (30 fish per tank) and fed the experimental diets for eight weeks. At the end of the trial, haematological parameters were determined using an automated analyser; histomorphometric measurements (muscle layers, villus parameters, and goblet cell counts) and histopathological evaluations were performed on intestinal samples. Furthermore, an intraperitoneal challenge with *L. petauri* was conducted to evaluate disease resistance. The HFD group exhibited significant suppression of leukocyte profiles (*WBC*, *LYM*, *MID*, *GRAN*) and a downward trend in HGB/HCT and erythrocyte indices. This group also displayed intestinal alterations consistent with muscular layer irregularities, submucosal oedema, and loss of goblet cells. In contrast, the HFD+Q group maintained more balanced haematological parameters and intestinal barrier indicators, contributing to the recovery of mucosal defence by increasing goblet cell counts compared to the HFD group. In the challenge trial, the survival probability was significantly higher in the HFD+Q group. In conclusion, quercetin is a functional feed additive with the potential to enhance resistance to *L. petauri* infection by supporting haematological homeostasis and intestinal integrity under high-fat feeding conditions. Further immunological and molecular studies are recommended to elucidate the mechanism of action and optimal dosage.

Keywords: *Oncorhynchus mykiss*, *Lactococcus petauri*, Quercetin, High-fat diet, Haematology, Intestinal histology



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Introduction

Ensuring sustainable production in aquaculture largely depends on maintaining the physiological health of fish (Oliveira et al., 2024; Awad, 2025). In this context, enriching the nutritional content of commercial fish feeds with functional ingredients has become a focal point of increasing interest in recent years (Onomu & Okuthe, 2024). Bioactive compounds of natural origin, in particular, have come to the fore in aquaculture nutrition due to their potential effects, such as reducing oxidative stress and supporting the immune system (Awad, 2025; X. Hu et al., 2025). The evaluation of physiological parameters is considered a fundamental approach to reliably determining the effects of such nutritional interventions on fish health (Oliveira et al., 2024; Onomu & Okuthe, 2024).

Physiological indicators are widely used to determine health status, stress response, and adaptation to environmental conditions in fish. Among these, haematological parameters are evaluated as reliable biomarkers reflecting the effects of nutritional practices, feed additives, and environmental stressors on the organism (Fazio et al., 2013; Seibel et al., 2021). It has been reported that various nutritional applications, such as plant-derived additives, prebiotics, and mineral supplements, can modulate physiological processes related to the immune system, and these effects are frequently observed through haematological changes (Czech et al., 2009; Jalali et al., 2009; Esmacili, 2021). Therefore, assessing physiological changes is of great importance for a holistic understanding of the impact of nutritional interventions on immune functions and overall fish health (Esmacili, 2021; Oliveira et al., 2024).

Intestinal histology represents a fundamental indicator of fish health. Histological investigations of the digestive system are widely regarded as reliable approaches for assessing metabolic status, overall health, nutritional condition, and the effects of environmental factors on fish (Matulić et al., 2020; Vatsos, 2021). The examination and monitoring of the histological architecture of the intestine and liver, which are the primary organs responsible for digestion and nutrient absorption, are commonly employed to evaluate the effects of feed formulations enriched with plant-derived ingredients (Raskovic et al., 2011). Given that intestinal tissue can undergo rapid and pronounced structural alterations in response to dietary components or stress-related factors, histomorphometric analyses enable the quantitative or semi-quantitative assessment of these changes, thereby providing valuable information on the impact of feed ingredients on intestinal function (Vatsos, 2021). Based on these considerations, it can be concluded that histomorphometric evaluations effectively

reveal diet-induced intestinal alterations and, when interpreted alongside other physiological parameters (e.g., haematological indices), allow for a more comprehensive understanding of the physiological responses to nutritional interventions.

Quercetin (3,3',4',5,7-pentahydroxyflavone), a member of the flavonoid family, is a common polyphenol naturally found in many fruits and vegetables (Bischoff, 2008). Studies have revealed that quercetin possesses antiviral, anticancer, antibacterial, anti-inflammatory, and immunomodulatory properties; it suppresses oxidative stress, supports the innate immune response, and exhibits hepatoprotective effects (Gasmi et al., 2022; Muderrisoglu et al., 2022; Duan et al., 2025; Köse, 2025). Due to these characteristics, quercetin is gaining increasing attention as a functional feed additive in aquaculture nutrition, particularly regarding its effects on fish physiology and immune-related parameters (Armobin et al., 2023; Y. Hu et al., 2025; Ming et al., 2025).

The regulation of immune responses and oxidative stress in fish plays a critical role not only in nutritional physiology but also in resistance against bacterial diseases. Lactococcosis, which causes significant economic losses in rainbow trout (*Oncorhynchus mykiss*) farming, is a globally widespread disease that progresses more severely under stress, immunosuppression, and inadequate farming conditions (de Ruyter et al., 2023; Egger et al., 2023). Although the disease was long associated with *Lactococcus garvieae*, recent studies have revealed that closely related species such as *Lactococcus petauri* can also be causative agents, a finding confirmed in *O. mykiss* isolates in Türkiye (Altinok et al., 2022; Vela et al., 2024). Considering previous studies that reported that plant-based additives such as *Capsicum annuum* (Yilmaz et al., 2024) and blends of organic acids and plant essential oils (Huyben et al., 2021; Balta et al., 2025) strengthen immune and antioxidant defence systems and increase survival rates against *L. garvieae* infections, it is clear that nutrition-based strategies are indispensable for disease management in *O. mykiss* aquaculture.

Although the physiological, immunological, and antioxidant effects of quercetin have been extensively investigated in various species, to the best of our knowledge, no studies have yet addressed the effects of this bioactive compound against *L. petauri*-induced infections, nor its role in haematological and intestinal histomorphometric responses in *O. mykiss*. In this study, the effects of low-fat (LFD), high-fat (HFD), and quercetin-supplemented HFD diets on physiological parameters related to the haematology and intestinal histology of *O.*

mykiss were evaluated, independent of growth performance. Furthermore, an experimental infection (challenge) trial was conducted against *L. petauri*, one of the causative agents of lactococcosis, to demonstrate the effects of quercetin-supplemented feeding on disease resistance. The findings provide holistic and up-to-date information for the literature regarding the role of quercetin as a functional feed additive in fish physiology and disease management.

Materials and Methods

Ethical Approval and Study Permissions

This study was conducted within the scope of project number FBA-2023-15, supported by the Scientific Research Projects Coordination Unit of Recep Tayyip Erdoğan University. Ethical approval was obtained from the Local Ethics Committee for Animal Experiments of Recep Tayyip Erdoğan University on June 13, 2023 (Decision No: 2023/30). All procedures involving the animals used in the study were carried out in accordance with the Experimental Animal Use Guide and standard operating procedures of Recep Tayyip Erdoğan University.

Fish Material, Feeding Conditions, Experimental Diets, and Experimental Design

Details regarding the fish materials, feeding regimen, formulation of the experimental diets, preparation methods, and experimental design have been presented in a previous publication (Köse, 2025). Briefly, fish were obtained from a private farm and transported to the Recep Tayyip Erdoğan University Iyidere R&D Unit, where they were acclimated to a commercial feed for 14 days. Following acclimation, fish were randomly assigned to three experimental groups (LFD, HFD, and HFD+Q). Each group consisted of three replicates with 30 fish per tank (initial mean weight: 38.37 g). The experiment was conducted in 100-L fibreglass tanks equipped with a flow-through system (0.2 L/sec) and aeration under natural daylight conditions. During the 8-week trial, fish were fed their respective experimental diets daily at a rate of 2% of their body weight, divided into three meals. Over the course of the experiment, the mean water temperature, pH, and dissolved oxygen were determined to be 14.2 °C, 7.74, and 7.72 mg/L, respectively.

The nutritional compositions of the experimental diets were as follows: Crude protein levels for LFD, HFD, and HFD+Q were 45.91%, 45.96%, and 45.94%, respectively; crude fat levels were 11.38%, 22.53%, and 22.33%; moisture levels were 9.89%, 9.54%, and 9.27%; ash levels were 7.69%, 7.87%, and 7.94%; and crude fiber levels were 1.79%, 1.82%, and 1.83%. Nitrogen-free extract (NFE) values were 23.34,

12.28, and 12.69, while gross energy (MJ/kg diet) levels were 19.69, 22.21, and 22.19, respectively. Distinct from the LFD and HFD groups, the HFD+Q group was supplemented with 0.20 g/kg of quercetin (3,3',4',5,7-pentahydroxyflavone; >95% purity, Sigma Chemical Co., USA). The quercetin dosage was determined based on effective dose ranges (150–800 mg/kg) reported for fish species such as *Channa argus* and *Cyprinus carpio* (Ghafarifarsani et al., 2022; Kong et al., 2022; Armobin et al., 2023), and levels reported to pose no toxicity risk in mice (250 mg/kg) (Cunningham et al., 2022). In the diets, fish meal, soy protein, and corn gluten were used as protein sources; fish oil and soybean oil (1:1 ratio) were used as lipid sources; and potato starch was used as a carbohydrate source. Diets were prepared at the Recep Tayyip Erdoğan University Iyidere R&D Unit Feed Laboratory according to protocols established in our previous publications (Köse et al., 2021, 2024; Kose & Karabulut, 2022).

Blood Sampling and Haematological Analyses

At the end of the trial, fish were fasted for 24 hours prior to sampling. Five fish were randomly selected from each tank and anaesthetized with clove oil at a dose of 2–5 mg/L. Blood samples were collected from the caudal vein using a sterile 2.5 mL syringe with a 22G needle. The collected blood samples were transferred to tubes containing EDTA-K3 and stored at +4 °C for approximately 4 hours until analysis.

For each blood sample, the following parameters were measured: leukocyte (*WBC*), lymphocyte (*LYM*), monocyte (*MID*), granulocyte (*GRAN*), erythrocyte (*RBC*), haemoglobin (*HGB*), hematocrit (*HCT*), mean corpuscular volume (*MCV*), mean corpuscular haemoglobin (*MCH*), mean corpuscular haemoglobin concentration (*MCHC*), platelet (*PLT*), and mean platelet volume (*MPV*). Analyses were performed using an automated haematological analyser, Prokan 6800VET (Prokan, China). Prior to the study, the instrument was calibrated specifically for fish blood, in accordance with the protocols outlined by Er et al. (2024). The operational principle of the analyser was detailed in a study by Minaz et al. (2022).

Histological Method and Sample Collection

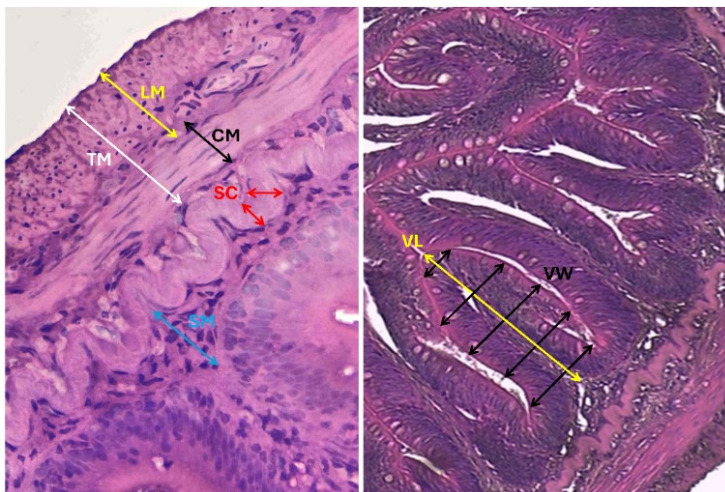
Intestinal tissues from the five fish previously selected for haematological analysis were used for histological examination. For fixation, samples were taken 0.5–1 cm distal to the portion identified as the second segment of the intestine. (Lokka et al., 2013).

Collected intestinal samples were fixed in Davidson's fixative for approximately 36 hours, followed by immersion in 70% ethanol for 24 hours. Tissue processing was carried out using

a LEICA TP1020 tissue processor, blocking with LEICA HistoCore Arcadia H, and tissue sectioning with a LEICA RM2125RT microtome (Leica Microsystems Limited, Switzerland). Hematoxylin-Eosin (H&E) staining was performed automatically using an MLT FS-16 Combo Automated Slide Stainer (MLT LLC, Dubna, Moscow, Russia).

The staining procedure involved the following steps: tissues were cleared of ethanol residues and passed through successive series of alcohol and xylene. Samples were incubated in a 65 °C paraffin bath, embedded in paraffin blocks, sectioned at 5 µm thickness, and mounted on slides. Slides were kept in an oven at 65 °C for paraffin removal, then stained with H&E, and finally coverslipped with Entellan™ (Luna, 1968). Histological images were acquired using a Leica DM500 light microscope integrated with a high-resolution camera (Taup-Cam YW3609EH, CMOS 4K UHD). Images were recorded via ToupTek ToupView software (version 4.11, ToupTek, Hangzhou, Zhejiang, China).

Intestinal tissues were evaluated in accordance with procedures previously described in the literature. Goblet cell count was calculated as the average number of goblet cells per villus. Villus absorption surface (*VA*) was obtained by multiplying villus length (*VL*) by villus width (*VW*). For this calculation, at least five measurement points were used for each villus (Baeza-Ariño et al., 2016; Köse et al., 2024) (Figure 1). Histopathological evaluation was confirmed by a blinded observer, with group information concealed for the samples.



In the histological micrograph, *TM* represents tunica muscularis; *LM*, longitudinal muscularis; *CM*, circular muscularis; *SC*, stratum compactum; *SM*, submucosa; *VL*, villus length; and *VW*, villus width (H&E, 40X)

Figure 1. Measurement points for intestinal wall and villus lengths in *O. mykiss*

Challenge Test with *Lactococcus petauri*

After sampling for other experimental parameters was completed, the tanks for each dietary group were pooled. Subsequently, fish randomly selected from each pool were used to establish challenge groups in triplicate (LFD, HFD, and HFD+Q; n=10 per replicate). One replicate from each group served as a control and was injected with sterile physiological saline solution (PSS). The mean body weights of the fish in the LFD, HFD, and HFD+Q groups were determined to be 100.11 ±2.14 g, 123.21 ±1.03 g, and 128.32 ±0.95, respectively.

Throughout the challenge trial, the mean water temperature, pH, dissolved oxygen, conductivity, and TDS were 15.95 ±0.35 °C, 7.90 ±0.08, 7.05 ±0.18 mg/L, 144.0 ±2.0 µS/cm, and 74.9 ±1.13 mg/L, respectively. The trial was conducted under natural daylight conditions, and the fish were hand-fed three times a day at a rate of 2% of their body weight. However, with the onset of disease symptoms and loss of appetite, feeding was carried out until the fish were satiated to prevent feed wastage.

The *L. petauri* strain (NCBI Accession number: JAQIFV000000000.1) used in the challenge study was obtained from the Fish Diseases Laboratory at the Faculty of Fisheries, Recep Tayyip Erdoğan University. The bacterial culture was incubated in Tryptic Soy Broth (TSB) medium for 24 hours, then centrifuged at 10000×g at 4°C for 5 minutes. The resulting bacterial pellets were diluted with physiological saline solution (PSS) to reach the McFarland 0.5 standard. Before infection, the bacterial concentration was determined to be 3.4×10⁷ CFU mL⁻¹ using the Plate Count Agar (PCA) method. The bacteria were injected intraperitoneally (0.2 mL per fish) into the fish using a sterile insulin syringe. Sterile PSS was administered to the control groups using the same method (Er et al., 2021; Köse et al., 2021, 2024).

Re-isolation was performed on the dead fish to confirm the relationship between the cause of mortality and the pathogen. Spleen, liver, and kidney samples were aseptically collected from dead individuals and inoculated onto tryptic soy agar (TSA) medium. After 48 hours of incubation at 22 °C, the obtained colonies were evaluated morphologically and biochemically. The phenotypic characteristics of the isolates, identified as Gram-positive, chained cocci, were found to be consistent with the *L. petauri* isolate used.

Cumulative survival rates were calculated using Kaplan-Meier analysis.

The following formula was used to calculate the survival rate:

$$S(t) = \prod_{t_i \leq t} \left(1 - \frac{d_i}{n_i}\right)$$

Where $S(t)$ represents the probability of survival at time t , t_i represents the time at which death events occurred, d_i represents the number of individuals who died at time t_i , and n_i represents the number of individuals at risk at time t_i .

Statistical Analysis

Data analysis and graphical representations were performed using the SigmaPlot 15.0 software package (AlfaSoft, Umeå, Sweden) and OriginLab Pro 2025 (OriginLab Corporation, Massachusetts, USA). All data are presented as mean \pm standard deviation (SD). The normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using the Brown–Forsythe test. To determine the effects of dietary treatments, a one-way ANOVA was applied to all parameters. The tank was considered the experimental unit ($n = 3$), and values for each tank were analysed based on the means calculated from five randomly sampled fish per tank. Tukey’s honestly significant difference (HSD) post-hoc test was used to identify differences between groups. Statistical significance was set at $p < 0.05$; however, relevant ANOVA results are reported with their actual p -values.

Results and Discussion

Haematological Analysis Findings

A significant portion of the haematological parameters in *O. mykiss* was influenced by the dietary treatments, resulting in statistically significant differences between groups ($p < 0.05$). These parameters were categorised into three groups based on their physiological functions: immune-related parameters (*WBC*, *LYM*, *MID*, and *GRAN*), oxygen transport-related parameters (*RBC*, *HGB*, *HCT*, *MCV*, *MCH*, and *MCHC*) and coagulation-related parameters (*PLT* and *MPV*).

All immune-related haematological parameters, including *WBC*, *LYM*, *GRAN*, and *MID*, exhibited statistically significant differences among the groups ($p < 0.001$). Significant decreases were observed in all of these parameters within the HFD group, suggesting a physiological state consistent with leukopenia, lymphopenia, and granulocytopenia ($p < 0.05$). In contrast, the mean values obtained in the HFD+Q group were found to be similar to those of the LFD group and were significantly higher compared to the HFD group ($p < 0.05$) (Figure 2).

Regarding haematological parameters related to oxygen transport, statistically significant differences were determined among the groups in terms of haemoglobin concentration (*HGB*), hematocrit (*HCT*), mean corpuscular volume (*MCV*) and mean corpuscular haemoglobin (*MCH*) ($p < 0.001$) (Figure 3). In contrast, no statistically significant difference was detected between the groups regarding erythrocyte count (*RBC*) and mean corpuscular haemoglobin concentration (*MCHC*) values ($p > 0.05$). Significant decreases in *HGB* and *HCT* values were observed in the HFD group compared to the LFD group ($p < 0.05$), and a significant reduction in *MCH* values was determined in parallel. These findings suggest a possible decrease in the oxygen-carrying capacity of erythrocytes in parallel with the decrease in *HGB*, *HCT*, and *MCH* without a distinct change in erythrocyte count (*RBC*) and show consistency with a hypochromic haematological profile. The downward trend observed in *MCV* values in the HFD group points toward microcytic changes. Conversely, *HGB*, *HCT*, *MCV*, and *MCH* values in the HFD+Q group were found to be significantly higher than those in the HFD group ($p < 0.05$) and showed statistically similar values to the LFD group regarding these parameters ($p > 0.05$). *RBC* and *MCHC* values remained similar among the group ($p > 0.05$).

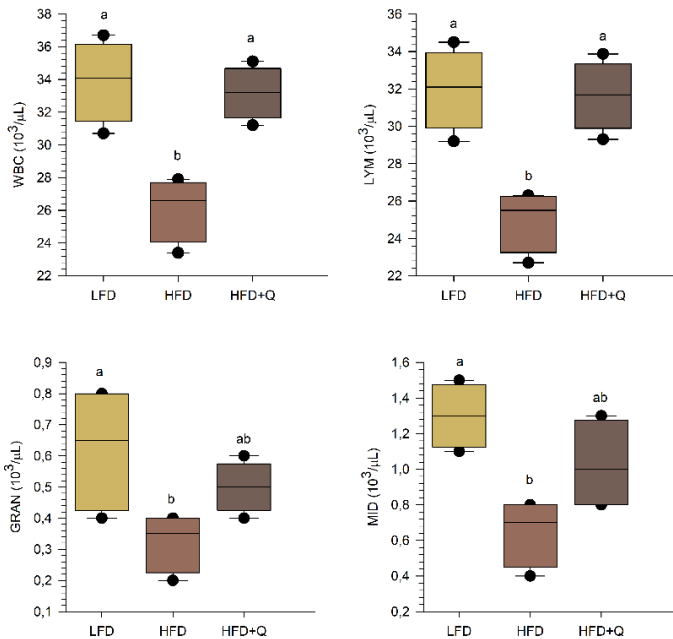
Regarding haematological parameters related to coagulation, no statistically significant difference was detected among the groups in terms of *PLT* and *MPV* levels ($p > 0.05$) (Figure 4). However, it was observed that *PLT* values in the HFD group showed a lower trend compared to the LFD group, while *PLT* values in the HFD+Q group followed higher levels compared to the HFD group. Similar distributions were observed in *MPV* values, and a wide range of variation was noted in the HFD+Q group.

Intestinal Histology

Morphometric findings

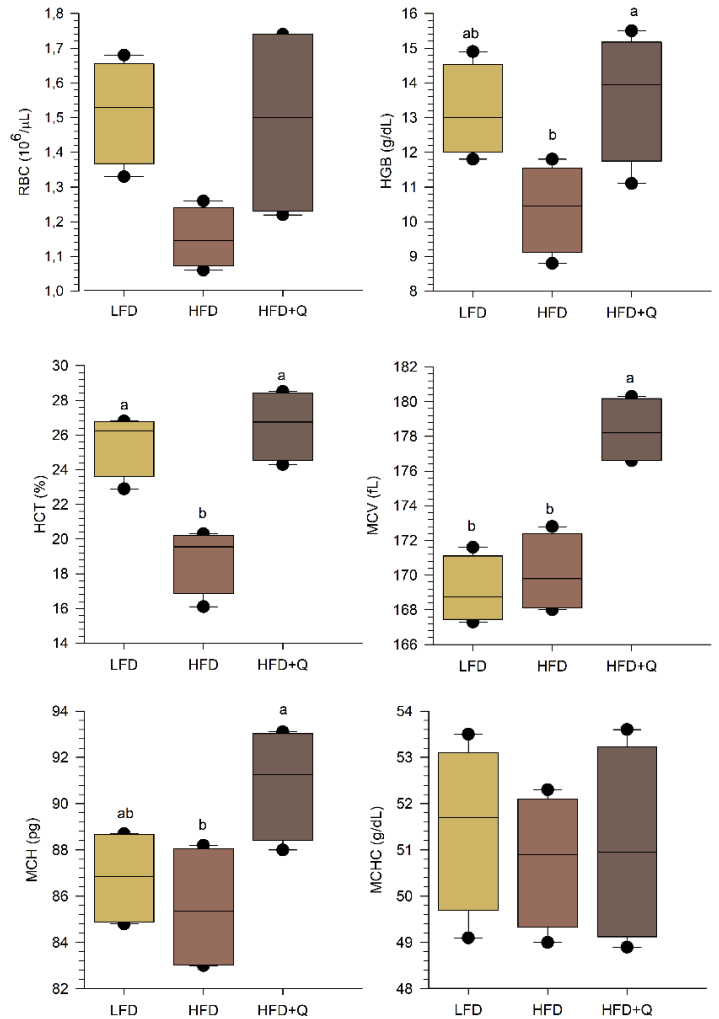
The intestinal histomorphometry evaluation revealed significant differences among the experimental groups for various structural parameters (Figure 5). Quercetin exhibited a trophic effect, significantly increasing the thickness of the longitudinal muscularis (*LM*) and tunica muscularis (*TM*) ($p < 0.05$). Accordingly, *LM* and *TM* thickness showed significant differences between groups, highest in the intestines of *O. mykiss* fed the HFD+Q diet and lowest in those fed the LFD diet ($p < 0.001$). In contrast, there was no significant difference in the circular muscularis (*CM*) thickness among the groups ($p = 0.288$), suggesting that this layer was not significantly affected by either dietary fat content or quercetin supplementation. The thickness of the stratum compactum (*SC*) was significantly increased in the HFD and HFD+Q groups

compared to the LFD group ($p < 0.001$). However, no significant difference was found between the HFD and HFD+Q groups for this parameter ($p > 0.05$). Similarly, the thickness of the submucosa (*SM*) was significantly higher in the HFD group compared to the other groups ($p < 0.001$). However, there was no significant difference between the LFD and HFD+Q groups for this parameter ($p > 0.05$).



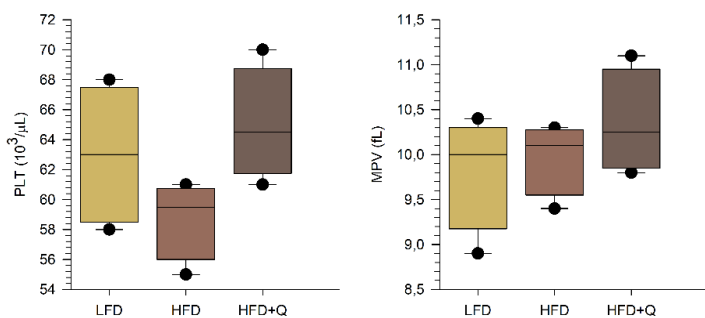
This figure illustrates the effects of different dietary treatments on immune-related haematological parameters, including white blood cell count (*WBC*), lymphocytes (*LYM*), granulocytes (*GRAN*), and mid-range cells (*MID*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD ($n = 3$ tanks per diet), with each value representing the tank mean calculated from five fish sampled per tank. Different lowercase letters indicate statistically significant differences among dietary groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$)

Figure 2. Effects of experimental diets on immune-related haematological parameters in *O. mykiss*



This figure illustrates the effects of different dietary treatments on oxygen transport-related haematological parameters, including red blood cell count (*RBC*), haemoglobin concentration (*HGB*), hematocrit (*HCT*), mean corpuscular volume (*MCV*), mean corpuscular hemoglobin (*MCH*), and mean corpuscular hemoglobin concentration (*MCHC*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD ($n = 3$ tanks per diet), with each value representing the tank mean calculated from five fish sampled per tank. Different lowercase letters indicate statistically significant differences among dietary groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$)

Figure 3. Effects of experimental diets on oxygen transport-related haematological parameters in *O. mykiss*



This figure illustrates the effects of different dietary treatments on coagulation-related haematological parameters, including platelet count (*PLT*) and mean platelet volume (*MPV*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD ($n = 3$ tanks per diet), with each value representing the tank mean calculated from five fish sampled per tank. Different lowercase letters indicate statistically significant differences among dietary groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$).

Figure 4. Effects of experimental diets on coagulation-related haematological parameters in *O. mykiss*

Measurements performed to evaluate the effects of experimental diets on villus morphology in *O. mykiss* revealed significant differences between the groups (Figure 6). Lamina propria (*LP*) thickness was significantly increased in both the HFD and HFD+Q groups compared to the LFD group, with the highest value observed in the HFD+Q group ($p < 0.001$). Similarly, a significant increase in villus length (*VL*) was detected in the HFD and HFD+Q groups compared to the LFD group, with the highest value observed in the HFD+Q group, which was significantly different from the LFD group ($p < 0.001$). Conversely, no significant difference was found among the groups regarding villus width (*VW*) ($p = 0.206$). The Villus absorption area (*VA*) was significantly increased in the HFD group compared to the LFD group. This increase was even more pronounced with quercetin supplementation in the HFD+Q group, which recorded the highest value. A significant difference was observed among all groups for this parameter ($p < 0.001$).

The high-fat diet (HFD) caused a decrease in goblet cell count (9.39 ± 0.93) in the HFD group, showing a significant difference compared to other groups ($p < 0.001$). Quercetin supplementation significantly increased the goblet cell count (11.73 ± 0.87) in the HFD+Q group compared to the HFD group ($p < 0.05$), while the LFD group showed a similar goblet cell count (11.07 ± 0.76) ($p > 0.05$) (Figure 7). These results indicate that HFD reduced goblet cell abundance, whereas quercetin supplementation restored it to levels comparable to LFD.

Histopathological findings

As shown in Figure 8, histological examination revealed distinct morphological differences between the experimental groups in both the intestinal muscle layers and the mucosal architecture of *O. mykiss*. In Figure 8A, corresponding to the LFD group, the circular (*CM*) and longitudinal (*LM*) muscle layers were clearly demarcated, and the tunica muscularis (*TM*) maintained a smooth and compact structure. No oedema or cellular infiltration was observed in the submucosal (*SM*) region, and the stratum compactum (*SC*) appeared thin and well-organised, preserving the muscular integrity. In contrast, Figure 8B shows that the HFD group exhibited pronounced disorganisation in the *CM* region, thickening of the *SC*, and eosinophilic staining within the *SM* layer, indicating expansion and oedema. These changes suggest hypertrophic remodelling of the muscle layers and submucosal inflammation induced by high-fat feeding. However, Figure 8C demonstrates that the HFD+Q group largely retained normal structural integrity. The *TM*, *CM*, and *LM* layers displayed compact organisation similar to the LFD group, and no signs of oedema were observed in the *SM*, confirming the protective effect of quercetin supplementation on intestinal wall morphology. The mucosal morphology also exhibited diet-dependent alterations. As shown in Figure 8D, the LFD group presented short and thick villi (*VL*) with irregular contours and localised oedema in the lamina propria (*LP*). Although slight epithelial irregularities were noted, goblet cells (*Gb*) were uniformly distributed and morphologically distinct.

In Figure 8E, the HFD group exhibited long and cylindrical villi with preserved epithelial continuity; however, goblet cells were sparse and less distinguishable, suggesting reduced mucus secretion and weakened mucosal defence. Finally, Figure 8F illustrates that the HFD+Q group exhibited partially regular villi with well-preserved epithelial integrity. The *LP* was compact with minimal oedema, and goblet cells were more numerous and evenly distributed compared to both other groups. These observations clearly indicate that quercetin supplementation mitigated the adverse effects of a high-fat diet, maintaining both muscular structure and mucosal defence integrity.

Lactococcus petauri challenge

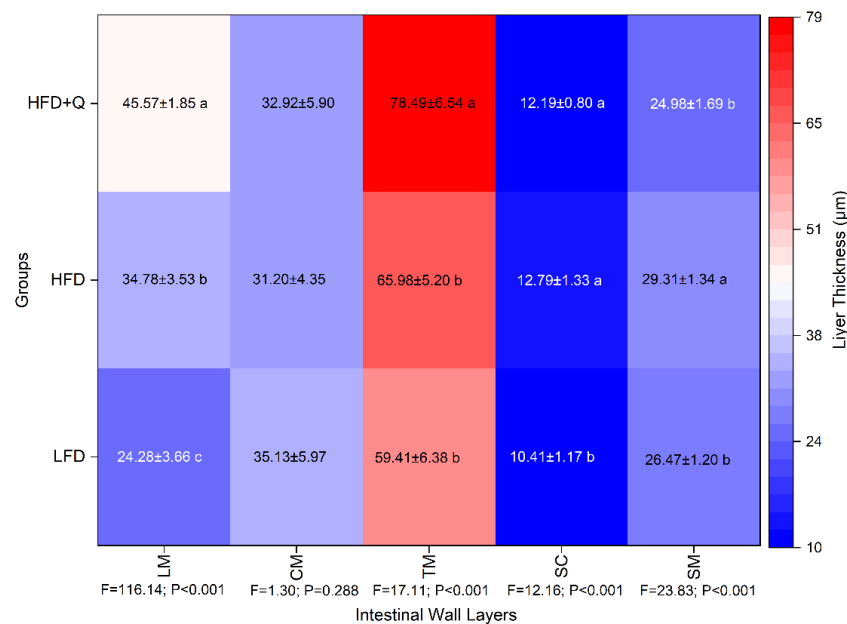
Environmental parameters, feeding conditions, and feeding regimen during the challenge trial were described in previous sections. The first death in the LFD group occurred on day 6 post-infection. The majority of deaths across all groups occurred between days 7 and 12. By day 15, the LFD group recorded the lowest survival probability at 20%, while the HFD group's rate was 45%. In contrast, the HFD+Q group had a

75% survival probability, with no further deaths occurring after day 10. Given the absence of additional mortality after day 15, this day was designated as the end of the experiment (Figure 9). The survival probability of the HFD+Q group was found to be significantly higher than both the LFD and HFD groups ($p < 0.05$). No mortality occurred in the control groups that received PSS throughout the trial. These results indicate that quercetin supplementation confers resistance against *L. petauri* infection.

Hematological Parameters

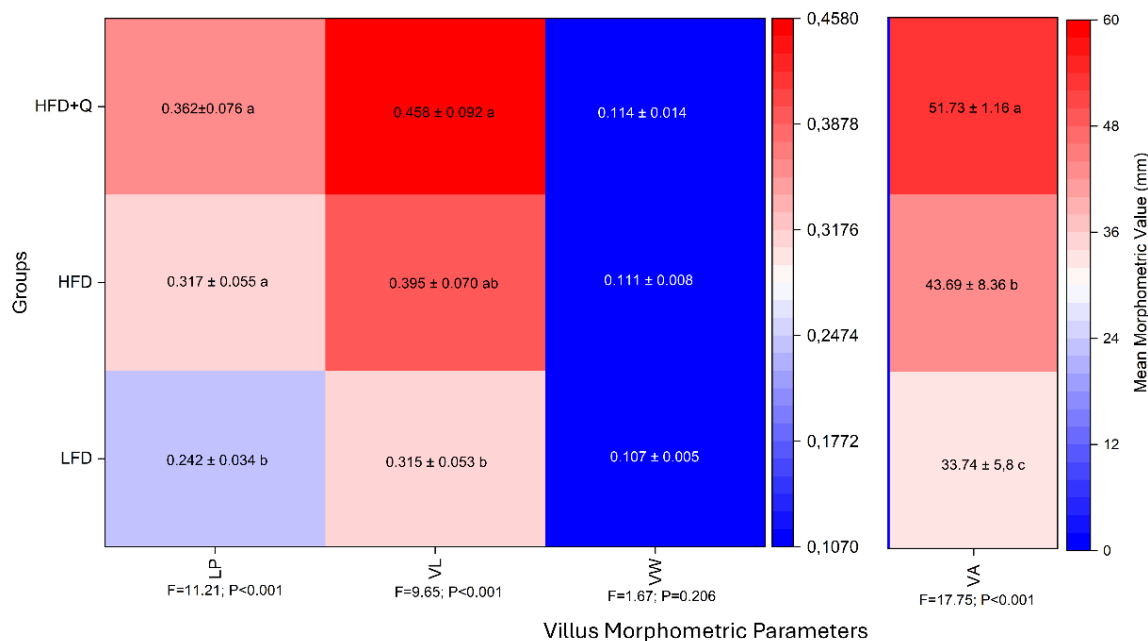
Haematological parameters are recognised as reliable biomarkers widely used to evaluate the physiological reflections of nutritional practices and environmental stressors in fish (Tavares-Dias et al., 2011; Fazio et al., 2013; Seibel et al., 2021). In particular, total leukocyte count (*WBC*), leukocyte subgroups (*LYM*, *MID*, and *GRAN*), erythrocyte indices, and platelet parameters can provide holistic information regarding the immune and physiological homeostasis of the organism. In the literature, it has been reported that diets with

high lipid content can adversely affect immune cell homeostasis in fish, potentially leading to decreases in total leukocyte count (*WBC*) and leukocyte subgroups. Specifically, long-term high-fat feeding regimens have been reported to disrupt immune homeostasis and negatively affect immune cell dynamics (Bujjamma & Padmavathi, 2018; Zahran et al., 2018). Conversely, it is reported that biologically active compounds such as plant-derived additives, prebiotics, and flavonoids can exhibit supportive effects on the immune response in fish, and this effect is frequently reflected through haematological parameters (Czech et al., 2009; Jalali et al., 2009; Esmacili, 2021). Consistent with this literature, the present study observed a decrease in *WBC* and leukocyte subgroups levels in *O. mykiss* individuals fed a high-fat diet; however, these parameters were maintained at levels similar to those in the LFD group in the HFD+Q group. These findings suggest that quercetin may play a balancing role in the immune cell profile under high-fat diet conditions and may contribute to the maintenance of immune homeostasis.



This figure illustrates the effects of different dietary treatments on the thickness of intestinal wall layers, including longitudinal muscularis (*LM*), circular muscularis (*CM*), tunica muscularis (*TM*), stratum compactum (*SC*), and submucosa (*SM*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean ±SD (n = 3 tanks per diet). Different letters in the columns indicate statistically significant differences among groups (one-way ANOVA with Tukey’s HSD post hoc test, $p < 0.05$)

Figure 5. Effects of experimental diets on intestinal wall histomorphometry in *O. mykiss*



This figure illustrates the effects of different dietary treatments on villus morphometric parameters, including villus length (VL), villus width (VW), lamina propria thickness (LP), and villus area (VA) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD (n = 3 tanks per diet). Different letters in the columns indicate statistically significant differences among groups (one-way ANOVA with Tukey’s HSD post hoc test, $p < 0.05$)

Figure 6. Effects of experimental diets on intestinal villus histomorphometry in *O. mykiss*

Dietary lipid levels and alterations in lipid metabolism have previously been reported to influence erythrocyte function and oxygen-carrying capacity in various fish species. Several studies have shown that high-fat diets may lead to reductions in *HGB* concentration, *HCT*, and erythrocyte indices such as *MCV* and *MCH*, while *RBC* count does not necessarily change in parallel with these alterations (Shiogiri et al., 2017; Witeska et al., 2023). Such discrepancies have been attributed to factors including feeding duration, lipid source, species-specific responses, and experimental conditions (Alami et al., 2024;).

Consistent with this body of literature, the present study demonstrated that exposure to a high-fat diet exerted suppressive effects on erythrocyte-related functional parameters represented by *HGB*, *HCT*, *MCV*, and *MCH*, without inducing a marked change in *RBC* levels. This pattern suggests that erythrocyte functional performance, rather than numerical integrity, was adversely affected. In contrast, the recovery observed in these parameters in the HFD+Q group indicates preservation of erythrocyte oxygen-carrying capacity. It has been previously reported that flavonoids, owing to their antioxidant properties, can mitigate oxidative stress-induced cellular damage and support haemoglobin synthesis, thereby

contributing to the maintenance of erythrocyte integrity (Pasdar et al., 2020). Moreover, a recent study conducted in *O. mykiss* reported that quercetin supplementation enhanced oxygen-carrying capacity and supported resistance against environmental stressors (Hoseini et al., 2025). In line with these findings, the recovery of erythrocyte functions observed following quercetin supplementation in the present study is consistent with the proposed regulatory role of flavonoids in maintaining haematological balance.

Parameters associated with coagulation, including platelet count (*PLT*) and mean platelet volume (*MPV*), have been reported to exhibit lower sensitivity to nutritional interventions compared with erythrocyte and leukocyte parameters (Witeska et al., 2023). Nevertheless, pronounced alterations in *PLT* and *MPV* values have been documented under conditions of prolonged stress, toxic exposure, or severe environmental disturbances (Zahran et al., 2018). Within this context, the absence of statistically significant differences in *PLT* and *MPV* among the experimental groups in the present study suggests that platelet dynamics may be relatively resilient to high-fat dietary interventions. However, the distributional trends observed in the quercetin-supplemented group imply that quercetin may assume a supportive, regulatory role in

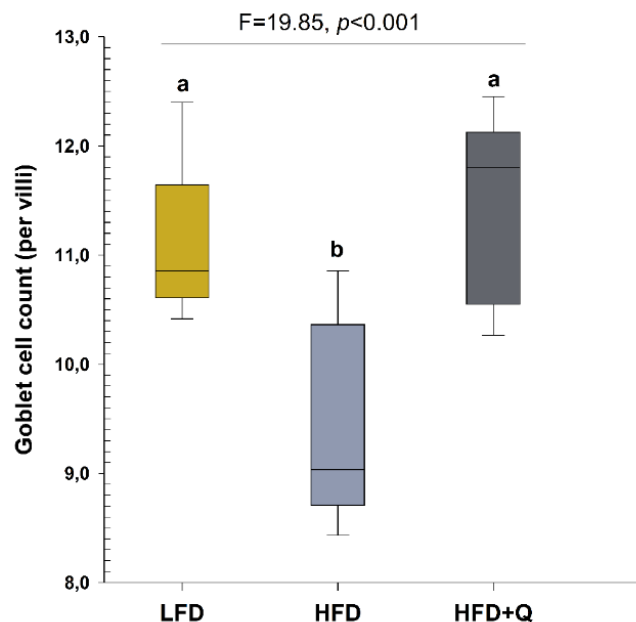
haematological homeostasis rather than exerting a direct effect on platelet-specific parameters.

Intestinal Histomorphology and Histopathology

Intestinal health plays a critical role in maintaining physiological homeostasis in aquatic organisms (Uyanga et al., 2021). It is well established that dietary composition directly affects intestinal structure and cellular organisation, thereby shaping the physiological capacity related to digestion and nutrient absorption (Hamedi et al., 2011). In this context, alterations in intestinal histomorphology provide important indicators for evaluating diet-induced physiological responses. Villus height (*VL*) and regularity are among the primary structural determinants of intestinal surface area (Awad et al., 2009), while mucins secreted by goblet cells play a central role in maintaining epithelial integrity and sustaining mucosal barrier function (Zahran et al., 2020; Feng et al., 2023). These structural and cellular characteristics are therefore considered to be closely associated with the maintenance of normal and healthy growth in fish.

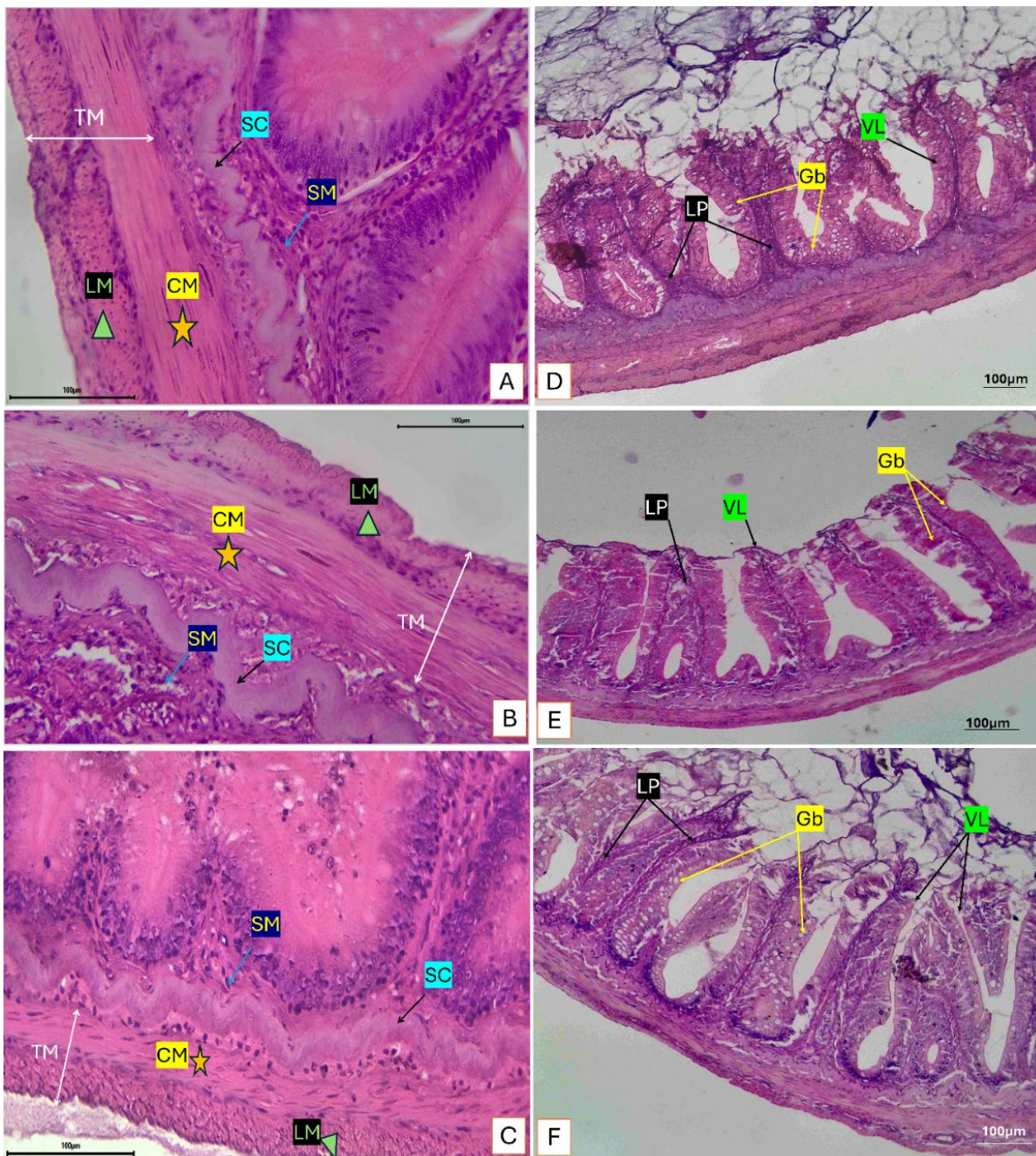
It has been reported that marked differences exist among fish species with respect to intestinal morphology and function. Herbivorous species, due to their adaptation to fibre-rich diets, are suggested to require less intensive peristaltic activity

(Nasruddin et al., 2014). In contrast, carnivorous species fed low-fibre diets may require more pronounced structural development of the muscular layers responsible for peristaltic movements to ensure efficient transport of intestinal contents. Accordingly, the thickness and organisation of intestinal muscle layers have been described as reflecting the structural basis of peristaltic activity (Nasruddin et al., 2014; Witeska et al., 2023). Within this framework, the increases observed in the thickness of the longitudinal muscularis (*LM*) and tunica muscularis (*TM*), particularly in the HFD and HFD+Q groups, suggest that the muscle layers responsible for peristalsis in *O. mykiss* may be structurally modulated in response to physiological demands. These structural alterations may represent a potential adaptive response aimed at supporting intestinal motility-related physiological capacity. When the studies demonstrate that dietary lipid levels can influence intestinal muscular layer thickness in *O. mykiss* (Liu et al., 2021) The findings indicate that remodelling of longitudinal and circular smooth muscle layers is closely associated with intestinal transport and motility capacity (Niessen et al., 2005; Khasanov et al., 2023), and the studies reporting the motility-regulatory effects of quercetin on the gastrointestinal tract (Kim et al., 2018; Modzelewska et al., 2021) When considered together, the present findings are strongly supported by the existing literature.



Values are presented as the mean \pm SD (n = 3 tanks per diet). Different letters above the bars indicate statistically significant differences among the groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$)

Figure 7. Effects of experimental diets on goblet cell counts in *O. mykiss*



Panels A–C show the tunica muscularis (TM), including the circular (CM) and longitudinal (LM) muscle layers, stratum compactum (SC), and submucosa (SM). Panels D–F display the villus (VL) structure, lamina propria (LP), and goblet cells (Gb). Representative micrographs illustrate: (A, D) normal muscular and epithelial organisation in the LFD group; (B, E) disorganisation of CM, thickened SC, submucosal oedema, and reduced goblet cells in the HFD group; (C, F) preserved structural integrity and restoration of goblet cell abundance in the HFD+Q group.

(A-C panels = 40X, D-F panels = 10X; scale bar = 100 µm, H&E)

Figure 8. Histopathological alterations in the intestinal structure of *O. mykiss* fed different experimental diets

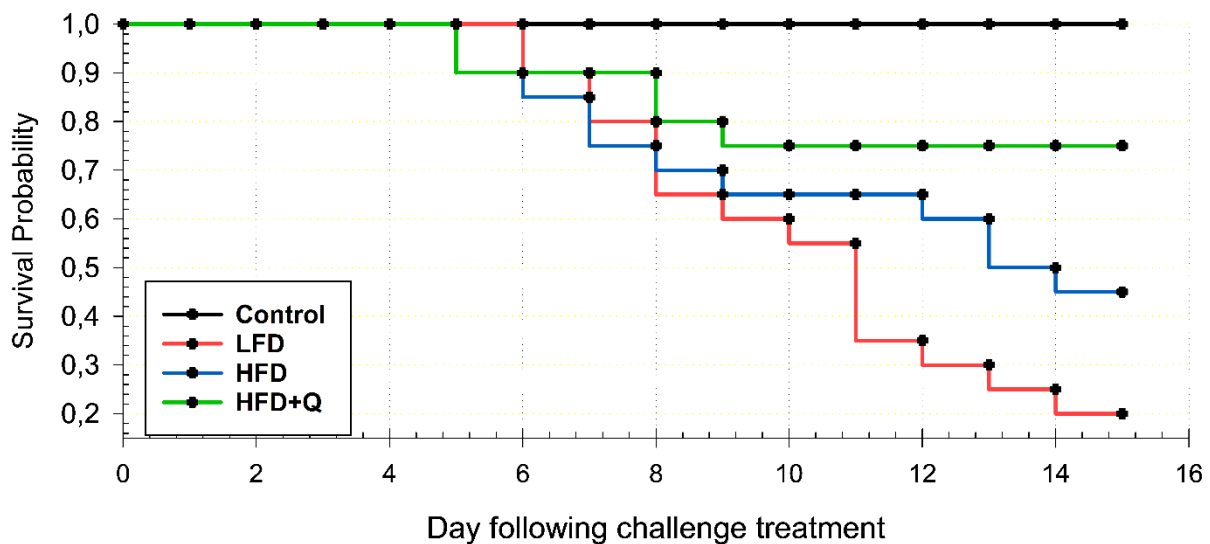


Figure 9. Kaplan–Meier survival curves of *O. mykiss* following *L. Petauri* infection after feeding with experimental diets

However, the effects of a high-fat diet on intestinal structure were not unidirectional. In the present study, histological impairments such as disorganised muscle layers, submucosal oedema, and a reduction in goblet cell number were observed in the HFD group. Goblet cell–derived mucins are known to play a critical role in maintaining mucosal barrier function, and reductions in these cells have been reported to compromise intestinal defence capacity (Zahran et al., 2020; Feng et al., 2023). Therefore, the loss of goblet cells observed in the HFD group may represent a risk condition associated with weakened mucosal integrity.

In the low-fat diet (LFD) group, although the muscle layers appeared regular, villi were notably shorter and thicker, and edematous areas were present within the lamina propria. This morphological profile suggests that intestinal surface area and tissue organisation may be physiologically constrained. Similarly, previous studies in different fish species have reported that dietary composition can be a determining factor influencing villus architecture and lamina propria integrity (Awad et al., 2009; Hamed et al., 2011; Hasan et al., 2024; Lee et al., 2024; Milián-Sorribes et al., 2024; Yan et al., 2024; Zhou et al., 2024).

In contrast, intestinal architecture was largely preserved in the HFD+Q group, as evidenced by more regularly organised muscle layers, longer and more homogeneous villi, and an increased number of goblet cells. These findings suggest that quercetin supplementation exerts a protective and regulatory effect on intestinal mucosal integrity and tissue organization. Although some studies have reported that high-fat diets do not always induce pronounced negative effects on intestinal

morphology in *O. mykiss* (Liu et al., 2021) The goblet cell loss observed in the HFD group in the present study indicates that mucosal defence mechanisms may be sensitive to dietary conditions. The ability of quercetin supplementation to compensate for this loss by increasing goblet cell density suggests a strengthening of the mucus layer and enhanced preservation of epithelial integrity. Similarly, the supportive effects of quercetin on intestinal mucosal integrity have also been reported in *O. niloticus* (Abdo et al., 2024).

Resistance Against *Lactococcus petauri* Infection

The challenge trial conducted with *Lactococcus petauri* provides a functional model for evaluating how dietary composition and the inclusion of alternative feed additives can shape physiological responses to infection in fish. In this context, experimental disease challenge trials are widely used to assess the effects of dietary interventions and feed additives on host resistance and host–pathogen interactions in fish (Austin & Zhang, 2006).

Haematological parameters in fish are influenced by multiple factors, including age, sex, life stage, seasonal variations, and nutritional status (Alcorn et al., 2003; Morgan et al., 2008; Seibel et al., 2021; Nabi et al., 2022). This multifactorial structure makes it difficult to establish reliable haematological reference intervals for fish species. Notably, the only study reporting reference intervals for *O. mykiss* in accordance with the standards of the American Society for Veterinary Clinical Pathology (ASVCP) was presented by Nabi et al. (2022). In the present study, although the total leukocyte (*WBC*) counts and leukocyte subgroups in the HFD group were lower than those in the LFD and HFD+Q groups, they

remained within the reported reference ranges. On the other hand, high-energy diets and polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA), have been previously reported to support faster and more effective immune responses (Calder, 2015; Cornet et al., 2018; Mendivil, 2021; Magalhães et al., 2023). In addition, adequate energy availability has been shown to be critical for sustaining host defence during infection, whereas insufficient energy intake may indirectly suppress immune responses (Austin & Zhang, 2006). In the present study, the gross energy content of the diets was higher in the HFD group than in the LFD group (22.21 MJ kg⁻¹ vs 19.69 MJ kg⁻¹). In this context, although statistically significant reductions in WBC and leukocyte subgroups were observed in the HFD group, these findings appear to be consistent with previously described pathophysiological mechanisms and host responses during lactococcosis in salmonids (Vendrell et al., 2006). Nevertheless, the higher survival rate observed in the HFD group compared with the LFD group may seem contradictory to the haematological findings at first glance. Evidence from fish challenge studies indicates that improvements in nutritional and energetic status, including lipid-related components, can enhance post-challenge outcomes such as survival. This highlights that infection outcome is shaped not only by single haematological indices but also by the host's metabolic capacity (Deng et al., 2013; Seibel et al., 2021; Nabi et al., 2022). This apparent discrepancy suggests that short-term survival during acute bacterial infections is determined not only by WBC counts or leukocyte abundance, but also by the host's capacity to meet the high energy demands associated with inflammation and tissue repair. Although the underlying biochemical mechanisms were not directly evaluated in the present study, the findings indicate that reduced lipid intake in the LFD group may have limited the ability of fish to develop an effective physiological response against *L. petauri* infection. Consequently, the higher survival rate observed in the HFD group, despite leukocyte suppression, may be attributed to improved infection tolerance supported by sufficient energy and lipid availability. This finding emphasises that host defence during infection is shaped not only by immune cell dynamics but also by metabolic and energy-related factors.

The survival advantage observed in the quercetin-supplemented HFD+Q group may be attributed to the combined effects of metabolic support provided by high energy and lipid availability and the regulatory role of this compound in maintaining haematological balance. The preservation of WBC and leukocyte subgroups in this group may have contributed to sustaining immune responsiveness during infection. Plant-

derived flavonoids have been reported to support host defence by modulating immune cell functions (Awad et al., 2015; Zhang et al., 2021). Furthermore, quercetin has been shown to exert antimicrobial activity by disrupting bacterial cell wall and membrane integrity (Wang et al., 2018). In this context, the effects of quercetin are considered to play a regulatory and supportive role in enhancing the physiological capacity provided by high-energy diets.

Given the increasing recognition of *L. petauri* as an emerging pathogen in salmonids, the survival advantage associated with quercetin supplementation observed in this study is particularly noteworthy. The reclassification of some outbreaks previously attributed to *L. garvieae* as being caused by *L. petauri* has necessitated a reassessment of the role of this pathogen in aquaculture (de Ruyter et al., 2023). In this context, evidence suggesting that plant-derived compounds such as quercetin can support physiological resistance to infection under high-fat dietary conditions offers an important perspective for the development of non-antibiotic strategies.

Overall, the findings of the present study indicate that dietary lipid level and quercetin supplementation can modulate physiological responses to *L. petauri* infection in fish. The positive effects of a quercetin-supplemented high-fat diet on survival may be associated with the regulatory role of this compound in maintaining physiological homeostasis. Nevertheless, further detailed immunological and molecular investigations are required to elucidate the underlying mechanisms responsible for the observed effects.

Conclusion

This study demonstrated that dietary lipid levels in *O. mykiss* exert significant effects on haematological homeostasis, intestinal structural integrity, and resilience against *L. petauri* infection. The high-fat diet (HFD) negatively impacted immune cell dynamics through a suppressive effect on the leukocyte profile (*WBC*, *LYM*, *MID*, and *GRAN*) and pointed toward a potential functional weakening of oxygen-carrying capacity by creating a downward trend in *HGB*, *HCT*, and erythrocyte indices (*MCV*, *MCH*) without distinct changes in erythrocyte count (*RBC*). Additionally, the HFD was associated with histopathological changes such as irregularities in intestinal muscle layers, submucosal oedema, and a reduction in goblet cells, which may weaken mucosal defence.

The quercetin-supplemented high-fat diet (HFD+Q) significantly limited these adverse effects of the HFD, presenting a more balanced physiological profile characterised by the preservation of leukocyte subgroups, more stable intestinal wall/mucosa organisation, and recovery of goblet cell density. These structural and haematological improvements

found a functional counterpart to the significant survival advantage observed in the HFD+Q group during the *L. petauri* challenge trial. In particular, the earlier cessation of mortality and the higher survival probability suggest that quercetin can support disease resistance.

In conclusion, quercetin supplementation can be considered a functional feed additive that supports haematological balance and intestinal barrier integrity under high-fat feeding conditions, and accordingly, carries the potential to increase resistance capacity against *L. petauri*, one of the causative agents of lactococcosis. However, due to the use of a single dose level and the fact that mechanistic endpoints (antioxidant/immune gene expression, mucosal immunity markers, etc.) were not directly measured, it is recommended to further detail the mechanism of action through dose-response designs and molecular/immunological validations.

Although the present findings support the functional potential of quercetin in aquaculture nutrition, further immunological and molecular studies are recommended to elucidate its mechanisms of action and to determine optimal dietary inclusion levels under different culture conditions.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The animal study was approved by Recep Tayyip Erdoğan University Experimental Animals Ethics Committee (decision number: 2023/30, date: June 13, 2023). The study was conducted in accordance with the local legislation and institutional requirements.

Data availability: The data will be made available upon request from the author.

Funding disclosure: This study was supported by the Scientific Research Projects Coordination Unit of Recep Tayyip Erdoğan University under grant ID: FBA-2023-15.

Acknowledgements: The author, Dr. Ö.K., would like to thank Dr. Salih KUMRU for proofreading the manuscript in its original language. Special thanks are also extended to Dr. Akir ER for his assistance with the challenge test protocol and to Yusuf Demir, our Data Collection Assistant, for his assistance with record keeping during data collection

Disclosure: -

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The role of *Eleutherine palmifolia* (Dayak Onion) as a natural immunostimulant for enhancing fish disease resistance in aquaculture systems

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Cite this article as:

Fahturohman, A.B., Takwin, B.A., Ristyningrum, W., Muna, Z., Feska, S. (2026). The role of *Eleutherine palmifolia* (Dayak Onion) as a natural immunostimulant for enhancing fish disease resistance in aquaculture systems. *Aquatic Research*, 9(2), 148-165. <https://doi.org/10.3153/AR26013>

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Submitted: 26.12.2025

Revision requested: 02.02.2026

Last revision received: 09.02.2026

Accepted: 10.02.2026

Published online: 30.03.2026

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ABSTRACT

The global food crisis, driven by population growth, climate change, and natural resource degradation, has intensified the need for efficient, affordable, and environmentally sustainable sources of animal protein. In aquaculture, this demand has accelerated the exploration of alternative feed ingredients and natural additives that enhance immune responses, suppress pathogens, and promote fish growth. *Eleutherine palmifolia* (Dayak onion), an endemic plant from Kalimantan, has emerged as a promising candidate due to its strong antibacterial activity and safety profile without chemical residues. Using a PRISMA-guided systematic review, this study synthesises current evidence on the phytochemical composition, immunological mechanisms, antibacterial activity, and application potential of *E. palmifolia* in sustainable aquaculture. The extract exhibits anti-inflammatory effects by modulating pro-inflammatory cytokines, enhancing TNF- α expression while reducing IL- β levels, thereby supporting a balanced immune response without excessive oxidative stress. Both ethanol and aqueous extracts effectively inhibit *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae*. The plant's secondary metabolites, including flavonoids, naphthoquinones, polyphenols, tannins, and saponins, demonstrate broad biological activities that improve fish health, growth, and disease resistance. Overall, *E. palmifolia* holds strong potential as an immunostimulant, antioxidant, and phytobiotic agent for environmentally friendly aquaculture practices.

Keywords: *Eleutherine palmifolia*, Immunostimulant, Antibacterial activity, Fish health, Sustainable aquaculture

Introduction

The global food crisis, driven by population growth, climate change, and natural resource degradation, has intensified the need for innovation in sustainable food production systems (Qu et al., 2024; Said et al., 2025). Aquaculture plays a strategic role in addressing this challenge by providing efficient and environmentally friendly sources of animal protein. According to FAO (2025), global fish production reached 223.2 million tons in 2022, reflecting a pronounced transition from capture fisheries to aquaculture. This shift is particularly evident in Asia, which contributes nearly 90% of global aquaculture output and positions the region as a central driver of food security and low-emission fisheries development (Chan et al., 2024). Nevertheless, the long-term sustainability of aquaculture remains constrained by recurrent disease outbreaks that cause substantial production losses and economic impacts (Subhashreedevasena et al., 2022; Patil et al., 2025)

In response to these challenges, global research efforts increasingly emphasise the development of alternative feed ingredients and natural functional additives that enhance immune responses, suppress pathogenic infections, and improve fish growth performance (Idenyi et al., 2022; Van Doan et al., 2023; Banu et al., 2025). Among these strategies, phytobiotics, bioactive compounds derived from medicinal plants, have gained considerable attention due to their multifunctional biological activities, lack of antibiotic residues, and environmental compatibility (Mabrouk et al., 2025). Consequently, locally sourced herbal plants are increasingly recognised as viable and eco-friendly alternatives to antibiotics in modern aquaculture systems (Banu et al., 2025).

Various local herbal plants, including garlic (*Allium sativum*), pennywort (*Phyllanthus niruri*), turmeric (*Curcuma longa*), and temulawak (*Curcuma xanthorrhiza*), have been widely evaluated as natural immunostimulants in aquaculture (Rezaei et al., 2022; Khieokhajokhet et al., 2023; Rosidi et al., 2025). However, the efficacy of phytobiotics strongly depends on dosage, extraction methods, and the stability of bioactive compounds during feed formulation and coating processes (Pudota et al., 2025). Moreover, many phytobiotic candidates still require dose optimisation and long-term toxicity assessments to ensure safe and effective application in intensive farming systems. By contrast, probiotics as biological additives often exhibit instability in microbial populations under fluctuating culture conditions, leading to inconsistent performance outcomes (Rahayu et al., 2024). Similarly, low-

dose vaccines show limited efficacy, as they frequently fail to provide long-term protection against diverse pathogen strains, particularly in high-density aquaculture systems (Miccoli et al., 2021).

One of the endemic plants from Kalimantan that has gained increasing attention is *Eleutherine palmifolia*, commonly known as Dayak onion. This species offers distinct advantages over other medicinal plants due to its strong antibacterial activity, which mimics antibiotic mechanisms while remaining safe and residue-free (Arbain et al., 2022). This characteristic highlights the importance of exploring Indonesia's endemic flora, which possesses broad bioactivity and ecological relevance. *Eleutherine palmifolia* contains diverse bioactive compounds, including alkaloids, flavonoids, naphthoquinones, saponins, tannins, and triterpenoids, that exhibit antibacterial, anti-inflammatory, antioxidant, and immunomodulatory properties (Chabib et al., 2018; Harlita et al., 2018; Masfria & Tampubolon, 2019; Annisa et al., 2020; Hongthongkham et al., 2025). The presence of these compounds supports the potential application of *E. palmifolia* as a phytobiotic in modern aquaculture systems. Mechanistically, the bioactive constituents of *Eleutherine palmifolia* enhance fish disease resistance through multiple innate immune pathways. These effects include increased phagocytic activity, stimulation of lysozyme production, and activation of non-specific immune signalling against major aquaculture pathogens such as *Aeromonas hydrophila* and *Vibrio haryeyi* (Hasim et al., 2023).

Previous studies have demonstrated that dietary supplementation with Dayak onion extract enhances phagocytic activity, total leukocyte counts, and post-infection survival rates in fish challenged with pathogenic bacteria (Nur et al., 2020; Hasim et al., 2023). Its ethanol extract also exhibits strong antibacterial activity against *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* by disrupting bacterial cell membranes and inducing protein denaturation (Harlita & Aina, 2023; Sirajuddin et al., 2025). Beyond its immunological and antibacterial effects, the complex carbohydrates and polyphenols present in *Eleutherine palmifolia* function as natural prebiotics, supporting gut microbiota balance and enhancing nutrient absorption through fermentative activity and improved intestinal functionality (Andriani et al., 2024). The synergistic integration of immunostimulatory, antibacterial, antioxidant, and growth-promoting effects positions *E.*

palmifolia as a promising phytobiotic capable of partially replacing antibiotics and synthetic additives in aquaculture feeds.

Despite growing interest in *Eleutherine palmifolia* as a natural immunostimulant in aquaculture, existing studies remain fragmented and largely descriptive. Most investigations emphasise isolated outcomes such as antibacterial activity, haematological responses, or survival rates without integrating these findings into a coherent mechanistic framework. Furthermore, immune modulation pathways are frequently inferred rather than experimentally validated, and standardised extraction methods, dosage regimes, and application strategies remain lacking. Notably, the performance of *E. palmifolia* in intensive aquaculture systems, including biofloc and recirculating aquaculture systems, has received limited attention. These knowledge gaps constrain the translational application of *E. palmifolia* as a phytobiotic alternative to antibiotics.

Accordingly, this article is designed as a PRISMA-guided systematic integrative review that synthesises existing evidence on the application of *Eleutherine palmifolia* as a phytobiotic in aquaculture systems. The novelty of this review resides in three principal contributions. First, unlike previous reviews that largely emphasise phytochemical identification or general pharmacological properties, this study systematically integrates aquaculture-specific evidence linking phytochemical profiles to functional immune, antibacterial, antioxidant, and growth-related outcomes in aquatic species. Second, this review explicitly distinguishes between direct experimental evidence and indirect mechanistic inference, thereby providing a critical evidence-weighted framework that clarifies the strength, limitations, and translational relevance of existing findings. Third, by contextualising current evidence within modern intensive aquaculture systems, including biofloc and recirculating aquaculture systems (RAS), this review extends applicability beyond laboratory-scale studies toward production-oriented scenarios. Through this integrative and critical approach, the present review aims to advance mechanistic understanding, identify methodological gaps, and support the rational development of *E. palmifolia* as a sustainable phytobiotic alternative to antibiotics in aquaculture.

Materials and Methods

Literature Search Strategy

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and reproducibility. A comprehensive literature search was performed using three major scientific databases: Scopus, Web of Science, and PubMed. The search was conducted between January 2016 and June 2025 using combinations of the following keywords: “*Eleutherine palmifolia*” OR “Dayak onion” AND “aquaculture” OR “immunostimulant” OR “phytobiotic” OR “antibacterial” OR “growth performance.”

Study Selection Process

All retrieved records were imported into reference management software, and duplicate records were removed. Titles and abstracts were screened for relevance, followed by full-text assessment based on inclusion and exclusion criteria. The study selection process followed the PRISMA flow diagram, resulting in the final dataset used for qualitative synthesis. The study selection process is summarised in the PRISMA flow diagram (Figure 1).

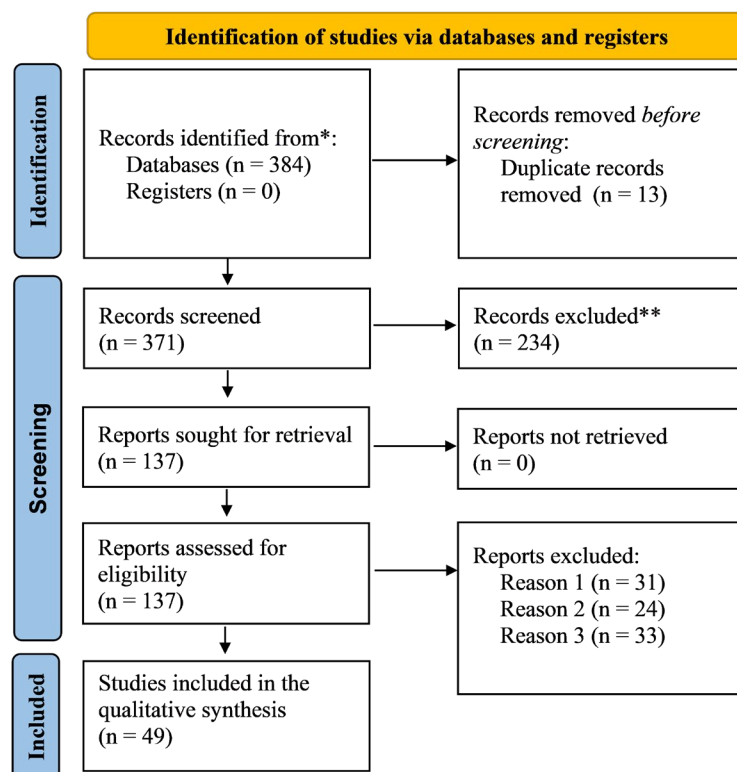


Figure 1. PRISMA flow diagram showing the literature search and selection process for studies on *Eleutherine palmifolia* in aquaculture applications

Due to the broad nature of keyword combinations, an initial title and abstract screening was applied to exclude records not directly related to *Eleutherine palmifolia* and aquaculture applications. All full-text articles considered relevant after title and abstract screening were successfully retrieved and assessed for eligibility. Full-text excluded with reasons (n= 88): not related to aquaculture species (n= 31), human studies only (n= 24), and insufficient experimental outcomes (n=33).

Inclusion and Exclusion Criteria

Inclusion criteria:

1. Original research articles or review papers
2. Studies evaluating *Eleutherine palmifolia* or its extracts
3. Research related to aquaculture species (fish or crustaceans)
4. Outcomes related to immunity, antibacterial activity, antioxidant capacity, growth performance, or stress resistance

Exclusion criteria:

1. Non-peer-reviewed articles, theses, or conference abstracts without full text
2. Studies focusing solely on human applications
3. Articles lacking clear experimental design or outcome parameters

Data Extraction and Synthesis

From each selected study, the following information was extracted: study type, aquaculture species, plant part and extraction method, dosage and application strategy, measured immune, antibacterial, antioxidant, or growth parameters, main outcomes, and reported limitations. Due to heterogeneity in experimental designs, species, and outcome measurements, a quantitative meta-analysis was not feasible. Therefore, a qualitative integrative synthesis was applied. Accordingly, this article is explicitly defined as a systematic-integrative review, combining a PRISMA-guided systematic literature selection process with an integrative analytical framework that connects phytochemical characteristics, immunological mechanisms, antibacterial actions, and physiological performance outcomes in aquaculture systems. This approach enables comprehensive evidence integration while maintaining methodological transparency and reproducibility.

Study Quality Assessment

To evaluate the methodological robustness of the included studies, a qualitative study quality assessment was conducted. Each study was appraised based on predefined criteria, including clarity of experimental design, description of extrac-

tion and application protocols, sample size adequacy, presence of control groups, and relevance of outcome parameters related to immunity, antibacterial activity, growth performance, or physiological responses. Studies were not excluded based on quality scores; instead, variations in methodological rigour were considered during evidence interpretation and synthesis. Given the heterogeneity in species, experimental durations, exposure routes, and measured endpoints, a formal quantitative scoring system was not applied. This approach aligns with integrative review methodologies, which emphasise contextualised interpretation of evidence rather than exclusionary quality thresholds, while still allowing critical evaluation of study reliability and comparability.

Critical Appraisal

A narrative critical appraisal was applied to identify methodological strengths, limitations, inconsistencies, and knowledge gaps across studies, with particular emphasis on dosage standardisation, molecular validation, and applicability in modern intensive aquaculture systems.

Risk of Bias and Limitations

Several sources of potential bias were identified across the reviewed studies. Selection bias was evident in studies employing single-dose designs or limited sample sizes, which may restrict the generalizability of reported outcomes. Performance and detection biases were also observed, as many in vivo experiments relied predominantly on haematological or survival parameters without molecular validation of immune signalling pathways. In addition, variability in extraction methods, solvent types, and plant parts used introduces heterogeneity that may influence bioactive compound composition and biological efficacy.

Publication bias cannot be excluded, as studies reporting positive effects of *Eleutherine palmifolia* were more prevalent than those documenting neutral or negative outcomes. Furthermore, most experiments were conducted under controlled laboratory conditions or short-term trials, limiting extrapolation to intensive aquaculture systems such as biofloc or recirculating aquaculture systems (RAS). These limitations were explicitly considered during evidence synthesis and highlight the need for standardised methodologies, long-term evaluations, and system-based studies to strengthen the translational applicability of *E. palmifolia* as a phytobiotic alternative to antibiotics.

Results and Discussion

Dayak Onion (*Eleutherine palmifolia*)

Dayak onion belongs to the genus *Eleutherine*, which is native to tropical America but has long been introduced and cultivated throughout Asia. A key botanical record dates to 1912, when Merrill documented its presence in the Philippines and classified it as *Eleutherine palmifolia* L. Merr., later recognised as a botanical synonym of *Sisyrinchium palmifolium* L. Over time, this species adapted successfully to Southeast Asian environments and acquired distinct cultural significance. In Kalimantan, its tubers have been used for generations by the Dayak people as traditional medicine to treat various health conditions. This close association with ethnomedical practices led to the widespread use of the local name “Bawang Dayak,” which was subsequently adopted in scientific literature and is now commonly used to refer to this species in Indonesia. Figure 2. Morphological characteristics of Dayak onion, showing the whole plant, bright red bulbs, and dried bulb slices.

Dayak Onion Phytochemical Content

Local communities have traditionally used dayak onion to treat various health conditions, including hypertension, hypercholesterolemia, diabetes, gastric disorders, constipation, and stroke, and is also consumed as a postnatal herbal drink. In addition, Dayak onion exhibits antifungal and antioxidant activities (Harlita et al., 2018). Phytochemical analyses have shown that Dayak onion extracts contain diverse secondary metabolites, including flavonoids, naphthoquinones, anthraquinones, alkaloids, saponins, tannins, triterpenoids, and steroids.

Anthraquinones are naturally occurring organic compounds belonging to the phenolic quinone derivatives and are widely distributed in medicinal plants. Anthraquinone extracts derived from *Rheum officinale* Baill. contain bioactive constituents such as emodin, chrysophanol, and rhein, and have long been used as immunostimulants (Huang et al., 1995). More recent studies have demonstrated that anthraquinone extracts

significantly enhance immune function, improve stress resistance, and promote growth performance in common carp and giant freshwater prawn (Xie et al., 2008). Findings by Liu et al. (2012) further revealed that dietary supplementation with 0.1% anthraquinone extract from *R. officinale* Baill. effectively increased the resistance of *Megalobrama amblycephala* to infection by the pathogenic bacterium *Aeromonas hydrophila*. This enhanced resistance was mediated through strengthened nonspecific immune responses, including increased lysozyme and alkaline phosphatase activities, reduced physiological stress responses (cortisol, AST, and ALT), and improved hepatic antioxidant capacity, as indicated by elevated catalase and superoxide dismutase activities accompanied by reduced malondialdehyde levels. In addition, the induction of hepatic *HSP70* gene expression before and during the early phase of infection highlights the role of anthraquinones in maintaining cellular homeostasis and stress tolerance during pathogenic challenge.

Saponins are glycoside-based secondary metabolites widely distributed in plants and are well known for their broad biological activities, including antibacterial, immunomodulatory, and antioxidant effects. In aquaculture, saponins have gained attention as phytogetic feed additives and non-antibiotic alternatives for disease control, particularly amid growing concerns about antibiotic resistance and chemical residues in aquatic products. Nevertheless, their efficacy and safety depend heavily on source, dosage, and application method, necessitating careful empirical evaluation. Boran et al. (2015) assessed the antibacterial activity of green tea (*Camellia sinensis*) seed extracts and their secondary metabolite saponin against five major fish pathogens in rainbow trout (*Oncorhynchus mykiss*). The results demonstrated that both saponin and watered tea seed (WTS) exhibited in vitro antibacterial activity, with statistically significant inhibition particularly against *Listonella anguillarum*. In vivo, fish fed saponin-containing diets showed significantly increased survival following challenge with *L. anguillarum*. However, mild and non-vital histopathological alterations were observed in the gills and liver, emphasising that the application of saponins requires careful consideration of dosage and safety.



Figure 2. Dayak onion: (a) whole plant, (b) bright-red bulbs, and (c) dried bulb slices (Harlita et al., 2018)

Recent evidence indicates that plant-derived polyphenols, such as tannic acid, can significantly enhance antioxidant capacity, improve liver and intestinal health, and increase fish survival, particularly under nutritional or metabolic stress conditions. Dietary supplementation with appropriate levels of tannic acid has been shown to elevate catalase activity, reduce malondialdehyde accumulation, decrease hepatic glycogen deposition, and alleviate histopathological alterations in the liver and intestine, thereby improving overall physiological resilience without endocrine manipulation (Zhang et al., 2022). Supporting this concept, Wang et al. (2024), reported that dietary tannic acid supplementation at 200–400 mg/kg in a high-carbohydrate diet enhanced antioxidant capacity, reduced oxidative stress, and improved liver and intestinal health in *Micropterus salmoides*, as evidenced by decreased MDA levels, reduced hepatocyte vacuolation, and improved intestinal morphology. However, higher supplementation levels suppressed growth performance due to inhibition of digestive enzyme activity

Triterpenoids are increasingly recognised as functional phytochemical compounds in aquaculture due to their immunomodulatory, antioxidant, and antimicrobial properties. Dietary supplementation with triterpenoid-rich plant extracts has been shown to enhance innate immune responses, improve epithelial and gut integrity, and increase resistance to bacterial infections in fish. Notably, triterpenic acid- and polyphenol-rich extracts from *Olea europaea* significantly enhanced systemic immunity and protection against furunculosis in Atlantic salmon smolts, demonstrating clear in vivo efficacy in aquaculture settings (Salomón et al., 2021). In addition, triterpenoid compounds such as glycyrrhizic and glycyrrhetic acids from *Glycyrrhiza glabra* have been reported to exert immunomodulatory, antioxidant, anti-inflammatory, and antimicrobial effects, contributing to improved health status and disease resistance in aquatic animals (Abasubong et al., 2024).

Steroid compounds have historically been used in aquaculture to enhance fish growth and reproduction; however, growing evidence indicates that their application may exert broad adverse effects, including suppression of immune function (reduced T-cell activity and B-cell proliferation), disruption of metabolic and digestive processes, impairment of reproductive systems, and detrimental effects on the hematopoietic system. These concerns raise serious issues regarding food safety and ecosystem health (Islam et al., 2024).

The phytochemical profile of *Eleutherine palmifolia* indicates the presence of multiple bioactive compounds with significant biological activities. These metabolites contribute to the antibacterial, antioxidant, immunomodulatory, and anti-

inflammatory properties of the extract. Several studies have reported that naphthoquinones and anthraquinones represent the dominant metabolite groups in Dayak onion bulbs, while flavonoids, alkaloids, tannins, and triterpenoids/steroids have been identified through qualitative phytochemical screening (Kamarudin et al., 2021; Mukti et al., 2023). Collectively, these findings highlight Dayak onion as a promising source of bioactive metabolites with potential applications in health-related fields and sustainable aquaculture. Table 1. Summary of major bioactive compounds identified in Dayak onion.

Table 1. Dayak Onion Phytochemical Content

Compound Name	References
Naphthoquinone	(Mutiah et al., 2020; Kamarudin et al., 2021)
Naphthalene	(Mutiah et al., 2020; Kamarudin et al., 2021)
Polyphenols	(Mutiah et al., 2020; Qureshi & Javed, 2022; Wahdaningsih et al., 2024)
Alkaloid	(Harlita et al., 2018)
Saponin	(Harlita et al., 2018)
Tannin	(Harlita et al., 2018)
Isoliquiritigenin	(Mutiah et al., 2020)
Sitosterol	(Saputra et al., 2016)
Glicoside	(Mukti et al., 2023)
Triterpenoid	(Gunawan et al., 2020)
Eleutherol	(Muti'ah et al., 2020)
Eleutherin	(Muti'ah et al., 2020)
Isoeleuterin	(Muti'ah et al., 2020)
Hongconin	(Muti'ah et al., 2020)
Elecanacin	(Muti'ah et al., 2020)
Isoeleutherol	(Muti'ah et al., 2020)
Dihydroeleuterinol	(Muti'ah et al., 2020)
1,3,6-trihidroksi-8-metilantrakuinon	(Muti'ah et al., 2020)
Eleuterinosida A	(Muti'ah et al., 2020)
6,8-dihidroksi-3,4- dimetoksi-1-metilantraquin-on-2-asam karboksilat metal ester	(Muti'ah et al., 2020)
Oxyresveratrol	(Muti'ah et al., 2020)
Isoliquiritigenin	(Muti'ah et al., 2020)
4-hidroksi-eleuterin	(Muti'ah et al., 2020)

The Role of Dayak Onion (Eleutherine palmifolia) as a Phytobiotic in Functional Fish Feed

Figure 3. Schematic illustration of the mechanisms by which *Eleutherine palmifolia* (Dayak onion) extract enhances physiological performance, immune responses, and fish growth. Bioactive compounds, including flavonoids, saponins, and tannins, act as natural antioxidants and immunostimulants that strengthen host defence systems. The synergistic antioxidant and immunomodulatory effects of Dayak onion extract increase phagocytic activity and total leukocyte counts, key indicators of enhanced non-specific immunity (Yuanita et al., 2023; Sirajuddin et al., 2025). Enhanced phagocytic function subsequently improves haematological parameters, including erythrocyte count, haemoglobin concentration, and hematocrit values, reflecting increased resilience to environmental stress. In addition, phenolic compounds from Dayak onion exhibit antibacterial activity against pathogenic bacteria, reducing the risk of secondary infections that impair productivity and indirectly improving feed efficiency and growth performance (Hasim et al., 2023).

The anti-inflammatory activity of Dayak onion extract is reflected in the modulation of pro-inflammatory cytokines, characterised by increased TNF- α and reduced IL- β expression, indicating balanced immune regulation without inducing excessive oxidative stress (Chabib et al., 2018; Mutiah et al., 2020). Its bioactive compounds also reduce malondialde-

hyde (MDA) levels and enhance the activity of key antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT) (Susilowati & Setiawan, 2020). The reciprocal interaction between enhanced antioxidant defences and improved immune function provides a strong scientific rationale for the use of Dayak onion extract as a functional feed additive (Fransira et al., 2020). Overall, Figure 3 illustrates that Dayak onion extract represents a promising natural alternative to synthetic antibiotics in aquaculture feeds, improving growth performance and disease resistance while supporting sustainable and environmentally friendly farming practices. The utilisation of local plant resources, such as Dayak onion, therefore, constitutes an important strategy for reducing reliance on synthetic antibiotics in aquaculture systems (Phaik Sim et al., 2019).

Research on Dayak Onion in Aquaculture

Studies evaluating Dayak onion as a bioactive feed ingredient have been conducted in several aquaculture species, including carp, tilapia, and tiger shrimp. These studies consistently demonstrate the potential of Dayak onion as an immunostimulant, antioxidant, and enhancer of physiological performance. Table 2. Summarises representative studies investigating the application of Dayak onion in aquaculture systems.

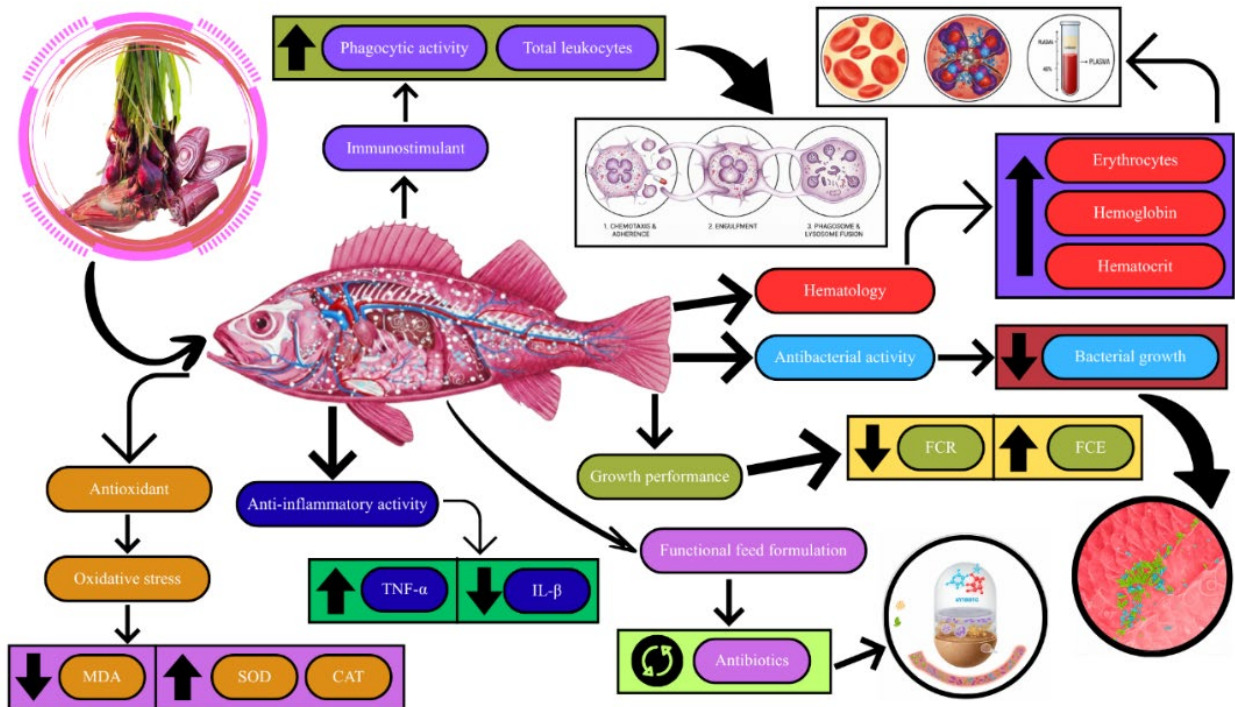


Figure 3. Mechanisms by which Dayak onion (*Eleutherine palmifolia*) extract enhances physiological and immunological performance in fish

Table 2. Research on the use of dayak onions in aquaculture

Biota	Plant Parts	Treatment	Result	Reference
Carp (<i>Cyprinus carpio</i>)	Extract+soaking	50, 60, 70 and 80 ppm	A crude extract of Dayak onion at a dose of 80 ppm was shown to improve tissue damage in carp infected with <i>Aeromonas hydrophila</i> in a dose-dependent manner.	(Maftuch, H. Suprastyani, et al., 2018)
Tilapia	Powder+feed	powders of 5% (P5), 10% (P10), and 15% (P15), while the extract was 0.5% (E05)	The addition of 15% powder and 0.5% crude Dayak onion extract significantly improved the immunity and blood of the fish. SR 50%, after being infected with <i>A. hydrophilla</i>	(Fauzi et al., 2024)
Tilapia	Extract+soaking	30 ppm extract (A), 50 ppm extract (B), 70 ppm extract (C)	Treatment C (70 ppm) SR 86.67%	(Fransira et al., 2023)
Tilapia	Extract+ application to wounds	(A: 10 ppt/ind), (B: 20 ppt/ind),	Treatment B (20 ppt/ind) SR 100%	(Akbar, 2017)

Notably, several studies reported enhanced haematological and immune-related parameters without a proportional increase in survival rate (SR), particularly under bacterial challenge conditions. This apparent discrepancy suggests that improved innate immune indicators alone may not be sufficient to guarantee survival, especially when infection pressure, pathogen virulence, or environmental stressors exceed physiological buffering capacity. Short experimental durations, single-dose designs, and the absence of molecular validation of immune signalling pathways may further obscure the relationship between immunological enhancement and survival outcomes. Moreover, suboptimal timing of phytobiotic administration relative to pathogen exposure could limit protective efficacy despite elevated immune indices.

Evidence Synthesis of *Eleutherine palmifolia* Applications in Aquaculture

The main characteristics, experimental designs, and key outcomes of representative studies evaluating *Eleutherine palmifolia* in aquaculture systems are summarised in Table 3.

As shown in Table 3, most in vivo studies report positive immunological and growth-related outcomes; however, methodological limitations such as short experimental duration, single-dose designs, and limited molecular validation remain

prevalent. Despite the consistently positive biological responses reported across studies, considerable variability remains in the effective doses of *Eleutherine palmifolia* applied to different aquaculture species. This interspecific variation can be attributed to differences in digestive physiology, metabolic rate, immune system sensitivity, and routes of administration. Fish species with faster metabolic turnover and higher feeding rates may require lower dietary inclusion levels to achieve immunostimulatory effects. In contrast, immersion-based applications often demand higher concentrations to ensure sufficient bioactive uptake through the gill and skin surfaces. In addition, species-specific tolerance thresholds and differences in gut microbiota composition may influence the bioavailability and efficacy of phytochemical compounds, resulting in divergent optimal dose ranges across taxa. Beyond their antibacterial effects, onion-based herbal therapeutics, including *Allium cepa* and *Allium sativum*, have also been shown to exhibit strong antiparasitic activity against major protozoan parasites in aquaculture, particularly *Ichthyophthirius multifiliis* and *Trichodina* spp (Özil, 2023; Gadallah et al., 2024). These findings suggest that onion-related phytochemicals may provide broader protective benefits encompassing both bacterial and parasitic disease management and support the potential antiparasitic relevance of *E. palmifolia*. However, direct experimental evidence remains limited and warrants further investigation.

Table 3. Summary of representative studies evaluating *Eleutherine palmifolia* in aquaculture systems

Study type	Species	Dose/ Application	Main Outcome	Limitation	Reference
In vivo immersion trial	Nile tilapia (<i>Oreochromis niloticus</i>)	30,50,70 mg/L (immersion)	Improved haematological parameters: ↑ erythrocytes, lymphocytes, Hb, Ht: ↓ leucocytes, monocytes, neutrophils	No molecular immune markers; no growth or feed data	(Fransira et al., 2020)
In vitro antibacterial assay	<i>Pseudomonas fluorescens</i>	Disk diffusion, MIC not stated	Inhibition zone formed; confirmed antibacterial activity of phenol, flavonoid, tannin, and saponin	No MIC quantification; no comparison with antibiotics	(Fransira et al., 2020)
In vivo immersion trial	Common carp (<i>Cyprinus carpio</i>)	50, 60, 70, 80 ppm (immersion)	Dose-dependent histopathological recovery in the gills, kidney, liver, and muscle; reduced necrosis, oedema, and congestion	No molecular immune markers; no haematology or survival data	(Maftuch, H Suprastyani, et al., 2018)
In vitro antibacterial assay (preliminary)	<i>Aeromonas hydrophilla</i>	Not specified	Confirmed antibacterial activity of Dayak onion extract	No MIC or inhibition zone data; not detailed in this article	(Maftuch, H Suprastyani, et al., 2018)
In vivo feeding & challenge trial	Tiger shrimp (<i>Penaeus monodon</i>)	6 ppm, 12 ppm, 18 ppm (extract applied in culture water; shrimp fed 4 x daily)	Improved growth (absolute weight & length), best survival at 12 ppm (43.34%); antibacterial effect against <i>V. harveyi</i>)	Short duration (1 month); limited immune/ hematology markers; survival rates are still relatively low	(Jumadi, 2019)
In Vitro protozoan fish parasite assay	<i>Ichthyophthirius multifiliis</i>	0.1, 0.25, and 0.50 ml/L use onion and garlic	Onion essential oil caused 94% mortality, while garlic essential oil caused 92% mortality after 60 minutes of exposure to the parasite <i>Ichthyophthirius multifiliis</i>	The results are based on short-term in vitro assays and require in vivo validation.	(Özil, 2023)

Antibacterial Activity of Bawang Dayak

Dayak onion contains a range of bioactive compounds with inherent antibacterial properties, including alkaloids, flavonoids, steroids, and tannins, which play key roles in inhibiting and eliminating pathogenic bacteria. Previous studies reported that a 100% ethanol extract of Dayak onion produced an inhibition zone of 12.33 ± 1.61 mm against *Staphylococcus aureus* (Warsiti et al., 2018). Similarly, ethanol and aqueous extracts effectively inhibited the growth of *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae*. These findings were further supported by Hidayah et al., (2021), who demonstrated that the K3M1 treatment yielded the largest inhibition

zones, measuring 15.47 mm against *Salmonella* spp. and 13.40 mm against *E. coli*.

The antibacterial activity of Dayak onion against aquaculture-related pathogens has also been investigated. Safratilofa & Sugihartono (2018) evaluated several extraction methods, including infusion, decoction, maceration with 96% alcohol, and maceration with 96% ethanol, against *Aeromonas hydrophilla*. Among these methods, alcoholic maceration produced the largest inhibition zone (3.5 mm). In addition, Fransira et al. (2019) reported that the minimum inhibitory concentration (MIC) of Dayak onion extract against *Pseudomonas fluorescens* was 100 ppm, representing the lowest effective concentration for bacterial growth inhibition.

Another research study. Extracts of *Eleutherine bulbosa* exhibit broad antibacterial activity attributed to diverse secondary metabolites, which effectively inhibit major aquatic pathogens such as *Vibrio harveyi*, *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Pseudomonas fluorescens* (Munaeni et al., 2021). In previous studies, we reported that crude extracts of *Eleutherine americana* exhibited antibiofilm and anti-quorum sensing activities against *Streptococcus pyogenes*. Furthermore, a partially purified fraction effectively inhibited methicillin-resistant *Staphylococcus aureus* (MRSA). The extract also demonstrated strong antibacterial activity against foodborne *S. aureus* isolates, with minimum inhibitory concentration (MIC) values ranging from 0.06 to 1.00 mg/mL (Mahabusarakam et al., 2010).

Mechanistically, phenolic compounds, quinones, and flavonoids disrupt bacterial cells by damaging cell walls and membranes, leading to protein denaturation and cell lysis (Haq et al., 2018). Increased phenolic content in *E. palmifolia* bulb extracts correlates positively with total phenol levels, thereby enhancing antibacterial efficacy. These phenolic constituents interact with microbial cell wall structures, reduce membrane permeability, and ultimately compromise bacterial survival.

Effects of Dayak Onion on Growth Performance

Fish growth is a critical determinant of aquaculture productivity. The application of Dayak onion as an immunostimulant is closely associated with improved growth performance, as its bioactive compounds enhance fish physiological responses and metabolic efficiency. Faramudhita (2024) reported that dietary supplementation with Dayak onion *simplicia* significantly improved growth parameters in Nile tilapia, including absolute length and weight gain, specific growth rate, feed intake, and feed conversion ratio. These improvements are primarily attributed to bioactive components in Dayak onion, particularly oligosaccharides and phenolic compounds, which function as prebiotics and immunostimulants. These compounds enhance digestive enzyme activity, modulate gut microbiota composition, and stimulate non-specific immune responses, including phagocytosis, lysozyme activity, and immune cell proliferation. Collectively, these mechanisms improve nutrient utilisation efficiency and promote accelerated growth. However, excessive inclusion levels may negatively affect performance. Oligosaccharide concentrations above optimal thresholds can disrupt feed formulation balance, as higher proportions of additives reduce the availability of essential nutrients such as proteins, lipids, carbohydrates, vitamins, and minerals. This imbalance lowers overall feed nutritional value, ultimately impairing growth

performance and feed conversion efficiency. This outcome aligns with fundamental principles of feed nutrition, which emphasise that non-nutritive additives must be incorporated within limits that do not compromise primary nutrient composition.

Beyond fish, the growth-promoting effects of Dayak onion have also been documented in crustaceans. In tiger shrimp (*Penaeus monodon*), dietary administration of 18 ppm Dayak onion extract increased growth by up to 4.58 g. The underlying mechanism parallels that observed in fish, involving immune stimulation, improved digestive tract function, and more efficient energy allocation toward growth (Jumadi, 2019). *E. bulbosa* extracts also contain functional oligosaccharides, including FOS, raffinose, inulin, and GOS, that promote probiotic growth and support gut microbiota balance, indicating their potential role as natural prebiotics. Furthermore, the presence of xanthenes, naphthoquinones, and anthraquinones confers strong antioxidant capacity, as reflected by low IC₅₀ values in DPPH assays. Collectively, these properties highlight *E. bulbosa* as a multifunctional phytotherapeutic agent with promising applications in sustainable aquaculture (Munaeni et al., 2021).

Dayak Onion as an Immune System Booster

Fish immune competence can be enhanced through dietary supplementation with immunostimulants that strengthen innate immune cell activity, regulate immune signalling pathways, and improve resistance to bacterial infections and environmental stressors (Citarasu, 2010; (Reverter et al., 2014). In aquaculture systems, such dietary strategies are particularly relevant because cultured fish are frequently exposed to pathogenic challenges and suboptimal environmental conditions that compromise immune performance and physiological stability.

Several studies have demonstrated the immunostimulatory potential of Dayak onion in aquaculture. Fauzi et al. (2024) reported that dietary inclusion of crude and extract forms of Dayak onion significantly enhanced haematological parameters, respiratory burst activity, and immune-related gene expression, including *IL-1 β* and *TNF- α* , in tilapia challenged with *Aeromonas hydrophila*. These findings indicate that *E. palmifolia* stimulates both cellular and molecular components of innate immunity during bacterial infection. Consistent evidence was provided by Faramudhita. (2024), who observed increased survival rates, improved haematological indices, enhanced phagocytic activity, and elevated intestinal lactic acid bacteria populations in fish receiving Dayak onion supplementation, suggesting a linkage between immune enhancement and gut microbiota modulation.

From a mechanistic standpoint, the immunological benefits of *E. palmifolia* are largely attributed to its bioactive compounds, particularly flavonoids, tannins, saponins, and terpenoids, which contribute to erythrocyte stability, antioxidant defence, and immune resilience (Izzah et al., 2022). Flavonoids have been shown to enhance macrophage and lymphocyte activity and support erythropoiesis through their antioxidant properties, thereby strengthening systemic immune capacity (Izzah et al., 2022). In addition, Dayak onion extracts have been reported to modulate pro-inflammatory cytokine expression, maintaining immune homeostasis and preventing excessive inflammatory responses that may impair growth and physiological performance (Moustafa et al., 2020).

A study by Supomo et al. (2019), demonstrated that *E. palmifolia* exhibits strong antioxidant activity, as evaluated using DPPH radical scavenging and Brine Shrimp Lethality Test (BSLT) assays. Among the tested fractions, the chloroform fraction showed very high antioxidant capacity, with an IC_{50} value of 20.29 ppm, which is categorised as a strong antioxidant and comparable to standard antioxidants. In addition, the BSLT assay indicated a moderate toxicity level ($LC_{50} \approx 527$ ppm), suggesting notable bioactivity while remaining within an acceptable safety range. These findings provide direct experimental evidence supporting *E. palmifolia* as a potent plant-based antioxidant source, reinforcing its relevance as a functional phyto-genic additive for mitigating oxidative stress in aquaculture systems.

These studies indicate that *E. palmifolia* acts as a multi-pathway immunostimulant, exerting its effects through haematological improvement, cellular immune activation, cytokine regulation, antioxidant protection, and microbiota support, rather than through a single immune mechanism. Such integrated immunomodulatory action aligns with current concepts of sustainable aquaculture, where long-term immune resilience is prioritised over short-term immune overstimulation (Citarasu, 2010).

Molecular Mechanism of Dayak Onion (*Eleutherine palmifolia*) Phytobiotics

Phytobiotics derived from the bioactive compounds of Dayak onion (*Eleutherine palmifolia*) have emerged as a key focus in the development of functional aquaculture feeds. Major constituents, including flavonoids, naphthoquinones, polyphenols, and tannins, exhibit multifaceted mechanisms that enhance non-specific immune responses, reinforce intestinal mucosal integrity, and inhibit pathogenic bacterial colonisation (Chabib et al., 2018; Harlita et al., 2018; Masfria & Tambubolon, 2019; Hongthongkham et al., 2025). A detailed mechanistic understanding of how these compounds act at cellular and tissue levels is essential for elucidating their collective contributions to fish health and disease resistance (Afandi et al., 2025). Figure 4 presents a schematic overview of the molecular interactions between Dayak onion bioactive compounds, pathogenic bacteria, and fish immune cells.

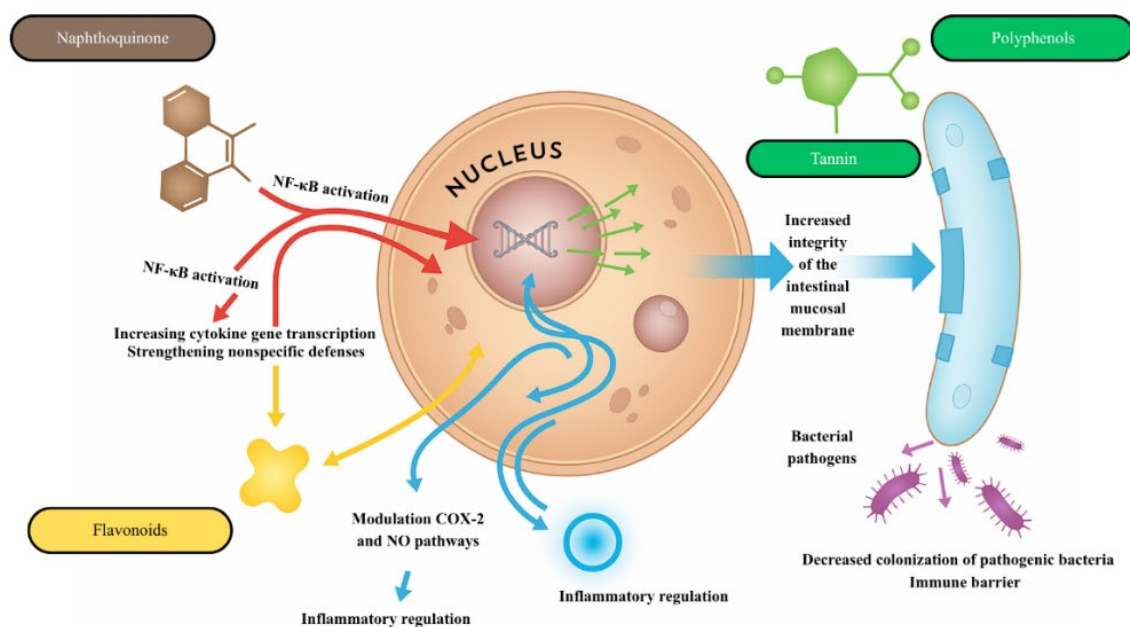


Figure 4. Molecular mechanisms by which bioactive compounds from *Eleutherine palmifolia* enhance fish immune responses

Figure 4 illustrates the mechanisms of action of major bioactive compounds in *E. palmifolia* that contribute to immune enhancement and protection against pathogenic infections in fish. On the left side of the schematic, naphthoquinones are depicted as key activators of the nuclear factor kappa B (NF- κ B) signalling pathway. Activation of this pathway induces the transcription of cytokine-related genes, thereby strengthening non-specific immune defences and enhancing phagocytic activity and the production of protective immune molecules (Firmino et al., 2021; Affandi et al., 2025). Flavonoids modulate inflammatory signalling by regulating cyclooxygenase-2 (COX-2) expression and the nitric oxide (NO) pathway, thereby preventing excessive inflammation, reducing tissue damage, and maintaining physiological stability during infection and environmental stress (Maleki et al., 2019; Ysrafil et al., 2023; Kari, 2025; Sahakyan, 2025).

Polyphenols and tannins enhance intestinal mucosal integrity by strengthening the epithelial barrier, thereby limiting pathogenic bacterial adhesion and colonisation (Molino et al., 2022; Andriani et al., 2024). Improved barrier function reduces infection risk and supports digestive health and nutrient absorption in fish (Mutiah et al., 2020; Kanika et al., 2025). Overall, Figure 4 highlights the synergistic interactions among Dayak onion bioactive compounds acting at molecular, cellular, and tissue levels to promote immune competence and disease resistance in aquaculture species.

Dayak Onion as an Anti-Stress Agent

Several bioactive compounds in Dayak onion exhibit potential as anti-stress agents during fish transportation. One dominant constituent is quercetin, a flavonoid known to reduce stress responses by inhibiting intestinal motility and decreasing capillary permeability in the peritoneal cavity. This mechanism physiologically reduces faecal excretion, thereby limiting the accumulation of toxic metabolites in the transport medium. In addition to quercetin, Dayak onion contains essential oil components that may lower fish metabolic rates, suppress digestive activity, and reduce waste production. Similar effects have been reported for lemongrass (*Cymbopogon citratus*) essential oil, which induces metabolic suppression during transport. Reduced metabolic activity resembles mild anaesthetic effects and has been shown to improve fish survival during transportation. A comparable response was observed in 3–5 cm botia fish treated with 1 mL/L lemongrass essential oil, which exhibited increased opercular movement, reduced activity, and diminished responsiveness to external stimuli, ultimately achieving a survival rate of 76% (Izzah et al., 2022). These findings support the potential application of Dayak onion-derived compounds as natural anti-stress agents to enhance fish survival under transportation-related stress.

Development Prospects

The development of Dayak onion (*Eleutherine palmifolia*) as a phytobiotic offers a promising strategy to reduce antibiotic dependence in aquaculture. Accumulating evidence indicates that Dayak onion functions as an effective immunostimulant and improves intestinal health, supporting its potential application as a functional feed additive. Owing to its complex and synergistic bioactivities, Dayak onion can be developed as a key component of immunological and performance-enhancing feeds for various fish and shrimp species in intensive farming systems.

Several strategic factors support its future development. Agronomically, Dayak onion exhibits strong environmental adaptability, allowing scalable cultivation to meet feed industry demands. Technologically, advances in extraction and processing techniques enable the targeted isolation of bioactive metabolite fractions, resulting in products with improved stability and consistent biological efficacy. From an industrial and regulatory perspective, the global shift toward natural and antibiotic-free inputs creates substantial opportunities for the commercialisation of Dayak onion-based phytobiotics. Moreover, formulation strategies such as microencapsulation and controlled-release systems offer promising approaches to enhance bioavailability and protect active compounds during feed processing.

From a research standpoint, further studies should focus on elucidating molecular mechanisms, including interactions with gut microbiota, immune signalling pathways, and oxidative stress responses. Integrating Dayak onion into sustainable aquaculture models—such as biofloc systems, recirculating aquaculture systems (RAS), and ecosystem-based aquaculture—also presents significant innovation potential. With robust standardisation, multidisciplinary collaboration, and strengthened local supply chains, Dayak onion has the potential to emerge as a flagship Indonesian phytobiotic, contributing to improved fish health, production efficiency, and the long-term sustainability of the aquaculture industry.

Conclusion

Eleutherine palmifolia (Dayak onion) represents a promising phytobiotic for sustainable aquaculture due to its rich phytochemical profile and broad spectrum of biological activities, including immunomodulatory, antibacterial, antioxidant, anti-stress, and growth-promoting effects. Accumulating evidence demonstrates that dietary supplementation with *E. palmifolia* enhances non-specific immune responses, improves haematological and physiological parameters, suppresses pathogenic bacterial infections, and increases survival and feed efficiency across diverse aquaculture species.

These beneficial effects are primarily mediated through the modulation of immune-related signalling pathways, reinforcement of intestinal mucosal integrity, enhancement of antioxidant defences, and inhibition of harmful microbial colonisation.

From a novelty perspective, this review represents the first PRISMA-guided systematic integrative synthesis that critically differentiates between experimentally validated outcomes and mechanistic inferences regarding the application of *Eleutherine palmifolia* in aquaculture. By consolidating fragmented evidence into a unified, mechanism-oriented framework and aligning biological effects with practical aquaculture contexts, this work moves beyond descriptive compilation toward evidence-informed interpretation. By integrating dispersed findings into a coherent analytical structure, this review advances current understanding of *E. palmifolia* as a functional feed additive. It provides a clearer basis for evaluating its translational potential in sustainable aquaculture.

Nevertheless, existing research remains constrained by fragmented experimental designs, inconsistent extraction and dosage protocols, and limited molecular validation, particularly under intensive aquaculture conditions. By integrating dispersed findings into a coherent, mechanism-oriented framework, this review advances current understanding of *E. palmifolia* as a functional feed additive and identifies critical knowledge gaps that must be addressed before large-scale application. Future research should prioritise standardised methodologies, molecular-level investigations, and system-based evaluations to fully realise the potential of *E. palmifolia* as an environmentally friendly alternative to antibiotics, thereby supporting resilient, efficient, and sustainable aquaculture production systems. From a methodological perspective, the PRISMA-guided systematic integrative approach employed in this review enables comprehensive synthesis while acknowledging variability and bias across existing studies, thereby providing a balanced and evidence-informed foundation for future experimental and applied aquaculture research.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures, with no animal experiments.

Data availability: All data generated or analysed during this study are included in this published article and its references.

Funding disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements: The authors would like to express their sincere gratitude to all researchers whose studies contributed to this review. No external funding was received for this study.

Disclosure: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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A preliminary study on the effects of seagrass wrack extract as biofertilizer on the growth, ice-ice disease, nitrogen, and phosphorus assimilation and carrageenan quality of eucheumatoid seaweed *Kappaphycus striatus*

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Cite this article as:

Tahiluddin, A.B., Terzi, E. (2026). A preliminary study on the effects of seagrass wrack extract as biofertilizer on the growth, ice-ice disease, nitrogen and phosphorus assimilation, and carrageenan quality of eucheumatoid seaweed *Kappaphycus striatus*. *Aquatic Research*, 9(2), 166-175. <https://doi.org/10.3153/AR26014>

ABSTRACT

Eucheumatoid seaweed farming is a significant aquaculture activity, not only supplying carrageenan to the global market but also serving as a vital livelihood source for many marginalised coastal communities, particularly in Tawi-Tawi, Philippines. However, the practice of applying chemical fertilisers in eucheumatoid seaweed farming to boost production by enhancing growth performance and mitigating ice-ice disease has become a contentious issue among local stakeholders in this region. This preliminary, proof-of-concept study investigated the potential of utilising seagrass wrack (*Thalassia hemprichii*) as a biofertilizer alternative, evaluating its effects on growth, ice-ice disease prevalence and intensity, nitrogen and phosphorus assimilation, and carrageenan quality (yield and gel strength) in the eucheumatoid seaweed *Kappaphycus striatus*. The experiment employed various concentrations of seagrass wrack extract (SWE): 0 mL L⁻¹ (control), 9 mL L⁻¹, 18 mL L⁻¹, and 27 mL L⁻¹. The results revealed no significant effects of SWE on growth or ice-ice disease prevalence and intensity (number and length of ice-ice spots per bundle) after 15, 30, and 45 days of cultivation. Nitrogen and phosphorus assimilation did not significantly impact SWE. Additionally, no impact on gel strength was observed after 45 days. Interestingly, a significant difference was detected in carrageenan yield, with the 27 mL L⁻¹ SWE treatment exhibiting a notably higher yield compared to all other treatments at the 45-day mark. While this study demonstrates the potential of SWE to enhance carrageenan yield, its lack of significant effects on *K. striatus* growth and health raises concerns about its overall suitability as a biofertilizer. Therefore, further research is warranted to explore the potential optimisation of the seagrass wrack extract, investigate the use of alternative seagrass wrack species or combinations, and identify strategies to improve the overall effectiveness of SWE as a biofertilizer.

Keywords: Biofertilizer, Carrageenan, Eucheumatoid seaweed farming, *Kappaphycus*, Seagrass wrack

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Submitted: 27.12.2025

Revision requested: 02.02.2026

Last revision received: 25.02.2026

Accepted: 01.03.2026

Published online: 31.03.2026

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<http://aquatres.scientificwebjournals.com>

Introduction

Kappaphycus is among the most widely cultivated eucheumatoid seaweeds globally, driven by the growing demand for carrageenan (Bindu & Lavine, 2011). Carrageenan, a polysaccharide derived from red algae like *Kappaphycus*, holds substantial commercial value across diverse sectors, including pharmaceuticals, food, cosmetics, printing, and textiles (Rupert et al., 2022). The cultivation of eucheumatoid seaweeds, particularly *Kappaphycus* and *Eucheuma*, is prevalent in tropical regions like Indonesia, the Philippines, and Malaysia, and in other parts of the world (Hayashi et al., 2017).

In the Philippines, seaweed farming, especially of *Kappaphycus*, provides vital livelihoods for many coastal and marginalised communities (Tahiluddin & Terzi, 2021a; Tahiluddin et al., 2023). Locals also consume these seaweeds as salads due to their nutritional benefits (Dumilag, 2019; Ajik & Tahiluddin, 2024; Tahiluddin et al., 2025). Consequently, seaweeds are the leading aquaculture species in the Philippines by volume, contributing significantly to the national economy (Tahiluddin & Terzi, 2021a). Despite this, farmers face challenges such as slow growth of farmed eucheumatoid seaweeds, particularly *Kappaphycus*, often attributed to poor seedling quality (Luhan et al., 2015) and increased susceptibility to diseases (Faisan et al., 2021; Tahiluddin & Eldani-Tahiluddin, 2024; Faisan et al., 2024; Tahiluddin & Terzi, 2024). Presumptive nutrient deficiencies in farms have led some farmers to use chemical fertilisers to enhance nutrient levels covertly (Tahiluddin et al., 2022a; Tahiluddin & Roleda, 2025).

Historically, nutrient enrichment in *Kappaphycus* farming was not practised, with farms relying solely on the natural nutrient availability. However, after decades of cultivation, nutrient depletion has become evident, leading to slow growth and frequent disease outbreaks among *Kappaphycus* cultivars (Tahiluddin et al., 2022a). Since 2012, inorganic nutrient enrichment has emerged as a practice in the southern Philippines, with farmers increasingly using commercial chemical fertilisers such as ammonium phosphate (16-20-0, N-P-K) and complete fertiliser (14-14-14, N-P-K) to enhance growth and mitigate ice-ice disease, thus enhancing production and profitability (Tahiluddin et al., 2022a). Nonetheless, the Philippine National Standard on “Seaweeds – Code of Good Aquaculture Practices (GAqP)” discourages the use of chemical fertilisers in seaweed farms (BAFS, 2021), highlighting the need for alternative solutions like biofertilizers.

Seagrass wrack presents a promising biofertilizer option. Research has demonstrated that biofertilizers derived from

seagrasses can effectively improve agricultural plants (Parente et al., 2013; Grassi et al., 2015; Mininni et al., 2015; Kavitha, 2017; Muniswami et al., 2021). Seagrass wrack, which poses environmental and economic challenges, can be composted and converted into biofertilizer for terrestrial plants, thus reducing reliance on inorganic fertilisers (Emadodin et al., 2020; Mainardis et al., 2021). Given that seagrass wrack is accumulating on the beaches of Sibutu, Tawi-Tawi, Philippines, and has not yet been explored as a biofertilizer for seaweed farming, investigating its potential use for *Kappaphycus* is valuable. This study serves as an exploratory study, offering the first preliminary evidence for the use of *Thalassia hemprichii* extract as a biofertilizer for *K. striatus*. Specifically, it aims to explore the use of seagrass wrack extract from *Thalassia hemprichii* as a potential biofertilizer for the commercial eucheumatoid seaweed *Kappaphycus striatus*, assessing its influence on growth, ice-ice disease prevalence and intensity (number and length of ice-ice spots per bundle), and carrageenan quality (yield and gel strength).

Materials and Methods

Study Site

The study was carried out in the seaweed farm of Sibutu, Tawi-Tawi, Philippines (Figure 1). This region is particularly well-suited for cultivating eucheumatoid seaweeds, such as *Kappaphycus striatus*, a practice that has been established since the 1970s.

Source and Preparation of Seedlings

Healthy seedlings of *K. striatus*, free from diseases and untreated with fertilisers, were sourced from a farmer in the study site. The seedlings were trimmed to weights of 50–55 g each and then attached to 5-m-long rope lines, spaced 25 cm apart. Each rope line had 20 attachment points. In total, 12 rope lines were prepared, representing three different treatments and a control, with three replicates for each.

Formulation of Seagrass Wrack Extract as Biofertilizer

Seagrass wrack (*T. hemprichii*) was manually collected from the coastal beaches of Sibutu, Tawi-Tawi, Philippines. The preparation of seagrass wrack extract as a biofertilizer followed the method outlined by Tahiluddin et al. (2022b). First, the collected seagrass was thoroughly washed to remove any epiphytes, sand, and debris. It was then sun-dried for 30 min to eliminate excess moisture before being cut into small pieces. The seagrass was mixed with distilled water in a ratio of 1:4 (0.5 kg of seagrass wrack to 2 L of distilled water) and boiled for 2 hr. After boiling, the crude aqueous extract was

cooled and filtered through muslin cloth. The newly formulated biofertilizer was then transferred to a clean bottle and stored in a cool, dry place.

Immersion of Seedlings in Biofertilizer Solution and Planting

The immersion of seaweeds in the biofertilizer solution was conducted in the late afternoon, between 4 and 6 pm, following the method outlined by Tahiluddin et al. (2022a) as practised by farmers in the study site. Four biofertilizer solutions were prepared for the treatments, with concentrations of 0, 9, 18, and 27 mL L⁻¹, by mixing the biofertilizer with seawater in a 20-L container. Three culture lines (n=3) were simultaneously immersed in each fertiliser solution for 30 sec, then left to sit overnight with a canvas covering. Prior to out-planting, the seaweeds were briefly rested for 10 min beneath the farmer's house to minimise stress before being transported to the farming site by small boat. The lines were set up randomly using the modified fixed-off bottom method (stakes at both ends with floaters), positioned 30 cm above the seabed.

Total Thallus Nitrogen and Phosphorus Determination

After the overnight enrichment of seaweeds, samples from each treatment were sun-dried for 3 days and then stored in ziplock bags. Total thallus nitrogen was measured using the Kjeldahl method (Kjeldahl, 1883), while the phosphorus con-

tent was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Hou & Jones, 2000). Chemical characterisation of the SWE (e.g., nitrogen, phosphorus, and bioactive compounds) was beyond the scope of this preliminary phase and remains a limitation of the study.

Monitoring of Growth Rate, Ice-Ice Disease, and Physicochemical Parameters

Growth sampling was conducted every 15, 30, and 45 days. To assess growth, five randomly tagged branches per line were removed, patted dry with a clean cloth, and weighed. The sampled seaweeds were then re-tied to their original positions. Specific growth rates (SGR) were calculated every 15 days utilising the formula provided by Luhan et al. (2015).

Ice-ice disease prevalence was checked every 15, 30, and 45 days through visual inspections. Each bundle in every line was examined for signs of the disease, which include soft thalli and whitish discolouration. The number of infected bundles per line was measured. Ice-ice disease prevalence (%) was calculated by dividing the number of infected bundles by the total number of bundles per line, then multiplying by 100 (Tahiluddin et al., 2022a). Ice-ice disease intensity was also measured by counting the number of ice-ice spots per bundle and measuring the length of each spot.

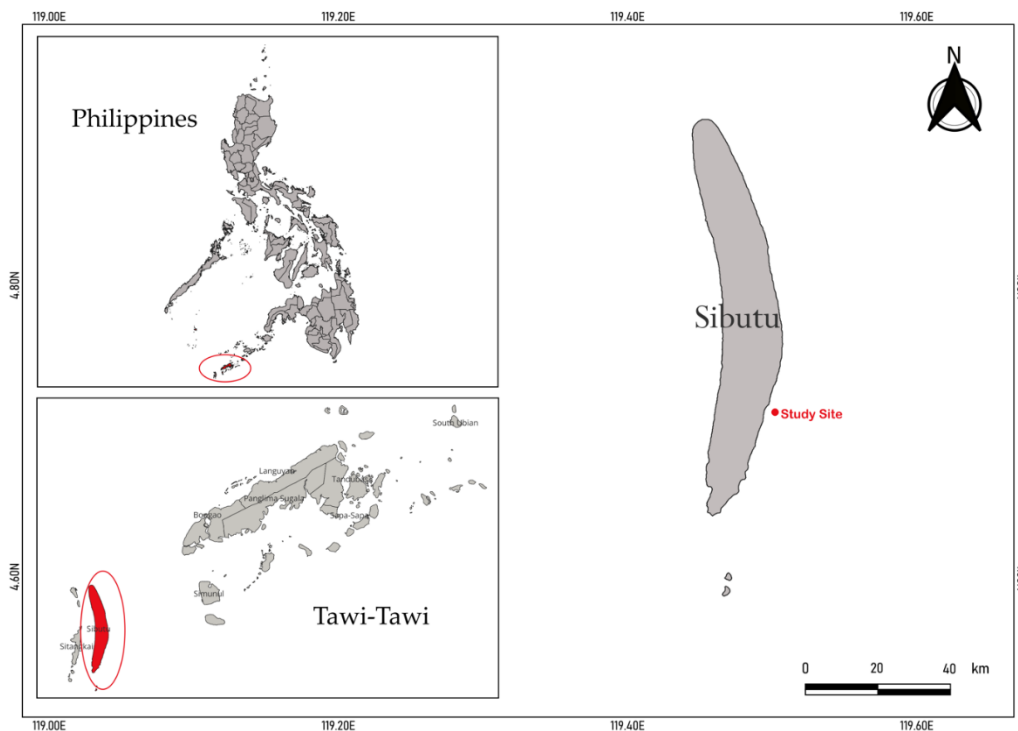


Figure 1. Study site (red dot) showing the location of the farm in Sibutu, Tawi-Tawi, Philippines

Physicochemical parameters, including temperature, salinity, pH, depth, water current, and wind speed, were recorded on days 0, 15, 30, and 45. Various instruments were used: a glass thermometer, a refractometer (Atago Master, Tokyo, Japan), a pH meter (Polsinelli, Kansas, MO, USA), a calibrated rope, a fabricated drogue, and a digital anemometer (BENETECH, Shenzhen, China). Additionally, the farm was maintained weekly by removing silt, debris, and predators from the seaweeds.

Carrageenan Yield and Gel Strength Determination

The seaweeds were carefully cleaned to remove any adhering foreign particles, sun-dried, and then chopped into small pieces. Gel strength and carrageenan yield were determined following the procedure outlined by Muyong & Tahluddin (2024).

Statistical Analysis

Data are presented as the mean \pm standard error (SE). The normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated with Levene’s test. Significant differences between treatments were evaluated through one-way analysis of variance (ANOVA). Given the exploratory nature of this preliminary, proof-of-concept study, Duncan’s Multiple Range Test (DMRT) was selected as the post-hoc test for mean ranking. DMRT was chosen to maximise the sensitivity for detecting potential differences among treatment groups—such as the observed impact on carrageenan yield—where more conservative tests might fail to identify significant effects in a dataset with low replication ($n=3$) and a single cultivation cycle. All statistical analyses were performed using IBM SPSS software (version 20; SPSS Inc., Chicago, IL, USA). While the replication ($n=3$) is standard for preliminary seaweed field trials, we acknowledge that this level of replication may limit the statistical power to detect subtle biological variations in nitrogen and phosphorus assimilation.

Results and Discussion

The present study represents the first attempt to explore the potential use of seagrass wrack (*Thalassia hemprichii*), which is abundant on beaches, as a biofertilizer for cultivating *Kappaphycus striatus*. While our results did not show significant effects on growth, ice-ice disease prevalence, or gel strength, they did reveal a notable increase in carrageenan yield.

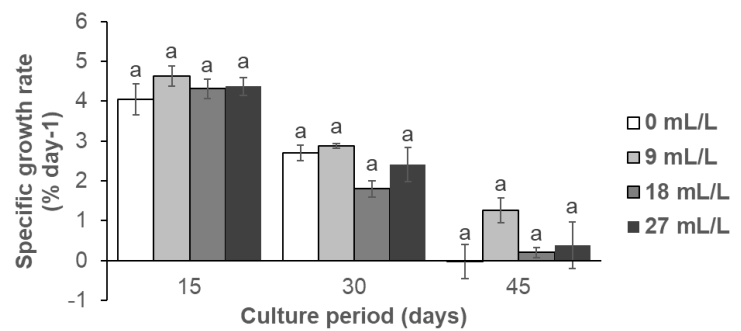


Figure 2. Specific growth rate (SGR, % day⁻¹) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 15

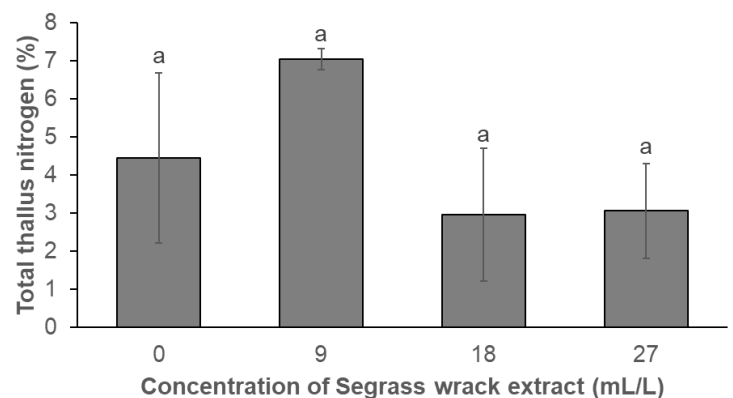


Figure 3. Total thallus nitrogen (%) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N=3



Figure 4. Phosphorus content (ppm) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

Previous research has demonstrated that chemical fertilisers such as ammonium phosphate (3.5 and 8.82 g L⁻¹) effectively enhance the growth of eucheumatoid seaweed (Tahiluddin et al., 2022a; Muyong & Tahiluddin, 2024) due to the high nitrogen uptake by the seaweed (Tahiluddin et al., 2021a). In contrast, our study found that using seagrass wrack (*T. hemprichii*) as a biofertilizer had no significant impact on *K. striatus* growth, even though the highest growth rates were consistently observed with a 9 mL L⁻¹ biofertilizer concentration on days 15, 30, and 45 (Figure 2). The lack of significant changes in total thallus nitrogen and phosphorus levels (Figures 3 and 4) likely explains the minimal growth impact. Without a chemical profile of the applied SWE, it is difficult to determine if the lack of growth response is due to low nutrient content in the extract or poor uptake by the seaweed. This suggests that seagrass wrack (*T. hemprichii*) is relatively ineffective in promoting the growth of *K. striatus*, although further studies are required. However, seagrass wrack has been recognised as a biofertilizer/biostimulant for crops (Muniswami et al., 2023) and coastal agriculture (Franzén et al., 2019). Additionally, previous studies have shown that brown seaweeds like *Sargassum cristaefolium* and *Turbinaria conoides*, or their combinations, can serve as alternative fertilisers for *K. striatus*, enhancing growth without affecting ice-ice disease occurrence (Tahiluddin et al., 2022b). Nonetheless, in this study, seagrass wrack did not yield significant results for *Kappaphycus* farming, suggesting that it may not be a suitable biofertilizer for *K. striatus*. Different extraction methods, such as fermenting the seagrass wrack and using various concentrations, were tested, but still resulted in non-significant findings (data not shown). Further studies are warranted to further explore the potential of seagrass wrack as a biofertilizer for *Kappaphycus*.

Ice-ice disease is a pathological condition in eucheumatoids, characterised by symptoms like softening and bleaching of the affected thalli (Faisan et al., 2021; Tahiluddin & Terzi, 2021b; Ward et al., 2022; Tahiluddin & Damsik, 2023; Tahiluddin & Terzi, 2024; Tahiluddin & Eldani-Tahiluddin, 2024). This disease is generally caused by a range of factors, including environmental and meteorological changes like temperature, salinity, irradiance, and rainfall (Tahiluddin & Terzi, 2021b; Ward et al., 2022; Tahiluddin & Terzi, 2024; Tahiluddin & Eldani-Tahiluddin, 2024), nutrient deficiencies such as sodium nitrate, ammonium, and ammonium phosphate (Luhan et al., 2015; Tahiluddin et al., 2022a; Tahiluddin & Terzi, 2024), or biological factors like pathogenic marine bacteria and marine-derived fungi (Tahiluddin & Terzi, 2021b; Tahiluddin et al., 2021b; Bermil et al., 2022). Nutrient enrichment has been shown to play a key role in reducing the occurrence of ice-ice disease. For instance, Tahiluddin et al.

(2022a) reported that ammonium phosphate fertiliser (8.82 g L⁻¹) significantly minimised the prevalence of ice-ice disease in *K. striatus*. However, it did not affect the severity of the disease in terms of the number and length of ice-ice spots/bundles. In the present study, the seagrass wrack extract biofertilizer (*T. hemprichii*) did not affect the occurrence or severity of ice-ice disease in farmed *K. striatus* throughout the sampling periods (Figures 5, 6, and 7). Similar findings were reported by Tahiluddin et al. (2022b), who found no significant impact on ice-ice disease prevalence when using brown seaweeds (*T. conoides* and *S. cristaefolium*) as biofertilizers. Additionally, Sarri et al. (2022) noted that inorganic nutrient enrichment (urea or phosphorus) had no impact on the prevalence of ice-ice disease. Environmental monitoring showed that pH levels rose from 8.63 to 9.20 over the 45-day study period. This high alkalinity is a significant physiological stressor that can trigger ice-ice disease by depleting the total inorganic carbon required for photosynthesis. Research demonstrates that when pH reaches such levels, seaweed yields can drop fivefold because the plants become carbon-limited, even if other nutrients are available (DeBusk & Ryther, 1984). For *Kappaphycus*, maintaining a stable environment near pH 8.0 is essential to prevent the stress and reduced growth associated with carbon depletion. Since all experimental groups were exposed to these identical high-pH conditions, the SWE did not appear to provide direct protection against these dominant environmental dynamics.

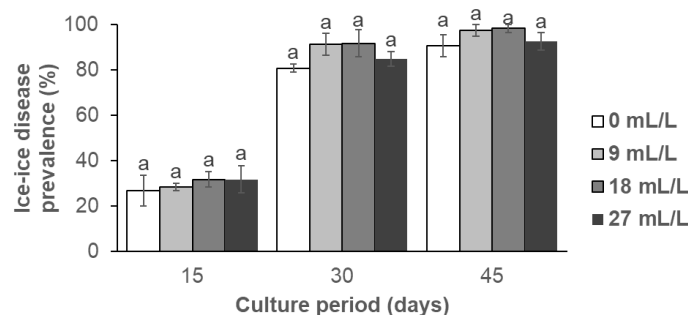


Figure 5. Ice-ice disease prevalence (%) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

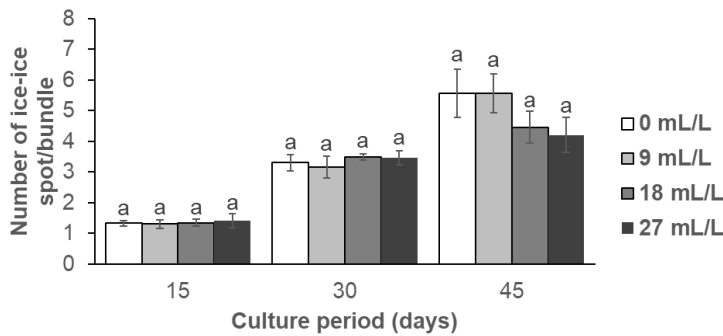


Figure 6. Number of ice-ice spots/bundle of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

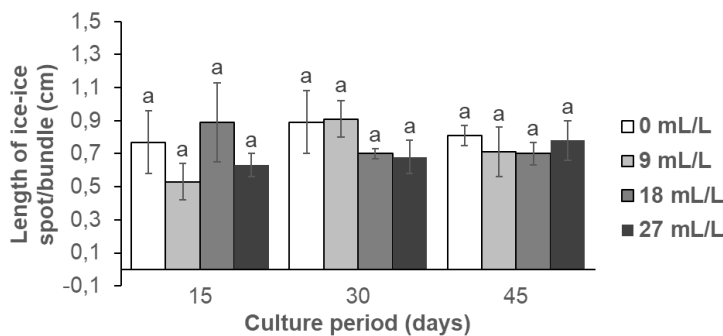


Figure 7. Length of ice-ice spots/bundle (cm) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

Table 1. Physicochemical parameters of the farm during the sampling period

Water parameters	Sampling Period (Days)			
	0	15	30	45
Temperature (°C)	30.00 ± 0.00	30.75 ± 0.14	32.00 ± 0.00	29.67 ± 0.08
Salinity (ppt)	34.00 ± 0.00	34.00 ± 0.00	34.00 ± 0.00	34.00 ± 0.00
pH	8.63 ± 0.09	8.80 ± 0.06	9.03 ± 0.03	9.20 ± 0.00
Water current (m s ⁻¹)	0.25 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
Wind speed (m s ⁻¹)	4.97 ± 0.15	1.07 ± 0.15	1.87 ± 0.03	0.03 ± 0.03
Farm depth (cm)	51 – 122			

The primary objective of *Kappaphycus* cultivation and processing is to extract carrageenan, a versatile polysaccharide widely used in the food industry (Rupert et al., 2022; Shafie et al., 2022). Carrageenan yield can vary based on species, location, and harvest time (Hurtado et al., 2009; Mendoza et al., 2006; de Góes & Reis, 2012; Sarri et al., 2022; Tahiluddin et al., 2022b), as well as various abiotic (temperature, salinity, irradiance, season, and nutrients) and biotic factors in the farm environment (Rupert et al., 2022). In the present study, carrageenan yield was significantly enhanced by the use of seagrass wrack biofertilizer, with the highest concentration of 27 mL L⁻¹ resulting in an elevated yield (Figure 8). This apparent decoupling between nutrient assimilation (which did not change) and carrageenan yield suggests that SWE may contain biostimulants or trigger metabolic shifts that prioritise polysaccharide synthesis over biomass production. This is supported by previous studies showing that biostimulants such as Acadian Marine Plant Extract Powder (AMPEP) significantly increase carrageenan yield while not affecting growth on day 20 and ultimately increasing both yield and growth on day 40 (Loureiro et al., 2014). Here, the biostimulant AMPEP acts as an elicitor, triggering a preventive stress response that prompts the seaweed to increase carrageenan production as a structural defence. Simultaneously, the extract contains antioxidant enzymes that neutralise oxidative stress, thereby allowing higher yields (Loureiro et al., 2012; Loureiro et al., 2014). According to Luhan et al. (2015), nutrient enrichment of *K. alvarezii* could increase carrageenan yield. This suggests that the seagrass wrack used in this study may have the potential to boost carrageenan yield. However, other studies have shown that biofertilizers made from seaweed liquid extracts of *S. cristaefolium* and *T. conoides*, or their combinations, did not impact carrageenan yield in *K. striatus* (Tahiluddin et al., 2022b). Similarly, the use of urea and phosphorus had no significant effect on the carrageenan yield of *K. striatus* (Sarri et al., 2022). Meanwhile, gel strength is a measure of how well a substance can hold its shape and resist falling apart when pushed or stirred (Islam & Hossain, 2021). In this study, the use of seagrass wrack biofertilizer did not affect the gel strength of *K. striatus* (Figure 9), consistent with Luhan et al. (2015), who found that sodium nitrate, as a chemical fertiliser, also did not influence the gel strength of *K. alvarezii*. It should be noted, however, that since the present study was conducted at a single site during one cultivation cycle, these results are specific to the seasonal and environmental conditions of the study period and should not be generalised to all *Kappaphycus* farming systems without further multi-site verification.

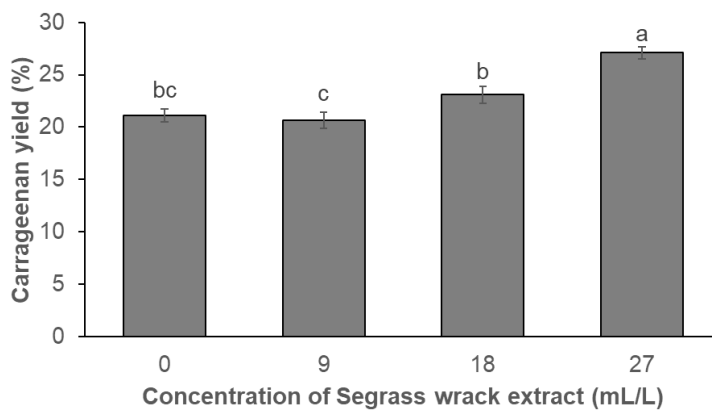


Figure 8. Carrageenan yield (%) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

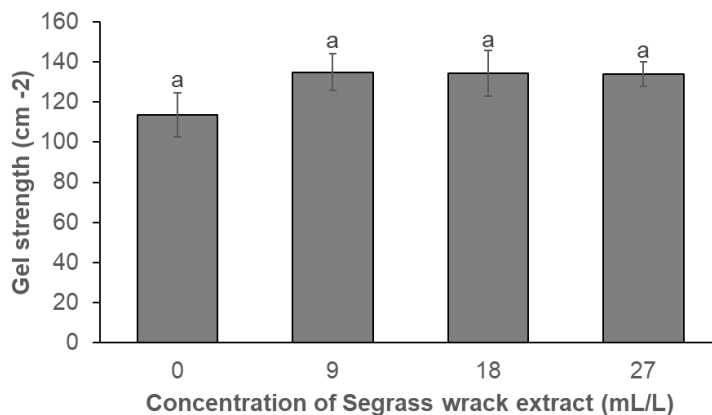


Figure 9. Gel strength (cm⁻²) of *Kappaphycus striatus* nutrient-enriched with different concentrations of seagrass wrack extract. N= 3

Conclusion

In conclusion, this study provides preliminary observations on the potential of seagrass wrack extract (SWE) in eucheumatoid seaweed farming. While SWE significantly boosted carrageenan yield, the findings for growth and disease resistance were largely non-significant. These results emphasise hypothesis generation regarding biostimulant effects rather than immediate application readiness, and further research with higher replication and chemical characterisation is required to validate these exploratory findings fully.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: Ethical committee approval is not required for this type of study.

Data availability: The data will be made available upon request.

Funding disclosure: This research received external funding from the Southeast Asian Regional Centre for Graduate Study and Research in Agriculture (SEARCA) PhD Research Scholarship with a Ref. No. GBG24-0889.

Acknowledgements: The authors are indebted to Rizal Jhunn F. Robles for his support during the conduct of the experiment.

Disclosure: This study is an output of the PhD Dissertation of Albaris B. Tahliluddin.

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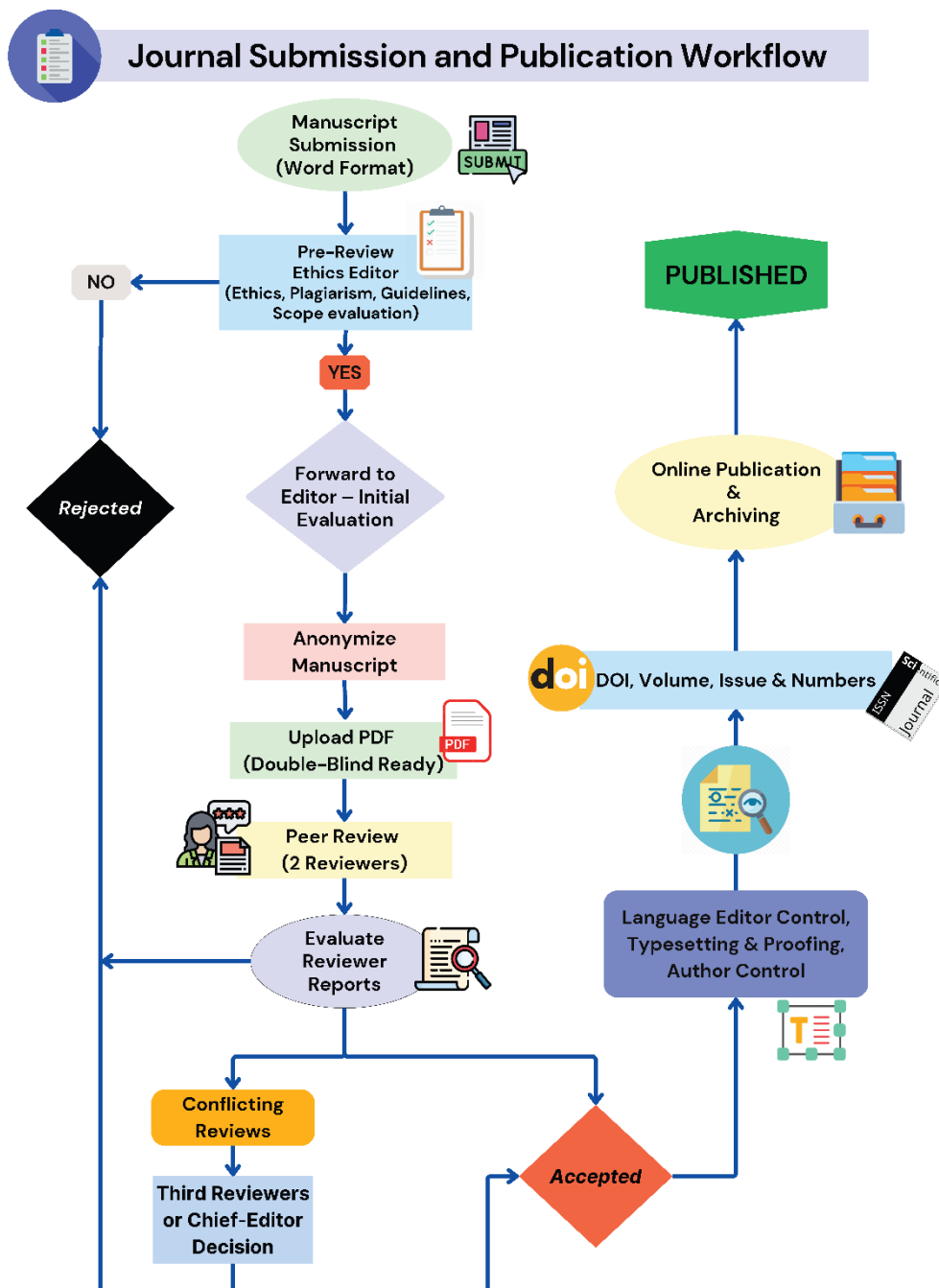
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Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking features of a previously published article. Articles on subjects within the journal’s scope that might attract the readers’ attention, particularly educational cases, may also be submitted as a “Short Communication”. Readers can also comment on the published manuscripts as a “Short Communication”. The main text should contain “**Title**”, “**Abstract**”, “**Introduction**”, “**Materials and Methods**”, “**Results and Discussion**”, “**Conclusion**”, “**Compliance with Ethical Standards**”, and “**References**” sections.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤30	200	40
Review Article	no limits	200	60
Short Communication	≤5	200	20

Tables

Tables should be included in the main document and presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be

placed above the tables. Abbreviations in the tables should be defined below them by footnotes (even if they are defined within the main text). Tables should be created using the “insert table” command of the word processing software and arranged clearly to provide easy reading. The data presented in the tables should not be a repetition of the data presented in the main text, but rather should support the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted through the submission system in the main document's Word files (in JPEG or PNG format). Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the primary document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in the USA), should be provided in parentheses in the following format: “Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA).”

All references, tables, and figures should be referred to within the main text and numbered consecutively in the order they are referred to within it.

Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

The citation style and methods that comply with the scientific standards required for the "Aquatic Research" journal are outlined below, as they pertain to the sources used by authors in their works.



Reference System is APA 6th Edition (with minor changes)

The APA style requires three types of information to be included in in-text citations. The author's last name and the work's publication date must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Bhujel, 2014).

....(Mol & Erkan, 2009).

....(Alofa et al., 2023).

....(Mol & Erkan, 2009; Bhujel, 2014; Alofa et al., 2023).

Citations for a Reference Section:

An article

Alofa, C.S., Olodo, I.Y., Chabi Kpéra Orou Nari, M., Abou, Y. (2023). Effects of the fresh and dried housefly (*Musca domestica*) larvae in the diets of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758): growth, feed utilisation efficiency, body composition, and biological indices. *Aquatic Research*, 6(1), 1-10.

<https://doi.org/10.3153/AR23001>

(if a DOI number is available)

A book in print

Bhujel, R.C. (2014). A manual for the tilapia business. CABI Nosworthy Way Wallingford Oxfordshire OX10 8DE UK, 199 p. ISBN 978-1-78064-136-2.

<https://doi.org/10.1079/9781780641362.0000>

(if a DOI number is available)

A book chapter

Craddock, N. (1997). Practical management in the food industry: A case study. In Food Allergy Issues for the Food Industry; Lessof, M., Ed.; Leatherhead Food RA: Leatherhead, U.K., pp 25-38. ISBN: 4546465465

A webpage

CDC (2020). Rift Valley Fever | CDC.

<https://www.cdc.gov/vhf/rvf/index.html> (accessed 20.08.2020).

Revisions

When submitting a revised version of a paper, the author must submit a detailed “Response to the reviewers” that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer’s comment, followed by the author’s reply and line numbers where the changes have been made) as well as an annotated copy of the primary document. Revised manuscripts must be submitted within 15 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal’s webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author, and their publication approval is requested within two days of their receipt of the evidence.