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Editor's Note

It is with great pleasure that we present Volume 26 (2025) of the Malaysian Fisheries Journal (MFJ)—the third and final special edition devoted to the 11th Diseases in Asian Aquaculture (DAA11) Symposium, held in Kuching, Sarawak in August 2022. MFJ, an annual publication by the Fisheries Research Institute (FRI), Department of Fisheries Malaysia, proudly supports this initiative proposed by the DAA11 National Organising Committee to spotlight research shared during this distinguished event. Especially aimed at empowering early-career researchers, this edition offers a platform to publish novel findings and foster collaborative growth within the aquatic animal health community.

As the final issue in this special edition series, we hope this collection not only informs but inspires on-going progress in aquatic animal health and sustainable aquaculture practices.

MFJ is freely available with special request to the Library FRIBM, 11960 Batu Maung, Pulau Pinang, Tel No: +6046263925/26

Australia's New National Aquatic Animal Disease Reporting System: Introducing AUSPestCheck®

YUKO HOOD[1]*, JOSHUA ALLAS[1] AND DAVID GALE[2]

[1]*Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, Australia*
[2]*Plant Health Australia, Canberra, Australia*

**Corresponding author: yuko.hood@aff.gov.au*

***Abstract*:** To fulfil Australia's international obligations and ensure effective national coordination of information, Australia developed a data-sharing and coordination software system, AUSPestCheck™. This system was initially created and managed by a joint government-industry non-profit organisation, Plant Health Australia (PHA), specialising in plant health surveillance. In collaboration with the Department of Agriculture, Fisheries and Forestry (DAFF), PHA initiated trials using AUSPestCheck™ as a data management system for terrestrial animal health surveillance. Recently, DAFF and PHA launched the "National Aquatic Animal Health Surveillance Trial" within AUSPestCheck™. The suitability of this trial program for compiling national aquatic animal disease data for international reporting is currently under evaluation. The benefits of this system have been demonstrated through trial implementation, including faster, improved uploading and data management processes, streamlined real-time reporting, enhanced visual outputs and better access to national disease intelligence supporting decision-making. AUSPestCheck™ replaces the previous Quarterly Aquatic Animal Disease (QAAD) online reporting system. Australia developed QAAD to collate information from all jurisdictions on reportable aquatic animal diseases. However, it has become outdated and no longer meets contemporary information security standards. Besides supporting Australia's domestic biosecurity and international reporting requirements, AUSPestCheck™ provides a model for countries seeking to modernise aquatic animal health reporting. Understanding the platform's governance structure, automation features and integrated data visualisation can help regional partners and international agencies adopt or adapt similar digital tools for national surveillance and global reporting. The presentation at DAA11 outlined Australia's aquatic animal disease reporting system, governance and functions of aquatic AUSPestCheck™. Case studies demonstrated how AUSPestCheck™ facilitates sharing, collation, and presentation of aquatic animal disease surveillance data for policy-makers.

Keywords: AUSPestCheck™, WOAH, NACA, disease reporting, surveillance, data visualisation

Abstrak: Untuk memenuhi kewajipan antarabangsa dan memastikan penyelarasan maklumat nasional yang berkesan, Australia telah membangunkan sistem perisian perkongsian data dan penyelarasan, AUSPestCheck™. Sistem ini pada mulanya dicipta dan diuruskan oleh organisasi bukan berasaskan keuntungan bersama badan kerjasama industri-kerajaan, Plant Health Australia (PHA), yang mengkhusus dalam pengawasan kesihatan tumbuhan. Dengan kerjasama Jabatan Pertanian, Perikanan dan Perhutanan (DAFF), PHA memulakan ujian menggunakan AUSPestCheck™ sebagai

sistem pengurusan data untuk pengawasan kesihatan haiwan darat. Baru-baru ini, DAFF dan PHA telah melancarkan "Percubaan Pengawasan Kesihatan Haiwan Akuatik Kebangsaan" dalam AUSPestCheck™. Kesesuaian program percubaan ini untuk menyusun data penyakit haiwan akuatik kebangsaan untuk pelaporan antarabangsa sedang dalam penilaian. Faedah sistem ini telah ditunjukkan melalui pelaksanaan percubaan, termasuk proses muat naik dan pengurusan data yang lebih pantas, pelaporan masa nyata yang diperkemas, output visual yang dipertingkatkan dan akses yang lebih baik kepada kecerdasan penyakit nasional yang menyokong pembuatan keputusan. AUSPestCheck™ menggantikan sistem pelaporan dalam talian Penyakit Haiwan Akuatik Suku Tahunan (QAAD) sebelumnya. Australia membangunkan QAAD untuk mengumpulkan maklumat dari semua bidang kuasa penyakit haiwan akuatik yang boleh dilaporkan. Walau bagaimanapun, ia telah menjadi lapuk dan tidak lagi memenuhi piawaian keselamatan maklumat kontemporari. Selain menyokong keperluan biosekuriti domestik dan pelaporan antarabangsa Australia, AUSPestCheck™ menyediakan model untuk negara yang ingin memodenkan pelaporan kesihatan haiwan akuatik. Memahami struktur tadbir urus platform, ciri automasi dan visualisasi data bersepadu boleh membantu rakan kongsi serantau dan agensi antarabangsa mengguna pakai atau menyesuaikan alat digital yang serupa untuk pengawasan nasional dan pelaporan global. Pembentangan di DAA11 menggariskan sistem pelaporan penyakit haiwan akuatik Australia, tadbir urus dan fungsi akuatik AUSPestCheck™. Kajian kes menunjukkan cara AUSPestCheck™ memudahkan perkongsian, pengumpulan dan pembentangan data pengawasan penyakit haiwan akuatik untuk pembuat dasar.

Kata kunci: AUSPestCheck™, WOAH, NACA, pelaporan penyakit, surveillan, visualisasi data

Introduction

Australia has established a national system for the surveillance and reporting of significant aquatic animal diseases. This system was established in 1998 and is coordinated by the Australian Government Department of Agriculture, Fisheries, and Forestry (DAFF). Since then, Australia has consistently reported to various international organisations, and this reporting continues to the present day.

Australia is a member of the World Trade Organization (WTO) and the World Organisation for Animal Health (WOAH). It is also a member country of the Network of Aquaculture Centres in Asia-Pacific (NACA). As a member country of the WTO and WOAH, Australia is required to report its status of WOAH-listed diseases to the World Animal Health Information System (WAHIS) (Chapter 1.1 of the WOAH Aquatic Animal Health Code) [WOAH 2024a]. The WOAH requirements specify the minimum standards necessary for Australia to fulfil its international obligations, which support domestic notification requirements. Some relevant aspects of Chapter 1.1 of the WOAH Aquatic Code include:

a. Article 1.1.2.: Member Countries shall make available to other Member Countries, through the WOAH, whatever information is necessary to minimise the spread of important diseases of aquatic animals and their pathogenic agents and to assist in achieving better worldwide control of these diseases.

b. Provisions of Article 1.1.3. to report information on listed diseases, including first occurrence, recurrence, occurrence of new strains, and changes in epidemiology.
c. Provisions of Article 1.1.5. to report emerging diseases.

Australia has also agreed to report on its status of regionally reportable diseases to the Asia-Pacific through NACA and the WOAH Regional Representation for Asia and the Pacific [NACA 2024; WOAH 2024b]. To report Australia's national status of significant aquatic animal diseases, DAFF, as the national government aquatic animal health authority, must coordinate and compile the information on the presence or absence of diseases within each jurisdiction. The information is gathered from state and territory government authorities, responsible for aquatic animal disease management within their jurisdiction.

Australia is a large continent with diverse environmental conditions and varied aquatic animal species, ranging from tropical species in the north to temperate species in the south. Consequently, aquatic animal species targeted by capture fisheries and farmed by aquaculture operators vary, from tropical barramundi and prawns in the north to salmon and abalone in the south. Each jurisdiction has different aquatic animal health controls, management, and legislation depending on their targeted aquatic animals, resulting in variable reporting arrangements, disease lists, and reporting and notification requirements. As the national aquatic animal health authority, DAFF coordinates the national response and is accountable for international reporting on the status of nationally significant diseases.

Effective surveillance of aquatic animal diseases is essential to safeguarding Australia's aquatic animal industries, protecting trade access, and ensuring early detection of biosecurity threats. However, traditional disease monitoring systems face challenges such as time lags in data collection, inconsistent reporting formats across jurisdictions, and limited capacity for real-time analysis. These limitations emphasise the need for more integrated, responsive reporting tools. In response, DAFF is implementing AUSPestCheck™, a digital platform to modernise aquatic animal disease surveillance. Its objectives include enabling real-time data capture and sharing, streamlining reporting processes across jurisdictions, improving data quality and transparency, and supporting national and international obligations for disease notification. AUSPestCheck™ aims to strengthen Australia's biosecurity system and improve its readiness and response to aquatic disease threats.

The development and implementation of AUSPestCheck™ also have broader relevance beyond Australia. By providing transparency in governance structures, data flow architecture, and visualisation capability, the system serves as a scalable model for other countries aiming to enhance national aquatic disease surveillance. Regional partners and international organisations can adopt insights from Australia's approach to improve their systems, harmonise data standards, and streamline cross-border reporting. The integration of automated data pipelines and tools such as Power BI for real-time analytics further exemplifies how digital transformation can strengthen both domestic and international disease intelligence networks.

1. Disease Surveillance in Australia

Aquatic animal disease surveillance in Australia is vital to the national biosecurity framework, supporting early detection, response, and ongoing management of significant aquatic animal health threats. The system incorporates both active and passive surveillance methods. Active surveillance involves structured sampling and testing programs, often targeted at specific diseases, species, or a combination of both. It is essential for verifying disease freedom or confirming known disease distribution during emergency disease response. However, passive surveillance plays a more prominent and indispensable role in Australia due to its vast geography, extensive coastline, and diverse aquatic ecosystems. It depends on routine reporting of disease events by industry stakeholders, researchers, veterinarians, and government officers. Given the scale and decentralised nature of Australia's aquatic industries, passive surveillance provides critical, real-time information that is often the first indicator of emerging health issues. When well-supported by communication, awareness, and diagnostic networks, this economical approach forms the foundation of Australia's aquatic animal health management system. To improve this system, digital technologies are increasingly employed to modernise and strengthen disease monitoring, enabling faster reporting, improved data accuracy, and real-time analytics.

2. AQUAPLAN 1998–2003

Australia's national aquatic animal health management priorities have been shaped by AQUAPLAN (Australia's National Strategic Plan for Aquatic Animal Health) [DAFF 2024], and collaborative efforts involving industry stakeholders, jurisdictions, and national government bodies. In 1998, the National List of Reportable Diseases of Aquatic Animals, and the Quarterly Aquatic Animal Disease (QAAD) system were developed as part of AQUAPLAN 1998–2003. These initiatives established nationally consistent protocols for disease reporting, supporting Australia's international reporting obligations.

3. National List of Reportable Diseases of Aquatic Animals (the National List)

The National List forms the basis of Australia's domestic and international reporting system for aquatic animal diseases. It contains a list of aquatic animal diseases, some exotic to Australia and some present in other parts of the region. In 1998, the Australian government and all state and territory governments agreed on the purpose and the concept of the National List, including criteria for listing and a strategy for permitting alterations to the list. The purpose, concept, criteria, and alteration strategy were endorsed by all governments in 1998. The Australian and all state and territory governments agreed that Australia would report internationally only on information provided by states and territories, which is consolidated and aggregated into the national report and then endorsed by all states and territories.

The National List was agreed to be a tool to compile and disseminate information on diseases of national importance. The term 'notifiable' was deliberately avoided due to its implications in some states and territories. It was agreed that reporting on the diseases on the National List should also:

a. Meet international disease reporting obligations
b. Provide a tool for negotiations in trade forums to support export certification and quarantine import policy
c. Enable international acceptance of disease-free 'zones'
d. Enhance the effectiveness of the control programs administered by individual states/territories by ensuring national awareness of the disease of concern in each state/territory
e. Support interstate movement, zoning, and translocation policies
f. Guide the further development of diagnostic tests and surveillance protocols to meet the needs of Australian aquatic industries
g. Guide the development of an aquatic animal disease surveillance and monitoring system.

3.1 Disease listing criteria

The National List comprises several diseases exotic to Australia, and some that occur in other parts of the region. To be listed, diseases must meet at least one of the following criteria:

a. A disease[1] listed by the WOAH in its Aquatic Animal Health Code (Aquatic Code);
b. A disease listed by the NACA reporting program that is clearly described by its aetiology (causative agent) and the relevant diagnostic method is available; or
c. A disease is of national and genuine concern to Australia.

For a disease to be listed because it is deemed to be "of national and genuine concern to Australia" (criterion c), the following criteria must apply:

a. A disease is exotic to Australia, or if it occurs in parts of Australia, vigilance is necessary to minimise its spread;
b. A disease would have significant socio-economic impacts if it occurred; and
c. A disease can be clearly described by its aetiology (causative agent) and the relevant diagnostic method is available.

Many aquatic animal diseases included on the National List are described as 'Infection with Pathogen X' to align with the disease naming convention of the WOAH Aquatic Animal Health Code. This is because reporting of disease to the WOAH is based on the confirmed detection of the disease, clinical or non-clinical infection with one or more pathogenic agents, where infection means active replication of the pathogen within the host, and not the detection of the pathogen alone.

3.2 Alternative strategy

DAFF and the state and territory government authorities have agreed to an alteration strategy for the National List:

a. The National List will be reviewed annually by the National Sub-committee on Aquatic Animal Health (SCAAH), with recommendations for any changes provided to the parental committee of SCAAH, Animal Health Committee for consideration.
b. Any disease deletion must not interfere with regional or international reporting requirements.

1 "Disease" under the listing criteria is defined as clinical or non-clinical infection with one or more pathogenic agents (the same definition as the WOAH Aquatic Animal Health Code).

c. Diseases delisted from international lists (WOAH, Asia Pacific QAAD reporting program) are not automatically delisted from the National List if they satisfy other listing criteria. For example, it may be beneficial for trade or quarantine purposes to continue to accumulate evidence of Australia's freedom from diseases.

Since its inception in 1998, the National List has been modified several times using some iteration of the above alternative process. The current version of the National List is presented in Table 1.

Table 1: Australia's National List of Reportable Diseases of Aquatic Animals

Australia's National List of Reportable Diseases of Aquatic Animals Endorsed by the Animal Health Committee (AHC) – May 2024			
Finfish			
Disease	Listed in the WOAH Aquatic Animal Health Code (2023)	Listed regionally (WOAH/NACA) (2023)	Exotic to Australia
1. Infection with epizootic haematopoietic necrosis virus	✓	✓	
2. Infection with infectious haematopoietic necrosis virus	✓	✓	✓
3. Infection with spring viraemia of carp virus	✓	✓	✓
4. Infection with viral haemorrhagic septicaemia virus	✓	✓	✓
5. Infection with Betanodavirus		✓	
6. Infectious pancreatic necrosis			✓
7. Infection with infectious salmon anaemia virus	✓		✓
8. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)	✓	✓	
9. Bacterial kidney disease (*Renibacterium salmoninarum*)			✓
10. Enteric septicaemia of catfish (*Edwardsiella ictaluri*)		✓	
11. Piscirickettsiosis (*Piscirickettsia salmonis*)			✓
12. Infection with *Gyrodactylus salaris*	✓		✓
13. Infection with red sea bream iridovirus	✓	✓	✓
14. Furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*)			✓
15. *Aeromonas salmonicida* - atypical strains			
16. Whirling disease (*Myxobolus cerebralis*)			✓
17. Enteric redmouth disease (*Yersinia ruckeri* – Hagerman strain)			✓
18. Infection with koi herpesvirus (Cyprinid herpesvirus 3)	✓	✓	✓

19. Infection with Singapore grouper iridovirus (ranavirus)		✓	✓
20. Infection with infectious spleen and kidney necrosis virus			✓
21. Infection with turbot reddish body iridovirus			✓
22. Infection with scale drop disease virus			✓
23. Infection with salmonid alphavirus	✓		✓
24. Infection with tilapia lake virus	✓	✓	✓

Molluscs

Disease	Listed in the WOAH Aquatic Animal Health Code (2023)	Listed regionally (WOAH/NACA) (2023)	Exotic to Australia
1. Infection with *Bonamia ostreae*	✓	✓	✓
2. Infection with *Bonamia exitiosa*	✓	✓	
3. Infection with *Mikrocytos mackini*			✓
4. Infection with *Marteilia refringens*	✓		✓
5. Infection with *Marteilia sydneyi*			
6. Infection with *Marteilioides chungmuensis*		✓	✓
7. Infection with *Perkinsus marinus*	✓		✓
8. Infection with *Perkinsus olseni*	✓	✓	
9. Infection with *Xenohaliotis californiensis*	✓	✓	✓
10. Infection with Abalone herpesvirus (Haliotid herpesvirus-1)	✓	✓	
11. Infection with ostreid herpesvirus-1			

Crustaceans

Disease	Listed in the WOAH Aquatic Animal Health Code (2023)	Listed regionally (WOAH/NACA) (2023)	Exotic to Australia
1. Infection with Taura syndrome virus	✓	✓	✓
2. Infection with white spot syndrome virus	✓	✓	*
3. Infection with yellow head virus genotype 1	✓	✓	✓
4. Gill-associated virus			
5. Infection with infectious hypodermal and haematopoietic necrosis virus	✓	✓	
6. Infection with *Aphanomyces astaci* (crayfish plague)	✓	✓	✓
7. Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)	✓	✓	
8. Infection with infectious myonecrosis virus	✓	✓	✓
9. Monodon slow growth syndrome		✓	✓
10. Infection *with Hepatobacter penaei* (necrotising hepatopancreatitis)	✓	✓	✓

11. Acute hepatopancreatic necrosis disease	✓	✓	✓
12. *Enterocytozoon hepatopenaei*		✓	✓
13. Infection with decapod iridescent virus 1	✓	✓	✓
Amphibians			
Disease	Listed in the WOAH Aquatic Animal Health Code (2023)	Listed regionally (WOAH/NACA) (2023)	Exotic to Australia
1. Infection with *Batrachochytrium dendrobatidis*	✓	✓	
2. Infection with *Batrachochytrium salamandrivorans*	✓		✓
3. Infection with Ranavirus species	✓	✓	
* Restrictions apply			

4. QAAD reporting system

The QAAD reporting system was established in 1998. It was an online Microsoft Windows SharePoint-based file sharing system developed to collate information from all jurisdictions regarding cases of reportable aquatic animal disease (Figure 1). A dedicated rapporteur in each state and territory compiles a quarterly submission. Then, each state or territory government has a designated authority for endorsement, usually its Chief Veterinary Officer (CVO). All jurisdictions, represented by aquatic rapporteurs, provide CVO-approved quarterly (three-monthly) reports on their status for diseases listed on the National List to Australia's national coordinator based in DAFF, through the DAFF-managed online database. National QAAD reports are compiled from individual jurisdiction reports (Figure 2). The QAAD report format includes provisions for reporting fish kill investigations and emerging diseases. The draft national QAAD reports are provided to all jurisdictions for endorsement prior to use in international reporting. The final national QAAD report is copied to all QAAD rapporteurs simultaneously with international submission.

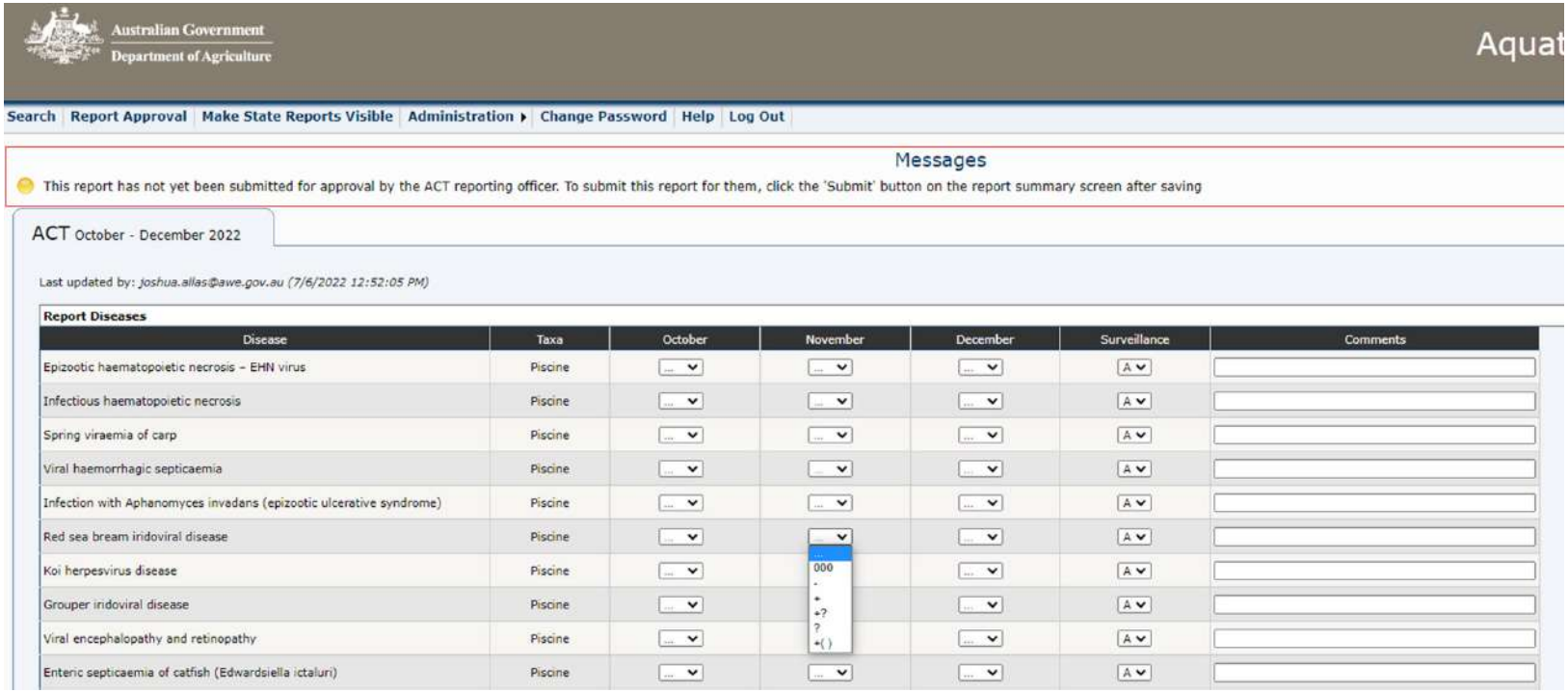

Figure 1. Example page in Quarterly Aquatic Animal Disease (QAAD) reporting system

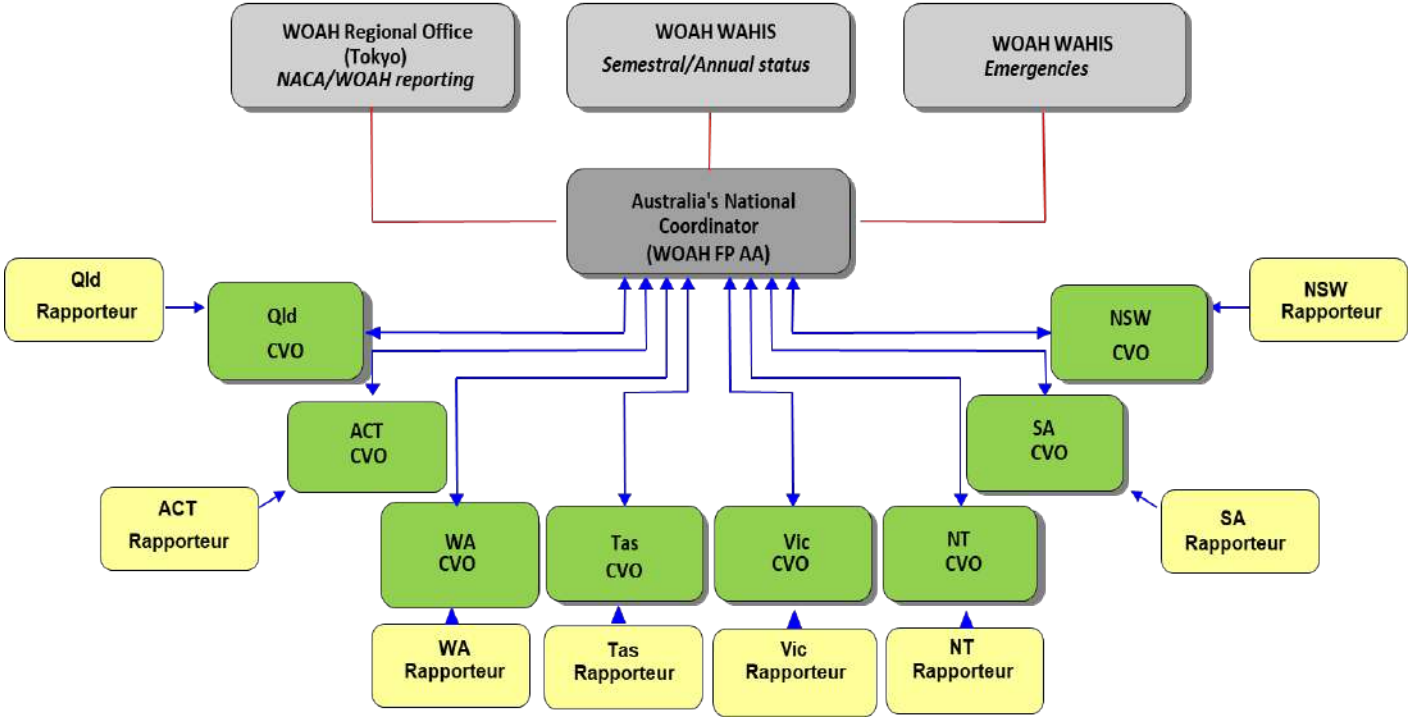

Figure 2. Quarterly Aquatic Animal Disease (QAAD) reporting flow diagram

Footnotes:

WOAH: World Organisation for Animal Health; WAHIS: World Animal Health Information System; WOAH FP AA: WOAH Focal Point for Aquatic Animals; Qld: Queensland

ACT: Australian Capital Territory; WA: Western Australia; Tas: Tasmania; Vic: Victoria; NT: Northern Territory; SA: South Australia; NSW: New South Wales

5. Development of AUSPestCheck®

In early 2021, the DAFF IT security team advised that the main SharePoint-based operational system for QAAD reporting had become outdated and was vulnerable to departmental security threats. To address this issue, DAFF initiated an evaluation of a new system, AUSPestCheck® [AUSPestCheck 2024]. AUSPestCheck® is a secure data aggregation, coordination, and visualization software system for pest and disease surveillance developed by Plant Health Australia (PHA), a joint government-industry non-profit organisation. It enables biosecurity surveillance data to be combined from multiple sources (e.g., different providers and/or systems), supporting data collection, mapping, and analysis of pest and disease distribution information. For example, AUSPestCheck® stores databases of surveillance results for plant pests, host species, detection methods, and temporal and spatial information, supporting pest/disease management and market access purposes. Several plant industries participate in AUSPestCheck®, and some have their surveillance data pages within the Plant tenancy (a program page). Originally developed for plant pest and disease surveillance, AUSPestCheck® was trialed as a data management system for terrestrial animal health surveillance by PHA in collaboration with DAFF in 2018.

5.1 AUSPestCheck® Aquatic Program trials

More recently, in 2022, the trials were expanded to create a new national aquatic animal disease reporting system (Aquatic Program) (Figure 3). During a series of tests, the suitability of this trial program for compiling national aquatic animal disease data for international reporting purposes was evaluated. The initial phase of development of AUSPestCheck® Aquatic Program conducted the following assessments:

a. Jurisdiction trial nationally standardised, user-friendly data entry using actual disease information data provided by jurisdictions (i.e., quarter 4 of 2021 and quarter 1 of 2022).
b. DAFF trialled national data transfer from the QAAD system.
c. DAFF conducted complete data transfer from the QAAD system and data aggregation for international disease reporting purposes.

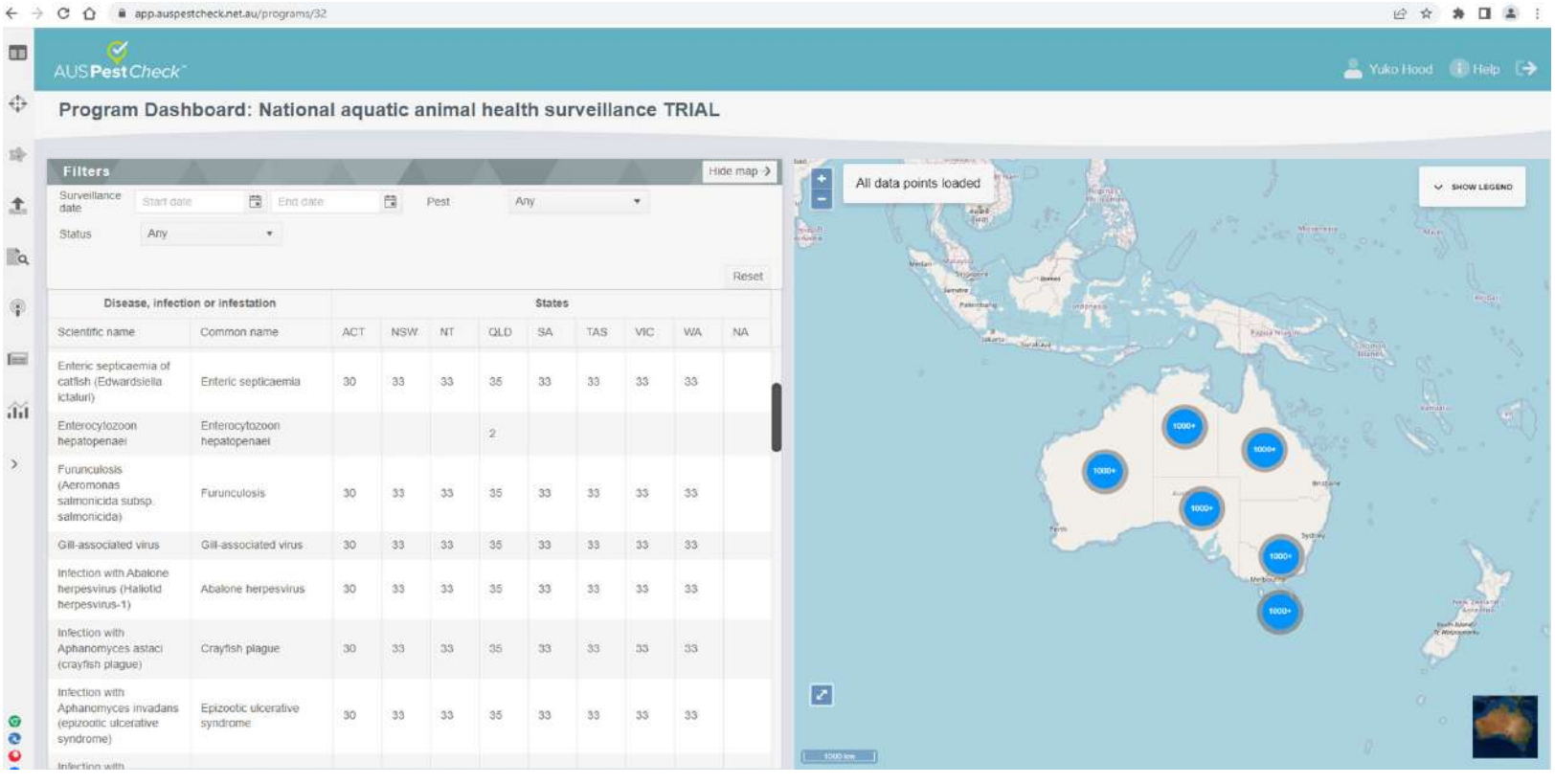

Figure 3. Example page of AUSPestCheck® Aquatic Program Trial

Note: As part of the trial, data from the legacy QAAD system were successfully migrated to the AUSPestCheck® Aquatic Program. This included the transfer of over 1,000 historical records, demonstrating the platform's capacity to manage and integrate large datasets while ensuring continuity of the national disease reporting system.

The first trial and data entry training for jurisdictions were conducted in May 2022, using spreadsheet-based CSV files for data transfer. Feedback from jurisdictional rapporteurs indicated that data entry using the Excel spreadsheet was not user-friendly and required workflow permissions from their CVOs. In response, the DAFF project team and PHA modified the Aquatic Program to include a web form for easier data entry and a workflow data staging area specific to the Aquatic Program. This staging area allows for CVO approval before jurisdictions submit their quarterly reports to the department.

During 2022, PHA contracted IT developers, and DAFF provided jurisdictional rapporteurs with multiple data entry training sessions. During this period, the old QAAD reporting system and AUSPestCheck® Aquatic Program were used in parallel to offer jurisdictions flexibility and to test the Aquatic Program's functionality by entering disease information for two data sets. In the AUSPestCheck® Aquatic Program, jurisdictions can manually submit their aquatic animal health data via a web form or an application programming interface (API) into AUSPestCheck®. The API will streamline and remove all manual data handling. Historical disease data sets since 1998 have been transferred from the old QAAD system to the AUSPestCheck® Aquatic Program, enabling jurisdictions to utilize the data for analysis and visualization.

Draft data standards for the AUSPestCheck® Aquatic Program have been developed by DAFF and reviewed by jurisdictions' aquatic rapporteurs. Trials conducted by jurisdictions using the AUSPestCheck® Aquatic Program for national aquatic animal disease information data entry and by DAFF for data transfer, storage, and aggregation have been successful. These trials highlighted the benefits of this new system. DAFF has fully implemented the AUSPestCheck® Aquatic Program since early 2024. Continuous updates to the system will occur based on ongoing user feedback even after full implementation. DAFF plans to organize further training with PHA for jurisdictional users, focusing on using Power BI functions within the AUSPestCheck® Aquatic Program for visualizing their data and aggregated national data.

5.2 AUSPestCheck® Aquatic Program data visualisation

Figure 4 shows a feature of AUSPestCheck® Aquatic Program data visualization. It is integrated with Power BI, meaning anyone who has access to this database can generate their own reports and dashboards using the data. Figure 4 is an example of infection with Perkinsus olseni, a WOAH-listed mollusc disease. The trial dashboard shows that Perkinsus olseni has been reported seven times in the last three years. Hovering over the bar graph reveals the location and timing (i.e., month and year) of the disease's detection. For instance, the January 2019 detection was from South Australia, and the February 2021 detection was from Western Australia.

The public-facing dashboard displays information at the state level. However, the state-only accessible dashboard can include more commercially sensitive details, such as specific farm locations. DAFF continues to test data integrations with Power BI visualization tools within the AUSPestCheck® Aquatic Program to improve its functionality.

5.3 AUSPestCheck® Aquatic Program system enhancement, data sharing and security issues

Significant system enhancement is underway for the AUSPestCheck® Aquatic Program, including IT developments to improve the PowerBI functionality, creation of a web form tool as an additional method of data upload, additional features for data entry users (e.g., auto-fill), and establishment of an approval process for jurisdictions to verify data within the system. The current focus is on delivering more mapping features to support the output of transmission zones, distribution maps, and the visualisation of surveillance activities at a regional level. Extensive testing of enhancements is ongoing to ensure functionality and performance in close collaboration with PHA and the DAFF project team.

Jurisdictional CVOs have raised concerns with AUSPestCheck® and data sharing, privacy, and security. Documents have been prepared to address these concerns, explaining how AUSPestCheck® will manage these issues. The DAFF project team and PHA have thoroughly consulted with all jurisdictions. The formal aquatic data-sharing memorandum of understanding will be developed once jurisdictions have agreed on the terrestrial version through the national animal health committee.

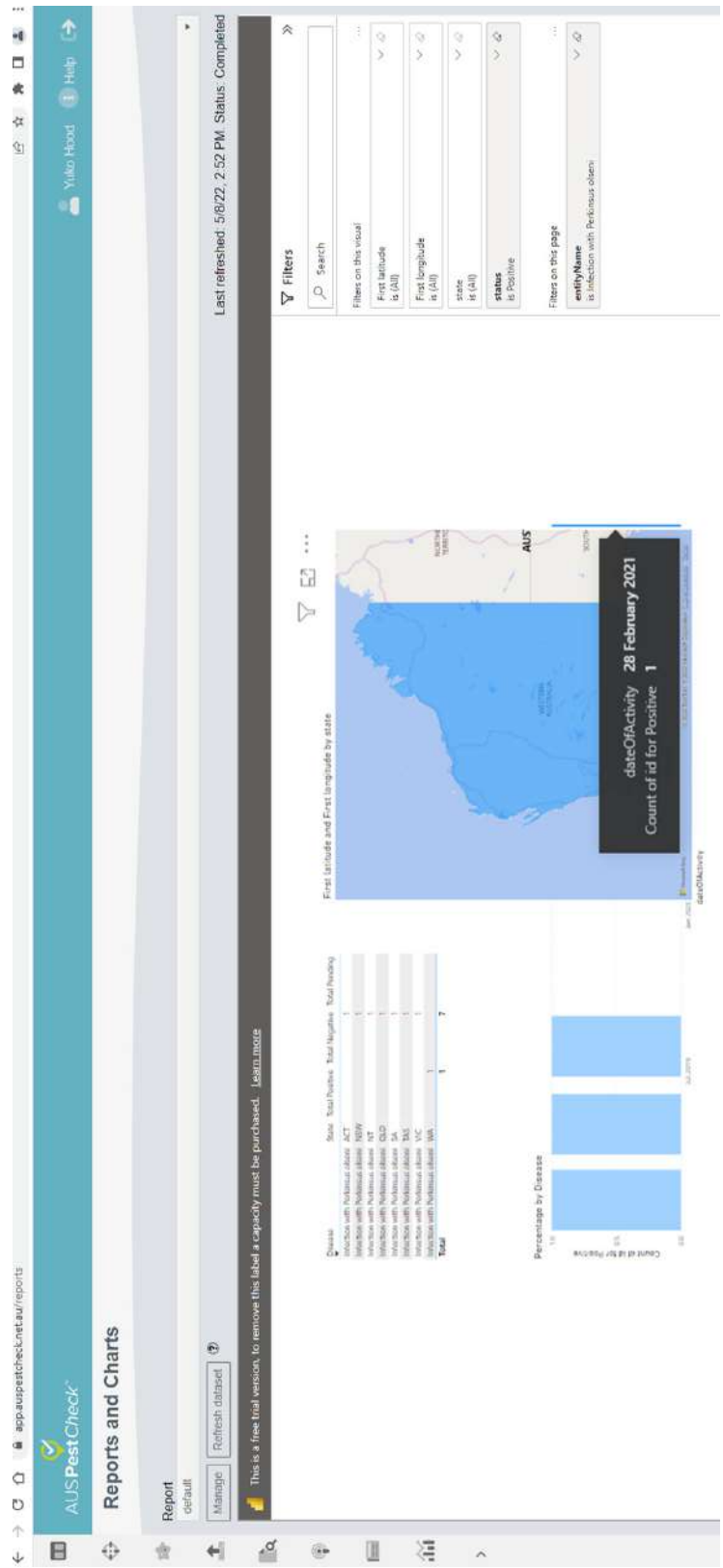

Figure 4. Visualisation example of AUSPestCheck® Aquatic Program data

Future Directions and Recommendations

The full implementation of the AUSPestCheck® Aquatic Program represents a significant advancement in Australia's national aquatic animal health reporting system. However, to remain effective, adaptive, and fit for purpose within an increasingly complex biosecurity environment, ongoing refinement and strategic development are essential. Future enhancements should prioritise expanding platform functionality and usability, particularly by increasing automation through API integration to reduce manual data handling, and embedding predictive analytics to support proactive risk management. Continued investment in user training, especially in advanced data visualisation tools such as Power BI, will enable jurisdictional users to derive actionable insights and contribute more effectively to national disease intelligence.

DAFF will maintain consistent stakeholder engagement through structured feedback loops and regular governance reviews. This includes finalising a formal data-sharing memorandum of understanding that addresses jurisdictional concerns regarding privacy, data ownership, and intellectual property, ensuring trust, clarity, and transparency across all participating entities. In addition, broadening the scope of the AUSPestCheck® Aquatic Program to incorporate new modules, such as wild aquatic animal disease surveillance, fish kill reporting, and interstate translocation testing, would significantly improve Australia's preparedness for emerging aquatic biosecurity threats. Strengthening collaboration with research institutions and international partners, including WOAH and NACA, will further align national surveillance strategies and contribute to regional and global aquatic animal health intelligence.

Conclusion

As Australia is a federal country, jurisdictions are required to provide DAFF with information on the presence or absence of all nationally listed diseases. DAFF is responsible for national coordination and compiling information for international reporting purposes. DAFF developed a new national aquatic animal disease reporting system, AUSPestCheck® Aquatic Program, to support this process. It is designed to assist stakeholders in collecting and submitting a jurisdictional disease report more quickly, efficiently, and accurately, promoting consistent and reliable disease reporting. Whilst the AUSPestCheck® Aquatic Program is streamlined, it retains a series of steps to ensure accuracy and appropriate jurisdictional authority over disease reports before the information is incorporated into official international reports.

AUSPestCheck® Aquatic Program has modern data security processes, and access to the program is restricted to authorised users. The use of data obtained from the program must comply with biosecurity, privacy, and intellectual property legislation. AUSPestCheck® Aquatic Program visualisation tools can be accessed by all stakeholders, including farms and veterinarians, who report disease events but do not receive regular feedback on diagnosed diseases and actions taken as a result of their reporting efforts. Visual data can provide more accessible passive surveillance feedback for these stakeholders.

The AUSPestCheck® Aquatic Program enables us to achieve our objectives by improving our disease intelligence analysis for national decision-making. It allows us to analyse data to demonstrate freedom from disease, support seafood trade, and justify our import biosecurity measures. Australia takes international disease reporting seriously, and the AUSPestCheck® Aquatic Program will significantly enhance our disease-reporting activities. As a WOAH member, Australia actively fulfils WOAH's international disease reporting obligations. As a NACA member country, Australia leads by example to support NACA and WOAH Regional Representation for Asia and the Pacific in improving regional aquatic animal disease reporting performance.

Australia has incorporated WOAH, NACA-listed diseases, and other significant diseases into the 'National List of Reportable Diseases of Aquatic Animals'. This list is designed to raise awareness among producers, aquatic animal health professionals, and jurisdictional government authorities regarding significant endemic and exotic diseases. It improves surveillance and monitoring capabilities, educates on the importance of early interventions for minimising disease spread, and reduces business risks of disease for local aquaculture enterprises. Historical reporting of the absence of disease demonstrates freedom for interstate and international trade. Our consistent and careful international disease reporting is critical for maintaining Australia's reputation as a transparent, accountable, and reliable trading partner and for demonstrating our commitment to regional disease management and biosecurity.

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Microsporidian Parasite, *Enterocytozoon hepatopenaei* Infection at Early Stage of Culture of Whiteleg Shrimps (*Penaeus vannamei*) in East Malaysia

ROHAIZA ASMINI YAHYA*[1], PADILAH BAKAR[1], WAN MUHAMAD HAZIM WAN SAJIRI[1], NUR SAMIHAH MAZLAN[1], NUR ASHIKIN ARBI[1] AND KUA BENG CHU[2]

[1]*National Fish Health Research Division (NaFisH), Fisheries Research Institute, 11960 Batu Maung, Penang, Malaysia*
[2]*Fisheries Research Institute, 11960 Batu Maung Penang, Malaysia*

**Corresponding author: rohaizaasmini@dof.gov.my*

Abstract: *Enterocytozoon hepatopenaei* (EHP) is a microsporidian parasite that infects the hepatopancreas of shrimp and causes various problems in aquaculture, often leading to increased operational costs. Early detection of EHP infection is essential, as it can help reduce the damage caused by the disease. Therefore, this study aims to determine the prevalence of EHP infection during the early stages of white shrimp (*Penaeus vannamei*) farming in Sabah and Sarawak. A total of 150 shrimp from five farms were sampled, and the presence of EHP in the hepatopancreas was analyzed using polymerase chain reaction (PCR) methods. The results indicated EHP prevalence ranging from 20% to 100% in the early stages of cultured whiteleg shrimp. A high prevalence of EHP (100%) was observed in shrimp under 20 days old, while 86% to 98% of EHP-positive shrimp demonstrated a specific growth rate (SGR) below the optimal body weight. The study also revealed a higher prevalence of EHP (87%) in Sabah compared to Sarawak (49%), which may be linked to the presence of EHP sources in the shrimp farming environment and suggests the potential role of vectors as carriers for disease transmission.

Keywords: whiteleg shrimp, *Enterocytozoon hepatopenaei,* prevalence, early-culture period

Abstrak: *Enterocytozoon hepatopenaei* (EHP) adalah sejenis parasit yang menjangkiti hepatopancreas udang dan menyebabkan pelbagai masalah dalam akuakultur, sering kali dikaitkan dengan peningkatan kos operasi. Pengesanan awal jangkitan EHP adalah penting, kerana ia dapat membantu mengurangkan kerosakan yang disebabkan oleh penyakit ini. Objektif kajian ini adalah untuk menentukan status semasa jangkitan EHP pada peringkat awal ternakan udang putih (*Penaeus vannamei*) di Sabah dan Sarawak. Sebanyak 150 ekor udang dari lima ladang telah diperiksa dan analisis kehadiran EHP pada organ hepatopankreas melalui kaedah tindak balas rantai polimerase (PCR). Sabah dan Sarawak melaporkan kejadian infestasi EHP pada peringkat awal pengkulturan udang putih, dengan kadar prevalens sebanyak 20% hingga 100%. Prevalen tinggi EHP (100%) ditemui dalam udang putih berusia kurang daripada 20 hari, manakala 86% hingga 98% pada udang positif EHP menunjukkan SGR kurang daripada berat badan yang optimum. Prevalen EHP yang tinggi (87%) dicatatkan di Sabah berbanding Sarawak (49%) yang mungkin berkait rapat dengan kewujudan sumber EHP dalam persekitaran ternakan udang dan secara tidak langsung menunjukkan potensi vektor sebagai pembawa untuk penyebaran penyakit.

Kata kunci: Udang putih, *Enterocytozoon hepatopenaei*, prevalen, tempoh awal kultur

Introduction

Shrimp is one of the protein sources in human nutrition, and its aquaculture supports significant livelihoods, particularly in Southeast Asia. In Malaysia, farmed whiteleg shrimp (*Penaeus vannamei*) contributed 78% of the total marine shrimp production in 2018, with a wholesale value of 186,588 USD (Annual Fisheries Statistics, 2018). Whiteleg shrimp is gaining popularity in Malaysia due to its fast growth and high market demand. It is currently the most extensively farmed species because of its advanced immune system, high tolerance to a wide range of environmental conditions, resistance to diseases, capability to be cultured in high density, high tolerance to salinity, and better feed conversion rate (Widanarni et al., 2019). Nonetheless, the worldwide culture of white shrimp has recently encountered significant challenges, particularly concerning the occurrence and intensity of various viral, bacterial, and parasitic diseases.

The Malaysian shrimp aquaculture industry has been challenged by current diseases such as white faeces syndrome (WFS) and acute hepatopancreatic necrosis disease (AHPND/EMS) (Kua et al., 2012; Wan Muhd Hazim et al., 2021). In addition, hepatopancreatic microsporidiosis (HPM) has become an increasing concern in shrimp aquaculture. *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite infecting the hepatopancreas (HP), was first reported in 2004 in growth-retarded *Penaeus monodon* in Thailand (Chayaburakul et al., 2004), and subsequently described in detail by Tourtip et al. (2009). Since then, EHP has emerged as a prominent intracellular pathogen in shrimp aquaculture across Asia, including China, Vietnam, Thailand, Indonesia, India, and Malaysia (Chaijarasphong et al., 2021). Although EHP infection is most commonly reported in *P. vannamei,* several other shrimp species are also susceptible. A suspected case in *P. japonicus* was reported in Australia (Hudson et al., 2001; Tourtip et al., 2009), while infections have been confirmed in *P. monodon* (Chayaburakul et al.,2004) and blue shrimp (*P. stylirostris*) (Tang et al., 2015). In Malaysia, the earliest reports of unidentified microsporidian infections in shrimp date back to Anderson et al. (1989); however, *P. vannamei* were not officially suspected of EHP infection until 2016 (NACA, 2016).

National surveillance led by Fisheries Biosecurity Malaysia between 2017 and September 2019 revealed no positive detections (NACA, 2017, 2018, 2019). However, EHP was confirmed during surveillance activities from October to December 2019, and subsequent cases were formally recorded in QAAD reports submitted to NACA (NACA, 2020). Later studies confirmed EHP presence in *P. monodon* (Marimuthu et al., 2021). The primary organ affected in shrimp is the hepatopancreas, which plays a vital role in growth performance and can result in significant variations in shrimp sizes at the end of the culture period. Horizontal transmission of EHP was reported through cannibalism (shrimp to shrimp), spores in fecal excretion, and infected water (Tangprasittipap et al., 2013).

The organic matter at the pond bottom provides a suitable ground for the survival of spores in the aquatic environment. Hence, benthic invertebrates and life feed polychaetes that live at the pond bottom as their natural habitat may become potential carriers that continuously infect or release spores to other aquatic animals/shrimps. Polychaetes are ubiquitous benthic invertebrates that naturally abound in the shrimp ponds and serve as natural food for shrimp (Varadharajan and Soundarapandian,

2013). According to Mukta and Paramveer (2018), EHP does not require an intermediate host for transmission. Little information is available on the current status of EHP, which was reported at an early stage of shrimp culture in East Malaysia. Therefore, this study aims to determine the status of EHP infestation in whiteleg shrimp at an early stage of culture in the pond.

Materials and Methods

Shrimp Collection

Shrimp was collected from five farms in Kudat, Tawau, Miri, Sibu, and Kuching using a one-time cross-sectional sampling method in 2019. These sites were selected based on their roles as leading shrimp-producing regions in East Malaysia. All shrimp were caught using a feeding tray. A total of 150 shrimp samples from seven pond cultures (DOC, less than 42 days) were analysed. The hepatopancreas of each sample was dissected and fixed in 95% ethanol for Polymerase Chain Reaction (PCR).

DNA Extraction

The genomic DNA from hepatopancreas tissue was extracted with a commercial DNA extraction kit (IQ2000TM GeneReach Biotechnology, Taiwan) following the manufacturer's instructions. The extracted DNA was then suspended in 100µl of TBE buffer.

Detection of EHP

The detection of the EHP gene sequence from hepatopancreas tissues of shrimps was performed by PCR. A unique spore wall protein gene specific to EHP (SWP-PCR), based on a method described by Jaroenlak et al. (2016), was used in this diagnosis. All the PCR products were electrophoresed on a 1.5% agarose gel prepared in TBE buffer at 100 V for 30 minutes, and the results were observed under the bio-imaging system (Syngene, Cambridge, UK). EHP was categorized into heavy and light infections based on the detection of amplicons at 514 bp (heavy) or 148 bp (light).

Data Analysis

Descriptive analysis was conducted to determine the mean and percentage of positive EHP infestation in two different groups of white shrimps (DOC), in relation to prevalence (%) and specific growth rate (SGR). The prevalence was classified into overall infection following Margolis et al. (1982). Under DOC, two periods, less than 20 days of culture and more than 20 days of culture, were selected to determine the initial occurrence of EHP. Comparison of SGR for the optimum weight gain in whiteleg shrimp was based on reported SGR by Dian et al. (2017) and Khademzadeh and Haghi (2017); meanwhile, the formula for SGR by body weight was based on Hidayah et al. (2020). Statistical data were analyzed using SPSS version 20. Statistically significant differences between EHP infestation and the parameters (specific growth rate and days of culture) were evaluated by the chi-square test and were considered significant if the p-value was less than 0.05.

Results and Discussion

EHP infestation, with a prevalence ranging from 20% to 100%, was detected in whiteleg shrimp from Sabah and Sarawak during the first 42 days of culture in the pond. In Sabah, prevalences of 100% and 73% were observed in whiteleg shrimp farms from Tawau and Kudat, respectively (Figure 1). Sarawak recorded a prevalence ranging from 20% to 100% in Miri, Kuching, and Sibu, respectively.

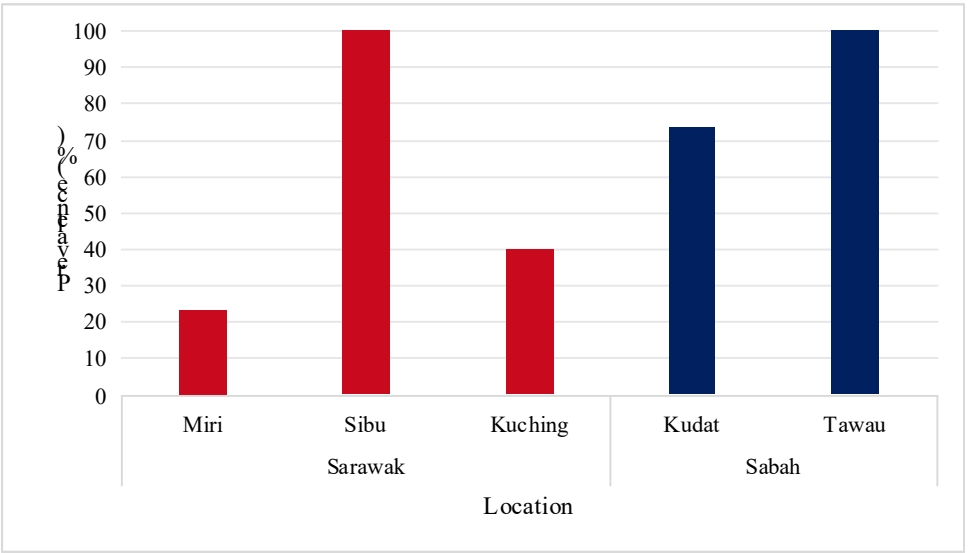

Figure 1. Prevalence of EHP occurrences in the early-stage culture of white shrimp in Sarawak and Sabah.

Further observation of the potential occurrence of EHP infection during the early-stage culture of whiteleg shrimp from two groups, in relation to SGR and sampling locations, showed corresponding rates of EHP infestation. Statistical analysis indicated a significant difference ($p \leq 0.05$) in DOC less than 30 days with the occurrence of EHP (Table 1). Shrimp cultures aged less than 20 days were more likely to be infected with EHP than those over 20 days, with a prevalence of 100% and 46%, respectively. Under the categorical parameter of SGR, the EHP infestation was significantly different in whiteleg shrimp of SGR, which was less than 4.9 (86%) compared with 46% in the other group of shrimps. Farms located in Sabah showed higher EHP infestation, with a prevalence of 87 compared to 49 in Sarawak (Table 1).

Table 1: The occurrence of EHP infestation in the early-stage culture of white shrimp from two different age groups.

Categories	Sample examined	Positive sample	Prevalence EHP (%)	X^2	p
Day of culture (DOC)					
Less than 20 days	50	50	100	42.2	0.000*
More than 20 days	100	46	46		
Specific growth rate (%)					
(a) Optimum SGR, 2.31% (Dian et al., 2017)					
Less than 2.31%	41	40	98	17.6	0.000*
More than 2.31%	96	150	64		
(b) Optimum SGR, 4.9% (Khademzadeh and Haghi 2017)					
Less than 4.9%	66	52	86	25.6	0.000*
More than 4.9%	84	39	46		
Location					
Sabah	60	52	87	22.2	0.000*
Sarawak	90	44	49		

The study highlighted that both farms of whiteleg shrimp in Sabah and Sarawak have a high prevalence of EHP (100%) infestation during the early stage of culture, less than 42 days in ponds. Nevertheless, a low prevalence of EHP (20%) was detected in Miri, Sarawak. These findings correspond with previous reports on EHP prevalence. Wan Sajiri et al. (2021) observed a prevalence ranging from 73.3% to 100% in a single production cycle of *P. vannamei* across three shrimp farms in the northern (Kedah and Penang) and southern (Johor) regions of Peninsular Malaysia. Similarly, Shen et al. (2017) reported a prevalence of 79.5% in whiteleg shrimp culture in earthen ponds in China, compared to 54.4% in greenhouse ponds. Meanwhile, in India, Rajendran et al. (2016) documented a high prevalence of 96.4% in pond-cultured *P. vannamei*, which was associated with white faeces syndrome. Many factors could contribute to the high prevalence of EHP in shrimp farms.

According to Tangprasittipap et al. (2013) and Newman (2015), EHP can be transmitted orally through infected live feeds such as polychaetes or horizontally via spores present in water and faeces from infected shrimp. Salachan et al. (2017) reported that EHP spores require approximately 14 days to initiate infection and can be easily transmitted among shrimp through cannibalism and cohabitation with infected individuals. Additionally, Mukta and Paramveer (2018) noted that EHP spores excreted through feces remain viable in water, posing a risk of pond-wide transmission. These findings suggest the potential for direct transmission from infected to uninfected shrimp. EHP infection has also been shown to spread progressively in rearing ponds and become widespread as cultivation continues (Tangprasittipap et al., 2013; Wan Hazim et al., 2021).

In this study, shrimp cultured for less than 20 days showed 100% infection compared to 46% in shrimp cultured for more than 20 days. These results suggest that whiteleg shrimp cultured for less than 20 days are more vulnerable to EHP infection compared to those cultured for over 20 days in

ponds. Potential vectors within the culture environments might contribute to disease transmission, despite the use of specific pathogen-free (SPF) post-larvae in this study, as reported by the farm owner. According to Wan Sajiri et al. (2023), macrofauna species from three phyla (Arthropoda, Mollusca, and Chordata) inhabiting farming ponds exhibited an average EHP prevalence of 82.93%, indicating their possible role as carriers. During the study period, all farms practiced chlorination and dechlorination of water in reservoir ponds to eliminate carriers or vectors. Although certain farms in Sabah and Sarawak implemented various biosecurity measures, there was a wide range of prevalence among locations during the survey. Sabah and Sarawak experienced different levels of EHP infestation, with Sabah showing a higher prevalence of EHP. It could be associated with changes in management strategies or the location of the farms.

The presence of EHP can negatively affect the growth of whiteleg shrimp, as it can reduce their growth rate. This study showed a strong association between early-stage culture of *P. vannamei*, DOC, SGR, and EHP infection rates. Dian et al. (2017) highlighted that the optimum SGR in Indonesia was 2.31%. A strong correlation was observed between the present SGR, less than 2.31%, and EHP. In the present study, 98% of shrimp with SGR less than 2.31% were infested with EHP, compared with 64% for uninfected shrimp. In another study by Khademzadeh and Haghi (2017), the optimum SGR for the whiteleg shrimp was less than 4.9%. Comparison of this SGR also showed similar results, while the SGR for shrimp infested with EHP was high (86%) in shrimp with an SGR of less than 4.9%, compared with only 46% of EHP-infested shrimp with an SGR of more than 4.9%. There was strong evidence of a correlation between SGR less than 4.9% and more than 4.9%. Besides, the present study indicated that despite the presence of EHP, the health of the shrimp was severely affected, and it could be considered that the affected shrimp continue to grow under optimum weight gain until the harvesting period. These findings are consistent with previous reports of growth retardation associated with microsporidian infections in shrimp. Similar observations were reported by Kevasan et al. (2016), who documented stunted juvenile whiteleg shrimp infected with EHP in India. Flegel et al. (1992) highlighted that infestation of EHP damages the hepatopancreatic tubular cells of shrimp, malfunctioning the digestive and absorptive capability of cells, leading to slow growth.

EHP generally does not lead to mass mortality. Nevertheless, biosecurity management measures such as seed screening, maintenance of good quality feeds, proper pond preparation, regular health monitoring, and adequate disposal of infected animals from shrimp farms should be practiced continuously by farmers. Apart from that, pond management and control must be strictly implemented to prevent the spread of disease from any potential carriers.

Conclusion

EHP infestation in whiteleg shrimp was detected in East Malaysia, with prevalence rates ranging from 20% to 100%. Factors such as different DOC, SGR, and geographic location significantly affected the prevalence of EHP in whiteleg shrimp in the present study. Shrimp aged less than 20 days exhibited a 100% EHP infection rate, notably higher than the 46% observed in shrimp older than 20 days. Similarly, shrimp with an SGR below the optimum threshold (2.31% or 4.9%) showed a markedly increased prevalence of EHP (86% to 98%) compared to those with higher SGR values (46%

to 64%). Regionally, Sabah farms recorded a greater EHP prevalence (87%) than those in Sarawak (49%), likely influenced by differing farm management practices. The present study highlighted the information gathered on the current status of the high EHP prevalence in East Malaysia and suggests the ongoing presence of EHP sources within the shrimp culture environment.

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BioDOF-Map System to Enhance Fisheries Biosecurity Monitoring

ELEANOR DANIELLA LOKMAN[1,2], NORSIDA MAN[1] AND SUHAIDA AINI[3*]

[1]Department of Agribusiness and Bioresource Economics, Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43400 UPM, Serdang, Selangor.
[2]Research Institute (FRI) Batu Maung, 11960 Pulau Pinang, Malaysia.
[3]Malaysian Space Agency (MYSA), Ministry of Science, Technology and Innovation (MOSTI), No.13, Jalan Tun Ismail, 50480 Kuala Lumpur, Malaysia

**Corresponding author: norsida@upm.edu.my*

***Abstract**:* The BioDOF-Map System is a web-based Geographic Information System (GIS) developed by the Department of Fisheries (DOF) Malaysia in collaboration with the Malaysian Space Agency (MYSA) to improve aquaculture biosecurity monitoring. It incorporates remote sensing, GIS, and information and communication technology (ICT) to support decision-making and resource management by providing real-time spatial data access, large-scale data processing, and collaborative tools via internet-connected devices. In Malaysia, the Department of Fisheries (DOF) employs a web-based GIS to improve fisheries biosecurity management, supported by collaboration with the Malaysian Space Agency (MYSA). This system combines remote sensing, GIS, and ICT to increase efficiency and effectiveness in monitoring and managing fisheries biosecurity activities. Key features include remote sensing integration for monitoring environmental changes and fish health using satellite imagery and drone data, real-time data access for immediate updates on critical parameters such as water quality and weather, and interactive mapping for visual representation and spatial analysis of aquaculture sites. Advanced data analysis methods such as buffering, overlay, and network analysis strengthen resource management. The user-friendly interface ensures accessibility for users of all technical levels, and comprehensive training and support, including sessions and manuals, promote proficient use. User Acceptance Testing (UAT) involves stakeholders in validating the system's operation and usability, ensuring it meets user requirements before deployment. This study finds that the key factors influenced the intention of MAFs to adopt BioDOF-Map. BioDOF-Map, prominently used by the DOF with MYSA's support, enhances fisheries biosecurity through historical data, remote sensing, and easy-to-use features, significantly improving decision-making and resource management.

Keywords: GIS, Remote Sensing, web-based, spatial.

Abstrak: Sistem BioDOF-Map ialah Sistem Maklumat Geografi (GIS) berasaskan web yang dibangunkan oleh Jabatan Perikanan (DOF) Malaysia dengan kerjasama Agensi Angkasa Malaysia (MYSA) untuk menambah baik pemantauan biosekuriti akuakultur. Ia menggabungkan penderiaan jauh, GIS, dan teknologi maklumat dan komunikasi (ICT) untuk menyokong pembuatan keputusan dan pengurusan sumber dengan menyediakan akses data spatial masa nyata, pemprosesan data berskala besar dan alatan kerjasama melalui peranti yang disambungkan ke Internet. Di Malaysia, Jabatan Perikanan (DOF) menggunakan GIS berasaskan web untuk menambah baik pengurusan biosekuriti perikanan, disokong oleh kerjasama dengan Agensi Angkasa Malaysia (MYSA). Sistem ini menggabungkan

penderiaan jauh, GIS, dan ICT untuk meningkatkan kecekapan dan keberkesanan dalam memantau dan mengurus aktiviti biosekuriti perikanan. Ciri utama termasuk penyepaduan penderiaan jauh untuk memantau perubahan persekitaran dan kesihatan ikan menggunakan imejan satelit dan data dron, akses data masa nyata untuk kemas kini segera pada parameter kritikal seperti kualiti air dan cuaca, dan pemetaan interaktif untuk perwakilan visual dan analisis spatial tapak akuakultur. Kaedah analisis data lanjutan seperti penimbalan, tindanan dan analisis rangkaian mengukuhkan pengurusan sumber. Antara muka mesra pengguna memastikan kebolehaksesan untuk pengguna dari semua peringkat teknikal, dan latihan dan sokongan yang komprehensif, termasuk sesi dan manual, menggalakkan penggunaan yang cekap. Ujian Penerimaan Pengguna (UAT) melibatkan pihak berkepentingan dalam mengesahkan operasi dan kebolehgunaan sistem, memastikan ia memenuhi keperluan pengguna sebelum penggunaan. Kajian ini mendapati bahawa faktor utama mempengaruhi niat MAF untuk mengguna pakai BioDOF-Map. BioDOF-Map, yang digunakan secara ketara oleh DOF dengan sokongan MYSA, meningkatkan biosekuriti perikanan melalui data sejarah, penderiaan jauh dan ciri yang mudah digunakan, meningkatkan dengan ketara dalam membuat keputusan dan pengurusan sumber.

Kata kunci: GIS, Penderian Jauh, laman sesawang, spatial

Introduction

The Malaysian ornamental fish business has a considerable impact on the country's economy, making a significant contribution to the global trade of ornamental fish. Malaysia ranks as the fourth-largest exporter of ornamental fish in the ASEAN region and the eighth-largest exporter worldwide. The output of ornamental fish in Malaysia in 2022 amounted to 200.34 million, with a total value of around MYR 453 million (USD 100 million). Penang, Perak, and Selangor are the main regions where the key production of goldfish occurs (Annual Fisheries Statistics, 2022; Devi, 2024). Goldfish is the dominant species and accounts for 60% of the total output (Zuridah and Zuridah, 2023). Web-based GIS is an effective tool that can be used to improve decision-making, increase operational efficiency, and promote collaboration in several areas, including urban planning, environmental management, transportation, and public health.

Access to spatial data and advanced mapping and spatial analysis over the internet is increasingly prevalent. Consequently, the role of an information system is to enhance decision-making capacity (Anjur et al., 2021). An interface system should be developed to assist users with searching, analyzing, and printing data. Web GIS, which stands for Web-based Geographic Information System, can be accessed through web browsers such as Google Chrome, Mozilla Firefox, or Internet Explorer.

The ArcGIS Server was employed to create and manage geographical data, GIS online services, and applications. It functions as ArcGIS Enterprise's backend server component, allowing geographic data to be shared with people inside an organization and, if requested, with anyone with an internet connection. It is specifically designed to make it easier to develop and deploy centralized GIS services and applications that meet various user needs. The GIS mapping system in this case is relevant only to goldfish culture and trade, ensuring accurate management, monitoring, and decision-making

in this sector. If applied to other commodities, the system must be tailored to the appropriate context, considering unique industry-specific factors. Organizations utilize ArcGIS Server to distribute maps and GIS functionalities through web-based mapping applications and services, enhancing internal workflows, providing essential information, and supporting stakeholder interactions. It enables the efficient sharing of GIS applications both within and outside an organization. Additionally, ArcGIS Server supports the integration of geographically enabled data into the broader IT environment, enabling the cost-effective sharing of spatial data advantages with a wider audience.

Materials and Methods

Data Collection

The study gathered secondary data through a comprehensive approach, utilizing both traditional and online sources. Information was obtained from library archives, reliable websites, governmental agencies, and the Department of Fisheries, ensuring accurate statistical information. Scholarly journals, past reports, seminars, and previous research provided valuable data on off-farm labor and rural challenges. The internet was instrumental in acquiring relevant GIS data, aiding in sampling procedures, validating study findings against regional or national datasets, and understanding study locations contextually.

Collaborating with authorized institutions like Jabatan Ukur dan Pemetaan Malaysia (JUPEM) enabled access to crucial spatial digital maps detailing roads, rivers, villages, paddy fields, and administrative boundaries. The Department of Agriculture (DOA) contributed important data on the geographical distribution of paddy farming and farmer-related information. The research methodology focused on examining academic publications, books, online resources, and governmental databases, emphasizing the collection of essential geographical data. This multifaceted approach ensures a thorough understanding of the topic and its complexities.

Data Verification and Integration

Data verification and integration are iterative procedures that require meticulous attention to detail, coordination between data sources and GIS analysts, and adherence to best practices in data management and quality control. GIS plays a crucial role in supporting well-informed decision-making, planning, and resource management in diverse sectors and disciplines by ensuring the accuracy and reliability of spatial data. Combining agricultural systems analysis with geographical data makes it possible to identify the interconnections between farming populations and their spatial features (Ghosh, 2023). This methodology integrates relevant socioeconomic and spatial data to examine the geographical factors that influence the formation of farming systems.

It is essential to verify reference projections when obtaining cadastral and digital maps to avoid errors and guarantee accuracy. Since its inception, Peninsular Malaysia has relied on the Malayan Revised Triangulation 1948 (MRT48) as its primary geodetic reference. MRT48, which is centred at Kertau, Pahang, uses the Rectified Skew Orthomorphic (RSO) projection and the Modified Everest

ellipsoid to minimize regional distortions and improve the precision of geodetic and mapping projects. To provide a consistent, earth-centered coordinate system that complies with international standards, the Geocentric Datum of Malaysia 2000 (GDM2000) was established in response to advances in geospatial technology and the growing use of GPS (Kadir, Mohamed, and Shariff, 2002). The network consists of 77 geodetic stations, 240 primary stations, 837 secondary stations, and 51 tertiary stations. Additionally, there are other triangulation sites that rely on conventional observations.

Digital cadastral maps in Shapefile format contain detailed information on each lot, including lot number, area, bearing, and boundary distance. These maps do not reveal any personal information. These maps are supported by supplementary digital layers, which enable thorough data integration. The locations of fish farms derived from cadastral maps are correlated with those obtained from remote sensing images and other primary or secondary sources. Surveyors match the names of farmers with their corresponding plots, thus updating farmer information collected via questionnaires in a centralised database. Data cleaning techniques are subsequently applied to ensure the quality and reliability of the data.

Database design, System Development and Operational procedures

The Malaysian Space Agency (MYSA) has acquired a server and ArcGIS server software, demonstrating its commitment to the advancement of interface systems. MYSA is responsible for developing and submitting system updates and ensuring consistent maintenance. The Department of Fisheries (DOF) Malaysia has created an interactive GIS web application using ArcGIS Server, Portal for ArcGIS, and Web AppBuilder. The application employs PHP and JavaScript programming languages. ArcGIS Server functions as a versatile platform that can host a wide range of web services and extensions. The services offered include cached and dynamic maps, geodata, geocode, geoprocessing, and image services. ArcGIS Pro also facilitates the administration of 3D scene data by providing scene services and vector tile services. This comprehensive software package enables users to create, manage, and display GIS data on the internet, addressing a broad range of mapping requirements on computers, mobile devices, and the internet.

Web-based GIS depends on the fundamental elements of efficient database architecture, systematic system development, and streamlined operational procedures. By integrating spatial data into web applications, organisations can maximize the potential of geospatial information, enabling users to make informed decisions based on actionable insights.

By combining geographic data with real-time monitoring and decision-making capabilities, web-based GIS enhances fisheries biosecurity monitoring in the BioDOF-Map System. By collecting and displaying spatial data on critical water quality indicators, including temperature, dissolved oxygen, pH, and salinity from aquaculture farms, the technology simplifies real-time environmental monitoring. This enables authorities and farm operators to detect any biosecurity threats early, including disease outbreaks or toxic algal blooms. Additionally, GIS spatial analysis is utilized for risk zoning and disease outbreak tracking, allowing the system to map high-risk areas for disease events. For example, suppose a Koi herpesvirus (KHV) outbreak occurs in goldfish farms. In that case,

the system can overlay spatial data to monitor the spread, identify nearby farms at risk, and enable containment measures.

Furthermore, spatial planning and farm management are improved through GIS tools, supporting site selection for new aquaculture farms by ensuring they are located in areas with optimal water quality, minimal pollution, and appropriate biosecurity measures. The system also assists in establishing biosecurity buffer zones, ensuring safe distances between aquaculture facilities and high-risk regions. GIS-based tracking of goldfish trade routes significantly improves supply chain and trade route monitoring beyond farm management. This enables authorities to monitor fish movements from farms to export hubs and ensure compliance with biosecurity regulations. GIS tools support authorities in effectively tracking and isolating affected shipments when flagged for potential disease risk. Furthermore, the BioDOF-Map System offers user-friendly data access and decision support through interactive GIS dashboards, allowing researchers, government organisations, and farmers to collaborate on effective aquaculture biosecurity programs, assess trends, and make well-informed decisions.

Results and Discussion

A BioDOF-Map GIS (Figure 1) system is an online platform that enables users to remotely access, analyse, and visualise geographic data. It integrates spatial data with web technologies to provide interactive mapping capabilities, allowing users to make well-informed decisions using geographical information. A web-based Geographic Information System (GIS) solution is essential in the Malaysian aquaculture setting for various reasons. Firstly, it enhances the effective management of aquaculture resources by providing up-to-date spatial information on water quality, land utilisation, and infrastructure.

The use of GIS technology is crucial, since it allows for a thorough understanding of spatial and temporal relationships within climatic data, which is vital for making well-informed decisions. GIS, unlike other information systems, offers a broad array of capabilities for geographical analysis and correlation. Additionally, it improves the decision-making processes by enabling stakeholders to select suitable locations for aquaculture development, monitor environmental impacts, and efficiently allocate resources. Furthermore, it encourages collaboration and the exchange of information among government agencies, researchers, and industry participants, thus supporting the sustainable growth and development of the Malaysian aquaculture industry.

In order to gain access to the system, users are required to meet particular minimum criteria. For maximum performance, it is advisable for users to have an internet connection speed above 520Kbps. Moreover, for optimal viewing of the system, it is recommended to use a computer resolution of 1024 x 768 pixels. To achieve an optimal user experience, it is recommended to set the computer resolution to 1024 x 768 pixels. This resolution enables users to fully understand and interact with the system's content, enhancing ease of use and accessibility Hobo Web (2023).

Figure 1. Interface of the bioDOF-Map System.

The bioDOF-Map system comprises five modules, namely, display, search, edit, measurement, and tools. Only registered users have access to these components. The login authentication system, a common component of web applications, provides security by allowing only registered users to access the website and its features that are special to members. Additionally, it enables the preservation of user information (Figure 2).

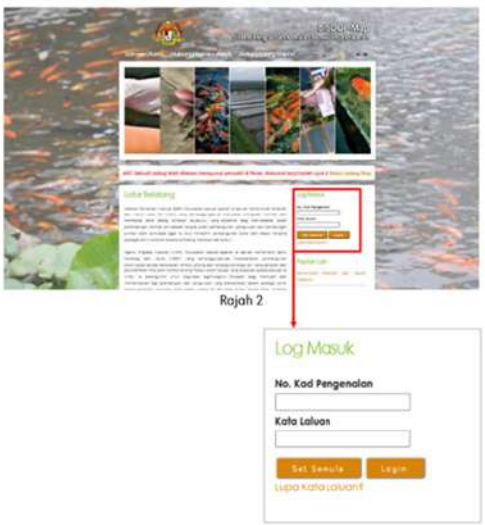

Figure 2. Accessing the BioDOF-Map System by entering login credentials.

The initial module involves observing the geographical locations of aquaculture facilities within the system. Users have the ability to access the system to collect information from specific farm facilities. The spatial data is classified into two types: fundamental data and aquaculture farm data within the system (Figure 3). The user browsing system has been improved with features such as zoom in, zoom out, home, my location, pan, full view, and bookmark (Figure 4).

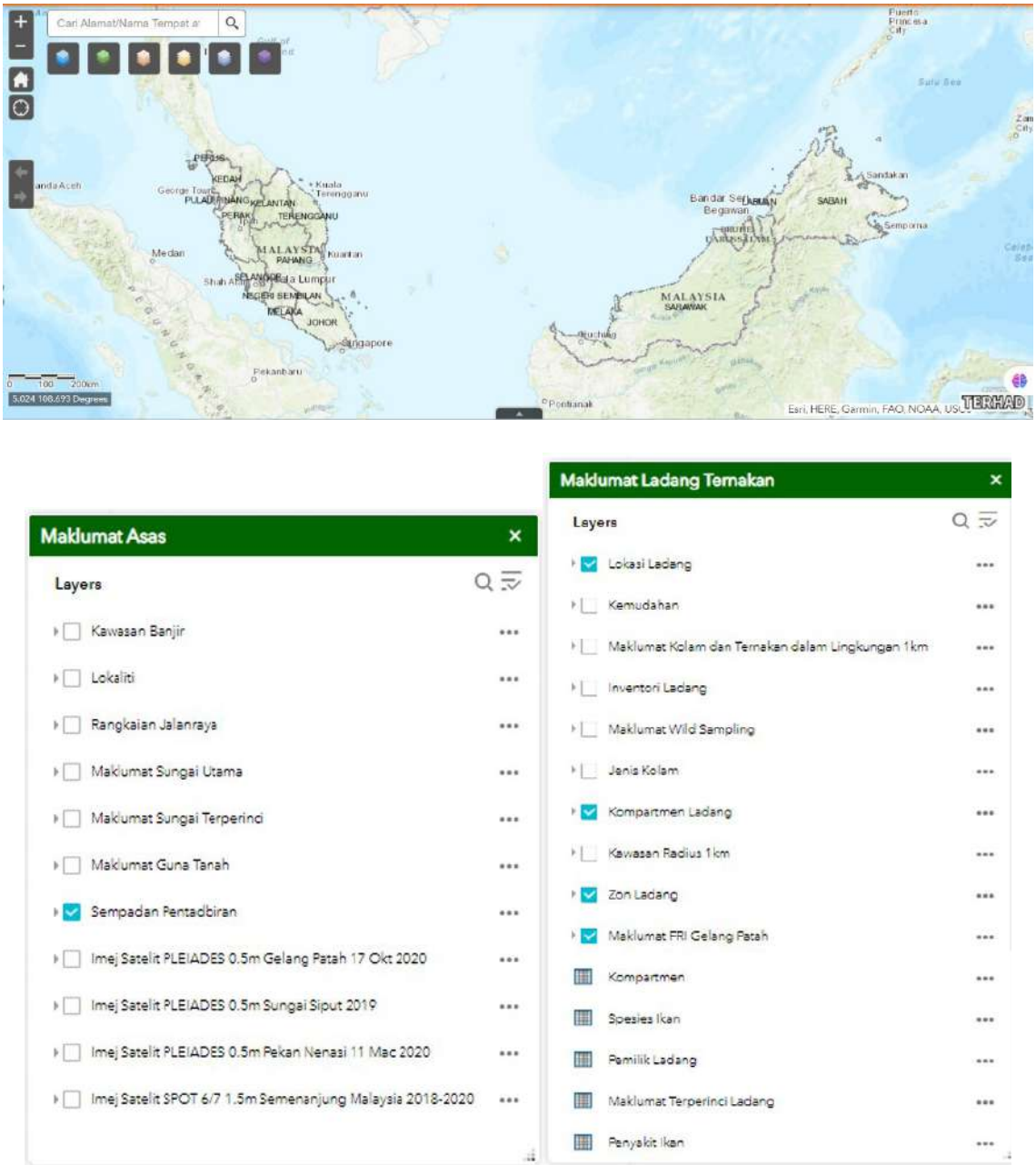

Figure 3. Spatial distribution of aquaculture facilities in the BioDOF-Map System, with a table displaying key data and information for each fish farm

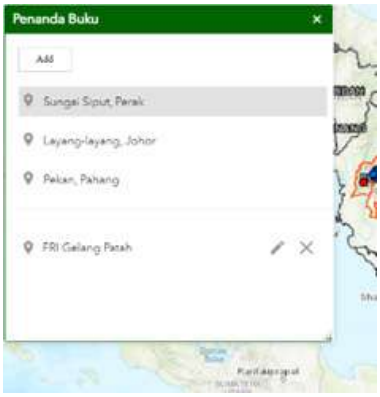

Figure 4. Bookmark panel.

The second module was categorised into two types of searches. The first is general search, which includes farm location and fish disease (Figure 5). The second is specific search, which involves latitude and longitude coordinates (Figure 6).

Figure 5. General search of the BioDOF-Map System

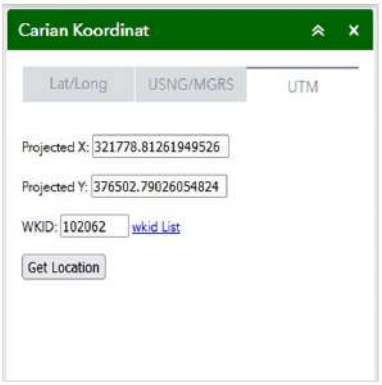

Figure 6. Targeted search of BioDOF-Map System

The Third Module is an edit widget that allows the administrator to modify data within the system. This widget is exclusively intended for those with permission to modify farm information (Figure 7).

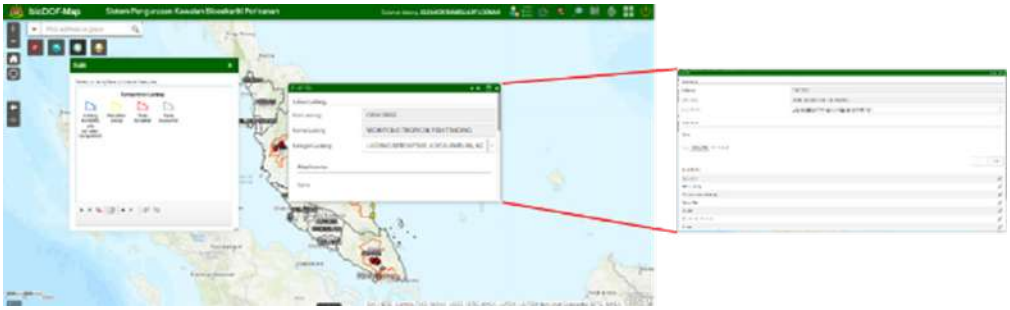

Figure 7. The Edit widget feature of the BioDOF-Map System

The Fourth Module is a measuring widget that allows the administrator to determine the size of a farm or area. It also allows measuring distances between two locations (Figure 8). This measurement method, known as distance measurement, is crucial in mapping activities. For instance, by measuring the distance between settlements and selected farm locations, it can significantly assist in Network Analysis.

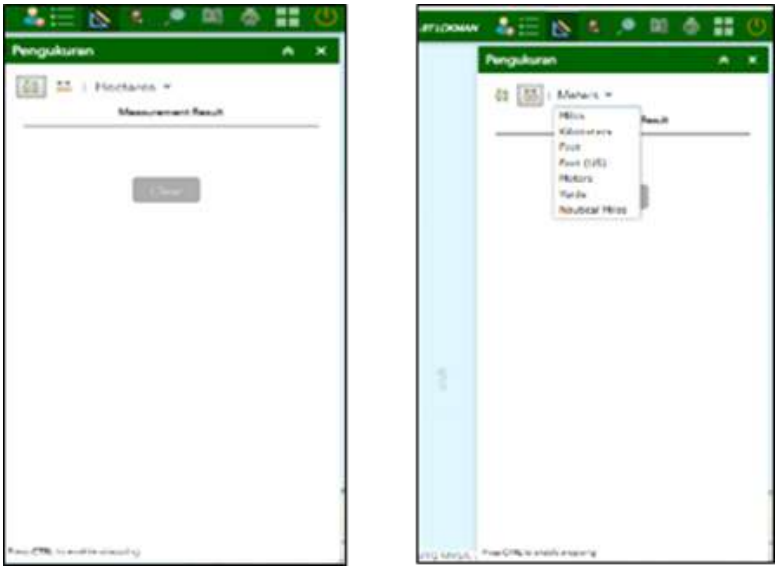

Figure 8. Measurement widget.

The final module comprises the printing widgets, which allow users to generate printed outputs of the study in the form of tables and maps (Figure 9). This system provides users with the option to choose the preferred basemap gallery type for visual presentation (Figure 10).

Figure 9. Printing result from BioDOF-Map System.

Figure 10. Basemap Gallery Option of the BioDOF-Map System.

The web-based system was built to maximise efficiency for all users, especially the officers from DOF and aquaculture farmers. A web-based GIS system designed specifically for aquaculture should incorporate many essential features and functionalities. The interface possessed an intuitive and user-friendly design, guaranteeing effortless navigation and clear understanding of the tools and instructions (GIS Mapping Software, Location Intelligence and Spatial Analytics | Esri, n.d.). The system has comprehensive data layers that include various types of spatial data related to aquaculture and could be expanded with additional parameters such as water quality, temperature, salinity, bathymetry, land use, and aquaculture infrastructure (Longdill et al., 2008). The interactive mapping tools developed are indispensable as they enable users to effectively access, analyze, and visualize spatial data. These tools provide features such as layer toggling, measurement tools, and thematic mapping capabilities. The integration of real-time data is highly important, involving the inclusion of current information on environmental conditions, weather forecasts, and aquaculture output indicators (FAO, 2013).

Thus, users are given the ability to customise their experience by selecting specific data layers, creating personalised maps, and saving configurations. The collaboration capabilities enable users to share information by exchanging data, engaging in collaborative mapping, and participating in discussion forums (Longdill et al., 2008). Ultimately, a feedback mechanism allows for continuous improvement by incorporating user input and meeting their requirements. This comprehensive methodology guarantees that the web-based GIS system optimises its usefulness and ease of use for all users engaged in DOF, especially top management and decision-making processes. GIS is a technique for efficiently collecting, storing, and organising geographical data, regardless of whether it has precise earth locations or not. This system also encompasses additional pertinent data linked to geographical information. Through the use of security protocols, GIS enables the prompt retrieval of required results by analysing this data. The implementation of strong security measures ensures the protection of sensitive data and user privacy by employing authentication, encryption, and access controls.

The programme can improve its functionality by utilising and displaying information on the latest advancements in smartphones, tablets, and smartwatches. Wireless technologies refer to communication networks that connect these devices in a flexible manner. Mobile devices enable the sharing of voice, data, and mobile applications. Furthermore, mobile compatibility ensures that the web-based GIS system can be accessed from any location, enhancing its usefulness and convenience. The system's usability is further supported by the availability of comprehensive training materials and user support resources (Longdill et al., 2008). Tablets and smartphones are both excellent devices for utilising GIS technology. However, the effectiveness of a mobile GIS primarily depends on three key factors: geographical functionalities, user interface design, and system performance (Al-Momani et al., 2019).

Although online GISs are extensively used for integrating geospatial resources and providing detailed information analysis to end users through geovisualization or animation via interactive web-based spatial portals, the availability of mobile portals is limited. Specifically, when using GIS applications and collecting data, both types of devices perform effectively. Collector for ArcGIS

enables users to use their smartphones for data collection and information updates while in the field. Smartphone and tablet applications use integrated GPS to enable the tracking and navigation of users (Ghose and Todri-Adamopoulos, 2016).

The BioDOF-Map system is capable of determining disease status distribution by utilising real-time updates from state fisheries officials. These updates are displayed on the dashboard through highlighted notifications. In the future, it is possible to enhance the system by incorporating an analysis module. The BioDOF-Map system enables clients to access and use a GIS dataset containing information about the farm, as well as other services and related data, without requiring extensive knowledge in GIS or the use of GIS software.

The timing of research is crucial, especially in GIS projects where the implementation process can be lengthy and costly. It is estimated that nearly 80% of the overall project expenses are related to timing (Smith, J., et al., 2019). The temporal dimension is essential in the design and implementation of several phases of the project, such as data gathering, analysis, modelling, and visualisation. Any delays in any of these phases might lead to increased costs and hinder the desired outcomes of the project. Hence, detailed planning and strict adherence to deadlines are essential to maximise resource efficiency and ensure prompt project completion.

Conclusion

The BioDOF-Map System represents a substantial advancement in improving biosecurity protocols for fisheries. This advanced solution combines GIS technology with a wide range of fisheries data, serving as an effective tool in protecting aquatic habitats. The BioDOF-Map System provides a comprehensive perspective of fisheries landscapes by integrating geographic data on water quality, habitat conditions, disease outbreaks, and aquaculture facilities. This broad viewpoint enables authorities to quickly detect and address potential biosecurity concerns accurately.

Therefore, the BioDOF-Map System excels in its capacity to support immediate monitoring and swift identification of invasive species, diseases, and environmental hazards. The system allows rapid identification of emerging hazards, enabling prompt response and effective containment measures. This helps to limit the spread of threats and reduce their impact on aquatic ecosystems. Furthermore, the system's collaborative features facilitate the exchange of information among individuals involved, encouraging synchronised endeavours in the management and enforcement of biosecurity.

GIS Web Mapping offers numerous benefits that significantly enhance the efficiency of the BioDOF-Map System. Firstly, it decreases the amount of time it takes to complete tasks by making data analysis and decision-making processes more streamlined. Furthermore, it promotes cooperation among diverse parties involved, enabling the sharing of information and joint resolution of challenges. In addition, GIS Web Mapping reduces the obstacles to entry by providing a cost-effective method for acquiring and using spatial data. The technology empowers users by integrating structured data from many sources, enabling real-time spatial analysis, and allowing remote access to web maps on any device. This supports informed decision-making and provides users with practical insights.

The BioDOF-Map System is vital in the Department of Fisheries Malaysia as it supports strategic decision-making and improves service delivery to stakeholders. The system enhances fisheries management and monitoring by offering critical insights, leading to optimized resource utilization and reduced human resource demands. Moreover, it serves as a key repository of essential information regarding fisheries, aquaculture, and fish diseases in Malaysia. This enables authorities to make well-informed policy decisions and implement precise interventions.

To summarise, the BioDOF-Map System is an innovative technology that significantly improves the protection of fisheries through enhanced biosecurity measures. The system allows authorities and stakeholders to effectively safeguard aquatic resources by integrating GIS technology, extensive data sets, and cooperative functions. The BioDOF-Map System plays a vital role in the sustainable management of fisheries and the conservation of aquatic ecosystems by providing real-time monitoring, rapid response, and informed decision-making.

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Microplastic-Loaded Parasites: A New Concern for Aquaculture and Fish Health?

MOHAMAD NAJIHAH[1*], KUA BENG CHU[2], ROHAIZA ASMINII YAHYA[3] AND KU KASSIM KU YAACOB[1]

[1]*Impact Assessment Research Center, Fisheries Research Institute (FRI), Department of Fisheries Malaysia, Jalan Batu Maung, 119600 Batu Maung, Penang, Malaysia*
[2]*Fisheries Research Institute (FRI), Department of Fisheries Malaysia, Jalan Batu Maung, 119600 Batu Maung, Penang, Malaysia*
[3]*National Fish Health Research Centre (NaFisH), Fisheries Research Institute (FRI), Department of Fisheries Malaysia, Jalan Batu Maung, 119600 Batu Maung, Penang, Malaysia*

**Corresponding author: najihah@dof.gov.my*

***Abstract*:** Microplastic (MP) accumulation in aquaculture systems raises concerns, particularly regarding its understudied association with fish ectoparasites. We examined microplastic loads in three ectoparasite species (*Zeylanicobdella arugamensis*, *Caligus* spp., and *Benedenia* spp.) found on groupers and pomfrets from commercial aquaculture operations in Pulau Pinang, Malaysia. Results revealed microplastic presence across all parasites, with *Caligus* spp. showing the highest concentration (1.4 particles/parasite), Z. *arugamensis* at intermediate levels (1.2 particles/parasite), and *Benedenia* spp. the lowest (0.6 particles/parasite). Parasite size strongly correlated with microplastic accumulation ($r = 0.78$, $p < 0.005$). The particles were predominantly fragmented (47%) or filamentous (44%), appearing mostly in blue, transparent, or black colors, with particles ≤300 µm being the most common. Polymer identification by FTIR spectroscopy confirmed the presence of polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), indicating both primary and secondary microplastic sources. The study reveals a complex relationship between parasites and microplastics in aquaculture environments. While parasite removal during routine management may inadvertently decrease microplastic loads, detached parasites could redistribute microplastics back into the environment. Furthermore, microplastic biofouling might reduce antiparasitic treatments, complicating fish health management. Parasites serve as mobile carriers for microplastic transport between fish hosts and across different aquaculture sites, potentially increasing contamination spread. These findings emphasize the importance of developing comprehensive aquaculture strategies that consider parasite-microplastic interactions and their implications for sustainable fish farming practices.

Keywords: Microplastics, Ectoparasites, Aquaculture, Fish Health, Marine Pollution, Microplastic Mitigation

Abstrak: Pengumpulan mikroplastik (MP) dalam sistem akuakultur menimbulkan kebimbangan, terutamanya mengenai perkaitannya yang kurang dikaji dengan ektoparasit ikan. Kami memeriksa beban mikroplastik dalam tiga spesies ektoparasit (*Zeylanicobdella arugamensis*, *Caligus* sp., dan *Benedenia* sp.) yang ditemui pada kerapu dan ikan bawal daripada operasi akuakultur komersial di Pulau Pinang, Malaysia. Keputusan mendedahkan kehadiran mikroplastik merentas semua parasit, dengan *Caligus* spp. menunjukkan kepekatan tertinggi (1.4 zarah/parasit), *Z. arugamensis* pada tahap

pertengahan (1.2 zarah/parasit), dan Benedenia spp. paling rendah (0.6 zarah/parasit). Saiz parasit sangat berkorelasi dengan pengumpulan mikroplastik ($r = 0.78$, $p < 0.005$). Zarah-zarah tersebut kebanyakannya berpecah-belah (47%) atau berfilamen (44%), kelihatan kebanyakannya dalam warna biru, lutsinar atau hitam, dengan zarah ≤300 μm adalah yang paling biasa. Pengenalpastian polimer melalui spektroskopi FTIR mengesahkan kehadiran polietilena (PE), polipropilena (PP), dan polietilena tereftalat (PET), menunjukkan kedua-dua sumber mikroplastik primer dan sekunder. Kajian itu mendedahkan hubungan kompleks antara parasit dan mikroplastik dalam persekitaran akuakultur. Walaupun penyingkiran parasit semasa pengurusan rutin mungkin secara tidak sengaja mengurangkan beban mikroplastik, parasit yang terpisah boleh mengagihkan semula mikroplastik ke alam sekitar. Tambahan pula, biofouling mikroplastik mungkin mengurangkan rawatan antiparasit, merumitkan pengurusan kesihatan ikan. Parasit berfungsi sebagai pembawa mudah alih untuk pengangkutan mikroplastik antara perumah ikan dan merentasi tapak akuakultur yang berbeza, yang berpotensi meningkatkan penyebaran pencemaran. Penemuan ini menekankan kepentingan membangunkan strategi akuakultur komprehensif yang mempertimbangkan interaksi parasit-mikroplastik dan implikasinya terhadap amalan penternakan ikan yang mampan.

Kata kunci: Mikroplastik, Ektoparasit, Akuakultur, Kesihatan Ikan, Pencemaran Marin

Introduction

Plastic pollution has emerged as among the most pressing environmental challenges of the 21st century, with millions of tons of plastic waste annually contaminating the world's oceans (Jambeck et al., 2015). Of particular concern are microplastics, which are defined as small plastic particles less than 5 mm (UNEP, 2019). This phenomenon is likely due to the degradation of larger plastic debris (secondary microplastics) or direct manufacturing at microscopic sizes (primary microplastics) (Song et al., 2024). The widespread presence of these particles has raised significant concerns about their impact on marine ecosystems, as they can be ingested by a wide range of organisms, such as zooplankton (Kvale et al., 2021), and potentially transferred through food webs (Pironti et al., 2021).

Although microplastic ingestion by fish and marine invertebrates has been extensively documented in Malaysia and globally (Cera et al., 2022; Kılıc, 2022), their interaction with fish parasites remains largely unexplored. This knowledge gap is particularly significant because parasites often serve as crucial bioindicators of ecosystem health and environmental contamination. Ectoparasites, which live on the external surfaces of fish, are ubiquitous in marine ecosystems (Wall, 2007) and occupy unique positions at the host-environment interface (Narvaez et al., 2024), making them potentially important agents in microplastic dynamics.

The ability of these parasites to accumulate microplastics could represent a previously unrecognized pathway for plastic particle transfer in marine food webs. This is particularly relevant, considering parasites can modify host behavior and physiology (Hughes and Libersat, 2019), potentially influencing the bioaccumulation and biomagnification of environmental contaminants. Furthermore, the presence of microplastics in parasites could alter their impact on host health by adding another layer of complexity to parasite-host relationships in polluted environments. Notably, the mobility of

ectoparasites across different fish species and their capacity to detach and reattach to new hosts (Chew et al., 2024) facilitates the transport of accumulated microplastics to various locations within and between aquaculture facilities, potentially spreading contamination across larger geographic areas.

In aquaculture settings where fish densities are high and parasite infections are common (Paladini et al., 2017), understanding the interaction between microplastics and ectoparasites becomes critically important for several reasons. First, the confined nature of aquaculture systems creates ideal conditions for parasite proliferation and microplastic accumulation, as contaminants and parasites remain within the farming environment with limited dispersion (Bouwmeester et al., 2021). Second, the high stocking densities typical of intensive aquaculture promote rapid parasite transmission between hosts, potentially increasing microplastic redistribution throughout the farmed population (Schmittmann et al., 2024). Third, routine aquaculture management practices, including feeding, water exchange, and equipment maintenance, may inadvertently introduce additional microplastic contamination while disrupting parasite-host dynamics. Malaysia, one of the major aquaculture producers in Southeast Asia, offers an ideal setting for investigating this interaction. This study focused on cultivated fish in Pulau Pinang, Malaysia, a region with significant aquaculture activity, to examine this interaction and its implications for aquaculture management and food safety.

Materials and Methods

Study Site and Collection of Fish Samples

Cultured groupers (*Epinephelus* spp.) and silver pomfret (*Pampus argenteus*) were sourced from a commercial fish farm in Pulau Pinang, Malaysia. Fifteen specimens of each fish species were examined for ectoparasites under standard laboratory conditions following established protocols (Scholz, 1999). Fish were randomly selected from three distinct cages (5 fish per cage per species) to ensure representative sampling across the farming operation. Sample size was determined through power analysis using G*Power 3.1 ($\alpha = 0.05$, power = 0.80), confirming sufficient statistical power to detect meaningful differences in microplastic accumulation among parasite groups.

Parasite Isolation and Processing

Parasites were systematically collected from both fish species using a standardized protocol. From each fish, all visible ectoparasites were carefully removed and identified to the species level using morphological characteristics and taxonomic keys (Kabata, 1979; Whittington et al., 2000). Three groups of ectoparasites (N=30 total) were isolated: crustacean parasite *Caligus* spp. (n=10, collected from both groupers and pomfrets); skin fluke *Benedenia* spp. (n=10, mainly from groupers), Moreover, marine *leech* Z. arugamensis (n=10, found on both species but more abundant on pomfrets). To ensure balanced representation, parasites were collected, including *Caligus* spp. (5 from groupers, 5 from pomfrets), *Benedenia* spp. (7 from groupers, 3 from pomfrets), and Z. *arugamensis* (3 from groupers, 7 from pomfrets), reflecting their natural distribution on the host species. Each parasite specimen was carefully removed using sterile forceps, and its length (mm) and weight (mg) were immediately measured in triplicate to ensure precision. The parasites were individually stored in sterile containers to prevent cross-contamination.

Microplastic Extraction

The parasites were subjected to a digestion protocol using 5% potassium hydroxide (KOH) solution at a ratio of 1:3 (parasite: solution by weight). While standard digestion protocol typically employs 10% KOH solution (Lusher and Hernandez-Milian, 2018; Karbalaei et al., 2019), we reduced the concentration to 5% due to the small size and delicate nature of the parasite specimens to prevent over-digestion while maintaining effective organic matter dissolution. The samples were then incubated at room temperature for 24 h to dissolve the organic matter while preserving the synthetic particles. The resulting solution was processed through vacuum filtration using a 1.0μm 47 mm GF/B filter to collect microplastic particles.

Microplastic Characterization

The filtered samples were examined using a stereomicroscope to confirm the presence of microplastics. Particles were categorized based on their physical morphology (fiber, fragment, and film), color, and size (Najihah et al., 2025). Polymer identification was conducted using Fourier Transform Infrared (FTIR) spectroscopy (Thermo Scientific Nicolet iS50) on a representative subsample (30% of all particles) to verify plastic composition and distinguish synthetic particles from natural organic matter. Size measurements were performed using ImageJ software calibrated with a stage micrometer.

Statistical Analysis

Data analyses were conducted using Microsoft Excel, version 16.63.1. Correlations between parasite body measurements and the occurrence of microplastics were evaluated. The relationship between parasite size and microplastic abundance was analyzed, with statistical significance set at $P < 0.005$. Sample size adequacy was assessed through power analysis using G*Power 3.1 ($\alpha = 0.05$, power = 0.80), confirming that 30 parasites ($n = 10$ per group) provided sufficient statistical power to detect meaningful differences in microplastic accumulation. The standard error of measurement (SEM) was computed for all quantitative data, and outliers were identified using Grubb's test ($\alpha = 0.05$).

Quality Assurance

Quality assurance measures were rigorously applied to ensure the reliability of microplastic quantification. Recovery rates were evaluated using spiked samples, which yielded high recoveries ranging from 85% to 95%, demonstrating the efficiency of the method for extracting and detecting microplastics. Additionally, positive controls utilizing commercial microplastic standards were included to validate the detection process and minimize analytical bias. These quality control measures collectively increase the reliability of the findings, ensuring reproducibility and accuracy of microplastic characterization across parasite groups (Gao et al., 2023).

Results and Discussion

Microplastics Abundance in Ectoparasites

Microplastic measurements were reported with 95% confidence intervals. The analysis results of 30 parasites isolated from grouper and pomfret (Figure 1) revealed a consistent presence of microplastics across all parasite groups *(Z. arugamensis*, *Caligus* spp. and *Benedenia* spp.). The average microplastic burden varied among species, with *Z. arugamensis* at 1.2 particles/parasite, *Benedenia* spp. at 0.6 particles/parasite, and *Caligus* spp. at 1.4 particles/parasite. This variation reflects differences in parasite feeding mechanisms and anatomical structures. *Caligus* spp., being active swimmers with rasping mouthparts that continuously scrape host tissues, encounter higher microplastic concentrations in the water column and host mucus. *Z. arugamensis*, as blood-feeding leeches with larger body cavities, can accumulate particles through direct ingestion and surface adhesion. *Benedenia* spp., primarily mucus feeders with smaller oral cavities, show lower accumulation rates consistent with their feeding behavior (Nagasawa and Cruz-Lacierda, 2004; Ribeiro et al., 2025 ANOVA revealed significant differences in microplastic abundance among parasite species ($F_{2,27}$ = 8.45, $p < 0.001$). Post-hoc analysis showed that *Caligus* spp. had significantly higher microplastic loads than *Benedenia* spp. ($p < 0.001$), while *Z. arugamensis* showed intermediate levels significantly different from other species ($p < 0.05$).

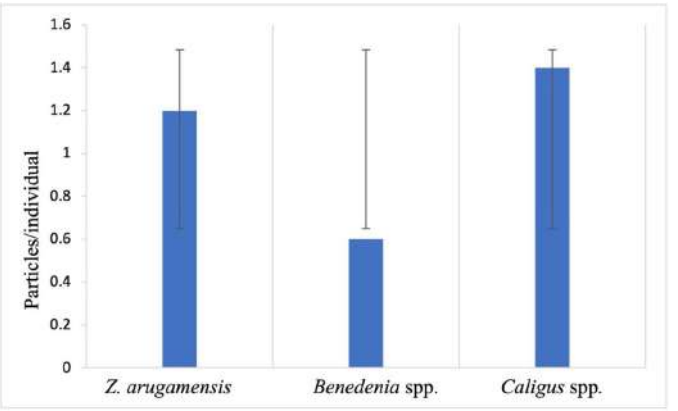

Figure 1. Microplastics abundance in ectoparasites according to species

A strong positive correlation was observed between parasite size and microplastic accumulation (Pearson's r = 0.78, $p < 0.005$). Triplicate measurements ensured reproducibility, and inter-observer reliability was high (Cohen's kappa = 0.85). Size-dependent relationships were analyzed using linear regression, yielding coefficients of $R^2 = 0.82$ (*Z. arugamensis*), $R^2 = 0.75$ (*Benedenia* spp), and $R^2 = 0.79$ (*Caligus* spp).

Polymer Identification and Morphological Analysis

FTIR spectroscopy confirmed that 89% of analyzed particles were synthetic polymers, with the remaining 11% classified as natural organic matter or unidentifiable materials. The dominant

polymer types were polyethylene (PE, 42%), polypropylene (PP, 28%), and polyethylene terephthalate (PET, 19%), consistent with common aquaculture equipment materials such as nets, ropes, and feeding pipes. These specific polymers indicate both primary microplastics (from equipment degradation) and secondary microplastics (from larger debris breakdown) within the aquaculture environment.

Morphological analysis in Figures 2 and 3 demonstrated the dominance of fragmented (47%) and filamentous (44%) microplastics. The prevalence of fragments suggests significant secondary microplastic formation through the breakdown of larger plastic debris within the aquaculture system, likely from nets, buoys, and other equipment subjected to constant mechanical stress and UV degradation (Wu et al., 2023). The filamentous particles predominantly originated from synthetic textiles and rope materials, as confirmed by FTIR analysis, which shows characteristic PET and nylon signatures. The filamentous particles predominantly originated from synthetic textiles and rope materials, as confirmed by FTIR analysis, which shows characteristic PET and nylon signatures.

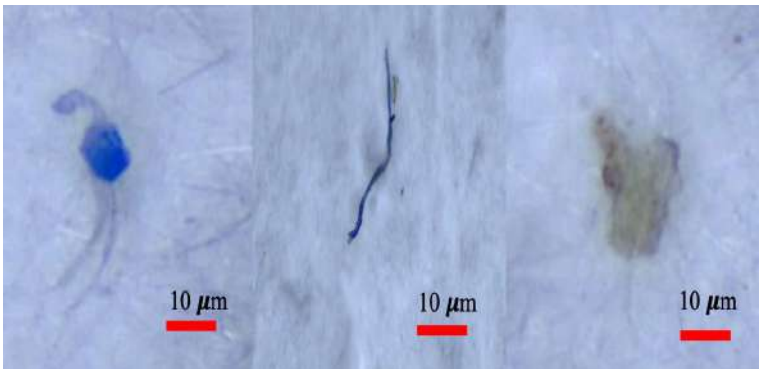

Figure 2. Common shapes found in ectoparasites, with the left most as a blue fragment, middle as filamented blue, and the right most as a transparent film.

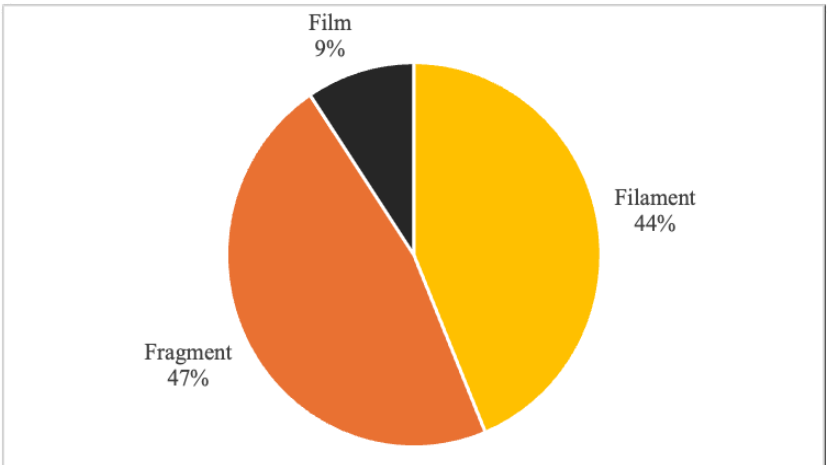

Figure 3. Microplastic analysis according to physical shapes

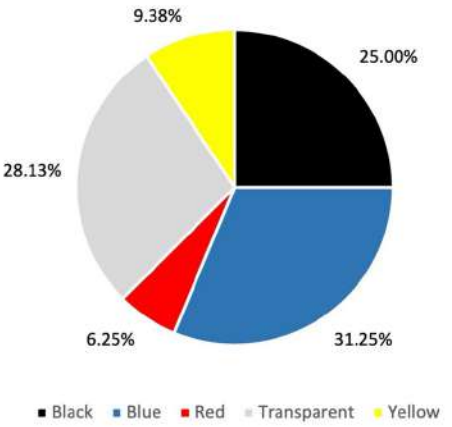

Figure 4. Microplastic classification into different colors based on microscopic examination

The dominant colors were blue (31.25%), transparent (28.15%), and black (25%). This color distribution reflects the prevalence of these colors in aquaculture equipment and local plastic waste streams. Blue corresponds to commonly used aquaculture netting and rope materials, while transparent particles likely originate from food packaging and feeding equipment. The abundance of black particles likely results from UV-degraded plastics that have lost their original coloration (Zhao et al., 2022).

Figure 5 illustrates the total microplastic particle count (mean ± standard error) detected in *Z. arugamensis*, *Benedenia* spp and *Caligus* spp, categorized into five particle size classes (≤20 µm, ≤50 µm, ≤100 µm, ≤150 µm, and ≤300 µm). Microplastic accumulation varied significantly among parasite groups and size classes ($p < 0.005$). This variation indicates that size differentiation was a key factor influencing microplastic retention.

Marine leeches exhibited the highest microplastic burden for ≤300 µm particles, followed by ≤20 µm and ≤50 µm particles, with lower counts observed for the other size categories. Parasite *Benedenia* spp demonstrated the lowest overall accumulation, with ≤300 µm particles being the most prevalent. In contrast, *Caligus* spp demonstrated the highest accumulation of ≤50 µm particles, followed by ≤300 µm and ≤100 µm, suggesting a size-dependent accumulation trend.

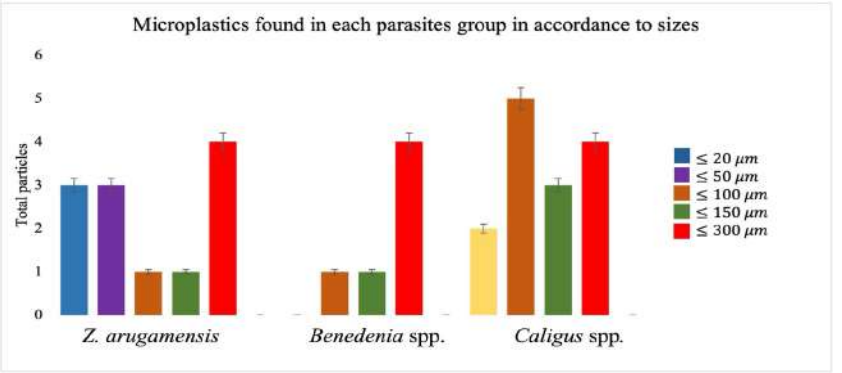

Figure 5. Microplastics found in each parasite group in accordance to sizes

The significant relationship between particle size and total accumulation highlights the influence of parasite morphology, feeding mechanisms, and attachment duration on microplastic retention. Error bars represent the standard error of the mean, showing the variability across parasite species and size classes.

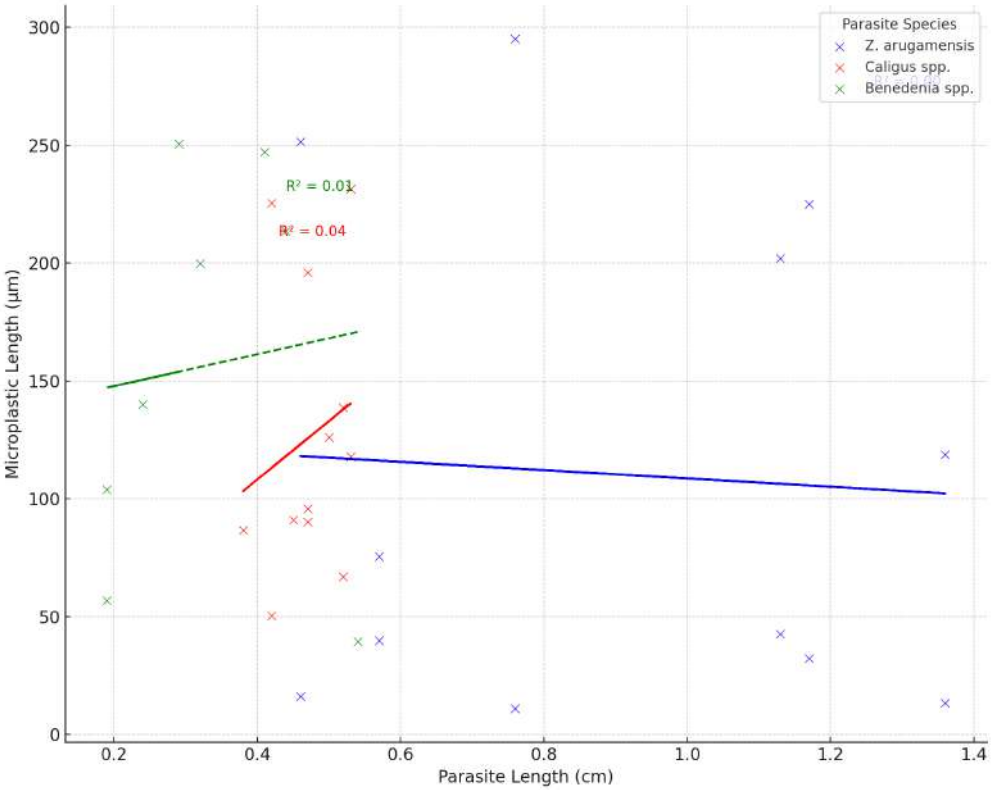

Figure 6. Correlation of parasite size and microplastic accumulation across species

A strong positive correlation was observed between parasite size and microplastic accumulation across various species, as presented in Figure 6 (Pearson's $r = 0.78$, $p < 0.005$). This correlation suggests that larger parasites tend to accumulate more microplastic. The analysis was based on triplicate measurements to ensure reproducibility, and high inter-observer reliability was confirmed (Cohen's kappa = 0.85). Linear regression analysis of the data yielded the following R^2 coefficients for each species: $R^2 = 0.82$ for Z. arugamensis, $R^2 = 0.75$ for Benedenia spp., and $R^2 = 0.79$ for Caligus spp. These findings indicate a size-dependent accumulation trend that is species-specific.

The anatomical and behavioral differences among parasite species explain the observed size-dependent accumulation patterns. *Caligus* spp., with their complex branched feeding appendages and active swimming behavior, efficiently capture smaller particles (≤50 µm) from the water column through filtration-like feeding mechanisms. *Z. arugamensis*, possessing larger oral cavities and expandable body segments, preferentially accumulate larger particles (≤300 µm) through direct ingestion during blood feeding. *Benedenia* spp., with their smaller, specialized feeding structures

adapted for mucus consumption, demonstrate limited capacity for particle retention across all size classes, explaining their consistently lower accumulation rates (Whittington et al., 2000).

Comparative Analysis

A global study has reported microplastic loads of 1.77 ± 1.79 particles/parasite (Aytan et al., 2023) in Turkish waters, whereas our findings (0.6 – 1.4 particles/parasite) indicate slightly lower contamination levels. This difference may reflect regional variation in microplastic pollution, with Malaysian aquaculture sites potentially experiencing lower ambient microplastic concentrations compared to heavily industrialized Mediterranean regions. Additionally, the parasite species examined in our study may have different accumulation capacities compared to Ligula intestinalis studied by Aytan et al. (2023). Few studies have examined microplastics in ectoparasites, but correlations between parasite infection and microplastic presence have been documented (Pennino et al., 2020; Minaz et al., 2024).

The microplastic size range (≤ 20 μm to ≤ 300 μm) is particularly concerning because smaller particles have a greater potential for translocation across biological membranes, which may enable uptake by host fish tissues. These size ranges fall within the critical window for cellular uptake, where particles can penetrate cell membranes and potentially reach systemic circulation (Campanale et al., 2020). This finding corresponds with Roslan et al. (2024), who reported microplastic sizes in humans ranging from 0.01 nm to 4812.9 μm.

Health Implication and Contamination Pathways

The presence of microplastics in ectoparasites raises significant concerns regarding fish health through several interconnected pathways. FTIR analysis revealed that 34% of particles exhibited characteristic peaks indicating the presence of adsorbed contaminants, including phthalates and polycyclic aromatic hydrocarbons (PAHs). These findings are important because microplastics can serve as carriers for persistent organic pollutants (POPs) and heavy metals, concentrating these toxins at parasite attachment sites and potentially delivering them directly to host tissues through parasite feeding.

The polymer types identified (PE, PP, PET) are known to readily absorb hydrophobic contaminants from seawater (Rochman et al., 2013). When parasites carrying contaminated microplastics attach to fish, they create localized contamination hotspots that may exceed the toxin concentrations found in surrounding water or sediments. This concentrated exposure pathway could lead to increased bioaccumulation in fish tissues, particularly at parasite attachment sites where tissue damage raises contaminant uptake rates.

Accumulated microplastics in parasites may have complex effects on fish health through multiple pathways. Parasites carrying microplastics can act as focused contamination points, leading to localized tissue damage or inflammation at attachment sites (Koelmans et al., 2016). Moreover, microplastic-laden parasites may worsen the mechanical damage typically associated with ectoparasite

attachment. If these microplastics contain adsorbed environmental contaminants such as persistent organic pollutants (Rodrigues et al., 2019) or heavy metals (EL-Hak et al., 2022), they could increase localized toxin delivery to fish tissues, creating a synergistic effect between parasitic infection and chemical contamination. Masud and Cable (2023) observed higher pathogen burdens in microplastic-exposed fish, contributing to increased mortality rates.

Furthermore, microplastic-contaminated parasites may act as reservoirs for secondary infections. The irregular surfaces of microplastic particles can harbor pathogenic bacteria and viruses, potentially introducing additional disease agents during parasite attachment and feeding (Zettler et al., 2013). This establishes a triple threat: mechanical damage from parasites, chemical toxicity from adsorbed contaminants, and biological contamination from pathogen-bearing particles.

Implications for Aquaculture and Microplastic Mitigation

Our findings suggest an intriguing potential for microplastic mitigation in aquaculture systems through parasite control. The conceptual framework in Figure 7 illustrates the role of ectoparasites in microplastic accumulation, highlighting how microplastics adhere to or are ingested by parasites attached to the fish surface. Since all examined ectoparasites accumulate microplastics, routine parasite removal is already a common practice in aquaculture (Buchmann, 2022), and could serve as a microplastic reduction strategy. The removal of each parasite eliminates multiple microplastic particles from the host-parasite system, decreasing localized contamination in fish tissues.

However, Figure 7 also suggests that ectoparasites may function as microplastic reservoirs that eventually release microplastics back into the aquatic environment. This release could occur naturally as parasites detach or degrade, or as a result of common aquaculture parasite treatments. The release pathway shown in the diagram raises concerns regarding the fate of microplastics following parasite removal methods. If parasite detachment causes microplastic release, then treatment methods that kill parasites without physically removing them may unintentionally return the accumulated microplastics to the environment. Future research should assess whether chemical treatments, mechanical removal, or biological controls effectively contain microplastics or whether they inadvertently promote microplastic redistribution in aquaculture settings.

Additionally, Figure 7 illustrates how the microplastic concentration within parasites might influence their local and systemic effects on fish health. The presence of microplastics in parasite tissues indicates a potential biological stressor that could affect immune function, disease susceptibility, or overall health. Importantly, the diagram implies a systemic effect where the accumulation of microplastics in fish tissues could cause broader physiological consequences, including altered metabolism, compromised immune responses, and reduced growth rates commonly observed in microplastic-exposed aquatic organisms (Lu et al., 2018).

Another key implication is the possible interference of microplastics with antiparasitic treatment. Studies have shown that biofilm formation on microplastic surfaces can create protective barriers that reduce the effectiveness of antimicrobial treatments (Zettler et al., 2013). In the context

of parasite control, microplastic-associated biofilms may similarly shield parasites from chemical treatments, requiring higher drug concentrations or prolonged treatment durations. This could lead to increased treatment costs, potential drug resistance development, and greater environmental impact from aquaculture chemotherapeutants.

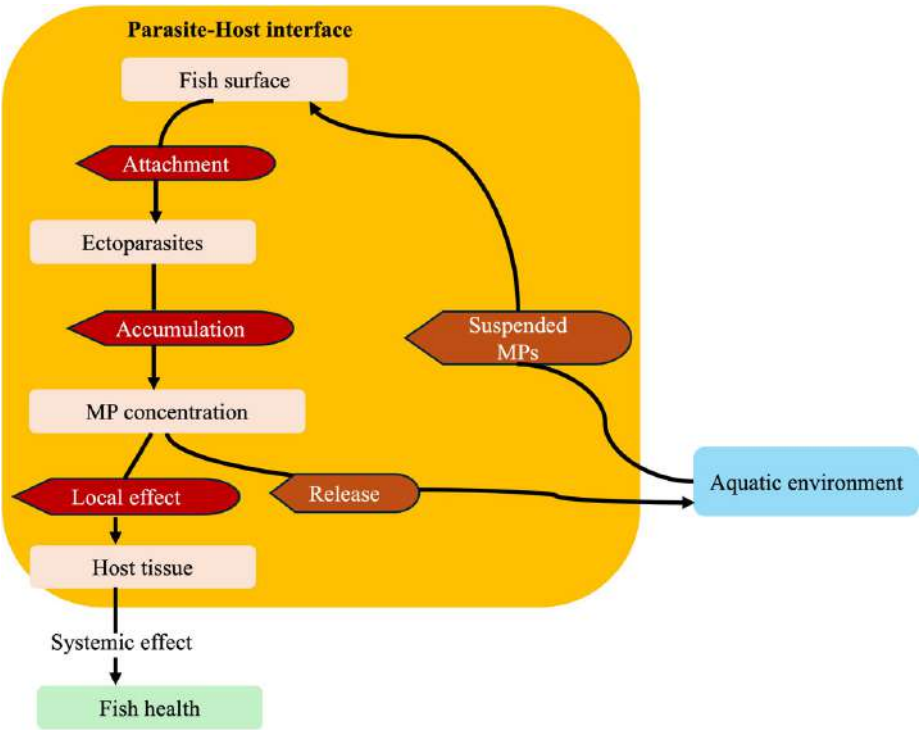

Figure 7. Pathway for accumulation and release of microplastics (MP) in fish and aquatic environments

The relationship between parasite size and microplastic accumulation in our study corresponds with the accumulation pathway, indicating that larger parasites may act as more effective microplastic carriers. This suggests that selective parasite removal, focusing on those with the highest microplastic loads, could improve microplastic mitigation efforts. However, this also highlights the importance of comprehensive monitoring; removal alone does not resolve the broader microplastic pollution in aquaculture environments.

Ultimately, this finding supports the idea that aquaculture systems are not isolated from microplastic contamination. The host-parasite interface constitutes a crucial and often overlooked pathway in microplastic cycling, emphasizing the need for continued research into how aquaculture health management strategies intersect with emerging microplastic pollution concerns. This study demonstrates that effective aquaculture management must consider the complex interactions between parasites, microplastics, and fish health to develop truly sustainable farming practices.

Conclusion

This study provides the first evidence of microplastic accumulation in fish ectoparasites in Malaysian aquaculture systems, with an average of 0.6 to 1.4 particles per parasite. FTIR spectroscopy confirmed the synthetic composition of accumulated particles, identifying polyethylene, polypropylene, and polyethylene terephthalate as dominant polymer types, indicating contamination from aquaculture equipment degradation and external sources. The predominance of fragmented and filamentous particles indicates secondary microplastic contamination, whereas the consistent presence across different parasite groups demonstrates a widespread phenomenon. The significant relationship between parasite size and microplastic occurrence suggests a size-dependent accumulation process that warrants further investigation.

Our findings highlight a previously undocumented pathway for microplastic transfer in aquatic ecosystems, especially in aquaculture settings. The discovery that parasites can concentrate contaminant-laden microplastics at specific sites on fish hosts raises important considerations for both fish health and food safety, since parasites may serve as additional vectors for microplastic and associated toxin accumulation in farmed fish tissues. Future research priorities should focus on: (1) live fish studies to determine whether parasite-associated microplastics increase contamination levels in fish tissues intended for human consumption; (2) investigation of the potential impact of microplastic-contaminated parasites on parasite-host interactions and fish immune responses; (3) assessment of whether microplastic accumulation affects parasite virulence or treatment effectiveness; and (4) development of parasite management strategies that optimize both fish health and microplastic reduction outcomes.

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Author Contributions: All authors have read the manuscript, agree that the work is ready for submission to the journal, and accept responsibility for the manuscript's contents. Najihah Mohamad contributed to conceptualization, data curation, formal analysis, investigation, methodology, and writing the original draft. Kua Beng Chu contributed to data supervision, investigation, writing, reviewing, and editing. Rohaiza Asminii Yahya and Ku Kassim Ku Yaacob contributed to data acquisition project administration and resources.

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Histological Markers in Hepatopancreas of Prawn (*Macrobrachium rosenbergii*): An Approach for Early Disease Detection in Aquaculture

IMELDA RANTTY[1*], SITI HAWA MOHAMAD ALI[1], KHO LI YUNG[1], SITI ROKHAIYA BIOLLAH[1], AHMAD FAUZI KHOSIM[1], GRACE HWANG MEI EN[1], PITTMAN KARIN [2,3] AND MERKIN GRIGORY[3]

[1]Fisheries Research Institute, Sarawak and Labuan Division, Jalan Perbadanan, Bintawa, 93744 Kuching, Malaysia
[2]Department of Biosciences, University of Bergen, 5006 Bergen, Norway
[3]QuantiDoc AS, Thormøhlensgate 53, 5006 Bergen, Norway

**Corresponding author: imelda@dof.gov.my*

Abstract: Timely and accurate disease detection in aquaculture is critical in providing prevention and intervention methods in aquaculture management. This study presents a novel quantitative method to identify early indicators of disease in the giant freshwater prawn (GFP) (*Macrobrachium rosenbergii*), an increasingly important species in aquaculture. Prawns were challenged with *Vibrio parahaemolyticus,* and hepatopancreatic changes were analysed over 72 hours. Histological sections were prepared using Periodic Acid Schiff-Alcian Blue staining, digitally scanned using MoticEasyScan Pro 6, and validated using mucosal mapping methodology (Veribarr), a validated quantitative histological assessment approach. Within 24 hours of pathogen exposure, significant alterations were reported in the hepatopancreas structure. Tubule size increased by approximately 25% ($p<0.05$, ANOVA), while tubule density decreased by about 30% ($p<0.05$, ANOVA), in challenged prawns compared to the control group. Vacuole density in tubule epithelia increased significantly, from 0.5 to 1.25 vacuoles per 1000 µm² ($p<0.05$, ANOVA). The lumen-to-tubule area ratio increased slightly, by about 10-20%, across all time points post-challenge. These structural changes were detectable within the first 48 hours of infection and serve as potential early biomarkers of disease. The optimal potential markers for quantifying the effect of the pathogen in GFP are the size and density of tubules, the lumen size, and the vacuole density in the hepatopancreas. These findings provide a foundation for developing rapid and objective diagnostic tools for prawn health assessment. The methodology presented has the potential to be adapted for other aquaculture species, contributing to more sustainable aquaculture practice.

Keywords: mucosal mapping, *Macrobrachium rosenbergii*, innate immunity, hepatopancreas, aquaculture

Abstrak: Pengesanan penyakit yang awal dan tepat dalam akuakultur adalah penting untuk menyediakan kaedah pencegahan dan intervensi dalam pengurusan akuakultur. Kajian ini memperkenalkan pendekatan kuantitatif yang baharu untuk mengenal pasti penunjuk awal penyakit pada udang galah *(Macrobrachium rosenbergii),* spesies yang semakin penting dalam industri akuakultur. Udang galah telah dicabar dengan *Vibrio parahaemolyticus* dan perubahan pada hepatopankreas dianalisis sepanjang tempoh 72 jam. Keratan histologi disediakan menggunakan pewarnaan

Periodic Acid Schiff-Alcian Blue, diimbas secara digital dengan MoticEasyScan Pro 6, dan dinilai menggunakan kaedah pemetaan mukosa (Veribarr), satu kaedah penilaian histologi kuantitatif yang telah disahkan. Dalam tempoh 24 jam selepas pendedahan kepada patogen, perubahan struktur yang ketara diperhatikan pada hepatopankreas. Secara khusus, saiz tubul meningkat kira-kira 25% ($p<0.05$, ANOVA), manakala ketumpatan tubul menurun kira-kira 30% ($p<0.05$, ANOVA) dalam kumpulan udang galah yang dicabar berbanding kumpulan kawalan. Ketumpatan vakuol dalam epitelium tubul meningkat dengan signifikan, daripada kira-kira 0.5 kepada 1.25 vakuol bagi setiap 1000 μm^2 ($p<0.05$, ANOVA). Nisbah keluasan lumen kepada tubul juga meningkat secara sederhana, sekitar 10–20%, merentas semua titik masa selepas cabaran. Perubahan struktur ini dapat dikesan dalam 48 jam pertama jangkitan dan berpotensi digunakan sebagai biomarker awal penyakit. Penanda terbaik yang berpotensi untuk mengkuantifikasi kesan patogen pada udang galah ialah saiz dan ketumpatan tubul, saiz lumen, serta ketumpatan vakuol dalam hepatopankreas. Penemuan ini menyediakan asas untuk pembangunan alat diagnostik yang pantas dan objektif bagi penilaian kesihatan udang galah. Metodologi yang dibentangkan di sini berpotensi untuk disesuaikan kepada spesies akuakultur lain, sekali gus menyumbang kepada amalan akuakultur yang lebih mampan.

Kata kunci: pemetaan mukosa, *Macrobrachium rosenbergii*, imuniti semula jadi, hepatopankreas, akuakultur

Introduction

Aquaculture is an important venture for ensuring food security. In 2022, worldwide aquaculture production exceeded 130.9 million tonnes, valued at USD 312.8 billion, constituting 59 percent of total global fisheries and aquaculture output (FAO,2022). That same year, aquaculture production of animal species exceeded that of capture fisheries, with inland aquaculture making a substantial contribution to total farmed aquatic animals (FAO, 2022). Farmed crustaceans are a significant contributor to the current expansion of the aquaculture sector and are anticipated to contribute more to the overall production of aquatic protein (Bondad-Reantaso et al., 2012a).

The *Macrobrachium* genus of freshwater prawns comprises more than 200 species in worldwide tropical and subtropical regions worldwide. The Indo-Pacific region is the native habitat of the giant river prawn, *Macrobrachium rosenbergii*. However, it has been introduced to every continent for aquaculture following the development of a mass postlarvae production technique (New and Nair, 2012; Hooper et al.,2022). *Macrobrachium rosenbergii* is susceptible to various disease-causing agents, such as bacterial, viral, fungal, and other eukaryotic pathogens. These infections can cause mortality or the production of a smaller or substandard animal with a lower market value, resulting in economic losses for the aquaculture industry (Pillai and Bonami, 2012).

Pittman et al. (2011) developed a new design-based stereological technique to measure the size, density, and position of mucous cells in fish skin. Mucosal mapping has enabled direct comparison of volumetric data reflecting intestinal dysregulation caused by diet (Torrecillas et al., 2015), infectious agents in gills, and skin responses to parasites (Pittman et al., 2013).

This is the pilot project to apply objective principles of mucosal mapping (Veribarr™) to standardized histological sections to determine the variability and trends in 3D composition of shrimp hepatopancreas in response to a bacterial challenge. This study aims to identify and evaluate quantitative histological markers in the hepatopancreas of *M. rosenbergii* that could serve as an early indicator of *Vibrio parahaemolyticus* infection. Standardized morphometric analysis techniques were employed to identify measurable parameters that demonstrate considerable changes within the first 24 hours of pathogen exposure, prior to the manifestation of clinical symptoms. The Veribarr technology was used to objectively measure tubule morphometry, luminal features, and vacuole dynamics, transforming subjective hepatopancreatic health evaluation into a quantified disease detection method. In addition, this study investigated whether dietary modifications might affect these histological reactions, providing information on potential dietary recommendations that pay potentially improve disease resistance in freshwater prawn aquaculture.

Materials and Methods

Experimental Design

Giant freshwater prawn juveniles (n=28) were reared in glass aquaria containing 10 litres of water and acclimatized for 30 days on a control diet before the start of the trial. The juveniles were then divided into four experimental groups: two groups exposed to the pathogen (one on a control diet and the other on an experimental diet) and two unexposed groups (one on a control diet and the other on an experimental diet). All groups were maintained under identical conditions at 30 ± 1 °C, with dissolved oxygen >5 mg/L, and a natural photoperiod. The rearing salinity was maintained at 0 ppt to reflect typical freshwater culture. Following the dietary acclimatization for 30 days, half of each diet group was challenged with the pathogen, *Vibrio parahaemolyticus,* on Day 0. *V. parahaemolyticus* strain was obtained from the National Fish Health Centre, cultured in nutrient broth (Merck, Darmstadt, Germany), enriched with 1.5% NaCl, and incubated at 31.5°C for 24 h. Bacterial density was then determined by measuring the optical density at 610 nm. The bacterial suspension was introduced directly into the aquarium water for a final concentration of ~1×10^6 CFU/mL. This exposure method was previously demonstrated to induce infection in crustaceans maintained in low salinity environments (Soonthornchai et al., 2010). The prawns were monitored for clinical signs over four time points (0h, 24h, 48h, and 72h), and histology samples were also extracted during those time points.

Histology

Two prawns per tank were randomly selected, and hepatopancreas samples were collected to investigate histopathological changes. Histological sampling followed the protocol described in Bell and Lightner (1988) with minor modifications. Juvenile prawn samples were injected with 10% of their body weight with Hartmann's fixative (Sigma-Aldrich/Merck KGaA, St. Louis, MO, USA). The samples were then fixed in Hartmann's solution for 48 h and later replaced with a 70% ethanol solution. Subsequently, samples were passed through an ethyl alcohol series (70%, 80%, 95%, 100%), followed by a xylene solution, and the tissues were then embedded in paraffin and sectioned at 3

μm. The sections were stained using Periodic Acid Schiff-Alcian Blue (Sigma-Aldrich/Merck KGaA, St. Louis, MO, USA) and digitally scanned using MoticEasyScan Pro 6 for analysis. Quantitative assessments were conducted on a single hepatopancreatic section per prawn to measure the relative area of functional components, such as the lumen, epithelial vacuoles, and unvacuolated epithelium, while counting the number of tubules. The volumetric density of vacuoles and the mean epithelial area per tubule were evaluated using validated Veribarr™ methodology. This objective approach aimed to provide insights into the structural responses of the hepatopancreas to pathogenic stress and dietary influences, establishing a foundation for future research in prawn health monitoring.

Quantitative Analysis

Quantitative analysis was conducted using the validated Veribarr™ methodology to assess various parameters of hepatopancreatic morphology as described in Pittman et al. (2011). This standardized approach enabled accurate measurement of tubule density, expressed as number per 1000 μm^2, providing insight into tissue architecture. Simultaneously, tubule size (μm^2) was measured to detect hypertrophic responses, while lumen area (μm^2) measurements captured changes in tubular function. Vacuole density (n/1000 μm^2) was also assessed as a potential early indicator of cellular stress or pathological response. Additionally, the lumen-to-tubule ratio was calculated to characterize the relative spatial relationship between these structures. Together, these complementary metrics formed a comprehensive profile of hepatopancreatic health status.

The Veribarr methodology was used to evaluate tubule morphometry, luminal features, and vacuole dynamics to convert the conventionally subjective evaluation of hepatopancreatic health into a quantitative method for disease identification.

Results and Discussions

Morphological Parameters of the Hepatopancreas

The hepatopancreas of crustaceans is structurally composed of branched tubules featuring a central lumen surrounded by epithelial cells containing characteristic intracellular vacuoles. Within the epithelium, distinct cell types, namely E-, R-, B-, and F-cells, perform specialized functions related to digestion, absorption, storage, and enzyme production. However, differentiation of these cell types remained beyond the scope of this study (Vogt, 2019). Pathological processes can induce either hypertrophy or atrophy of the hepatopancreatic tissue, making early detection of alterations in cellular dimensions and compartmental areas essential for accurately identifying environmental or pathogenic stressors (Lightner and Redman, 1994; Franceschini-Vicentini et al., 2009). These measurable histological responses can persist over extended periods, and repeated exposure to stressors may lead to increased sensitivity or tolerance development (Stentiford et al., 2012). Healthy hepatopancreas (Figure 1) can be affected by physical and microbial challenges and parasite attacks. In general, the hepatopancreas of the prawns shows a normal appearance with a star-shaped lumen in the tubules. However, a challenge by a pathogen can result in collapsed and necrotic tubules (Nadella, 2016), a larger lumen (Jiang, 2021), and/or an increase in the number of vacuoles (Jiang, 2021).

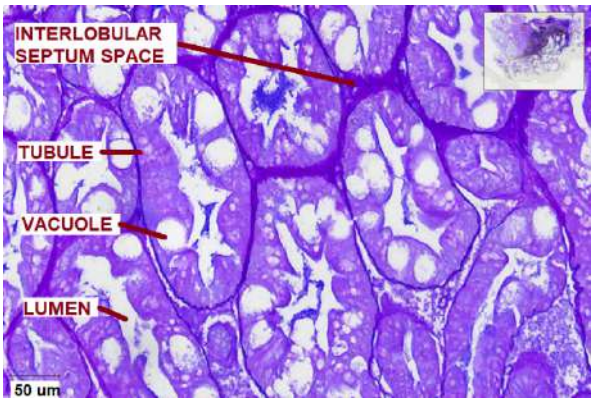

Figure 1. Histological structures of the hepatopancreas in *Macrobrachium rosenbergii*, stained with PAS-AB, which served as the reference for the initial quantification of hepatopancreatic components in healthy and pathogen-challenged individuals reared in the laboratory.

Luminal Characteristics

The lumen is the central cavity within each hepatopancreatic tubule where digestive fluids and particulate matter accumulate before absorption. A significant enlargement ($p<0.05$, Figure 2) of the hepatopancreatic tubule lumina was observed 24 hours after exposure to *V. parahaemolyticus*. The most substantial luminal measures (>1960 μm²) were primarily noted in challenged specimens (5 out of 6 cases). All prawns exhibiting the smallest lumina (<650 μm²) were from the non-challenged group (Figure 4). This suggests early epithelial remodeling consistent with pathogen-induced stress (Lightner and Redman, 1994). Dietary treatment had no significant impact on luminal size (Figure 3, $p>0.05$), indicating that early luminal expansion results from pathogen exposure rather than nutritional status.

Tubular Density and Morphometric Analysis

Hepatopancreatic tubules density (n/1000 μm²) was significantly higher in non-challenged specimens at 24 hours post-exposure ($p<0.05$, ANOVA, Figure 5). While the challenged group maintained consistently lower tubule counts during the experimental period, the non-challenged group progressively decreased tubule density over time. Significant tubule enlargement was observed in pathogen-exposed prawns 24 hours post-challenge ($p<0.05$, Figure 6). Unchallenged specimens exhibited higher tubule densities and smaller lumen, indicating an inverse relationship between tubule density and luminal size, particularly within the first 48 hours (Figure 4).

Lumen-to-Tubule Ratio

The ratio of luminal area to total tubule area exhibited a progressive increase in pathogen-exposed specimens at all timepoints. However, statistical significance was only observed at 72 hours

in the diet comparison ($p<0.05$ ANOVA, Figure 7). This implies that early morphological changes occur in individual structures, but proportional tissue adjustment architecture develops progressively over time. This ratio is an integrated indicator of both lumen dilation and epithelial thinning.

Dynamics of Vacuoles

The hepatopancreatic vacuoles exhibited the most consistent and rapid response to pathogen challenge. Infected specimens showed a significantly higher vacuole density than non-challenged controls within 24 hours of exposure ($p<0.05$, ANOVA, Figure 8). The volumetric density of vacuoles ranged from 0.25 to 1.75 per 1000 µm² of tubule tissue, with the lowest densities (<0.5/µm²) recorded only in non-challenged specimens (Figure 9). Although the vacuole size (up to 300 µm²) was similar across treatment groups (Figure 10, Figure 11), the significant difference in density serves as a reliable parameter for the early detection of pathological changes.

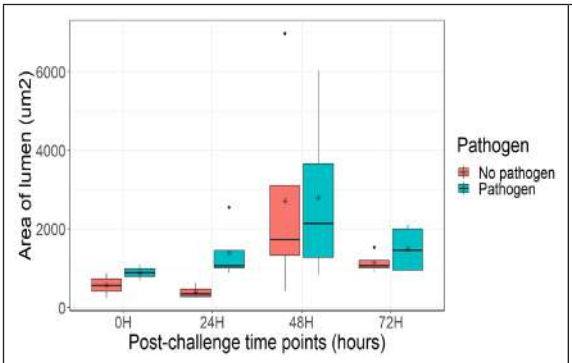

Figure 2. Hepatopancreas measurements of *M. rosenbergii* (n=28) categorized by pathogen exposure; the relative (calculated) mean area of lumen per tubule (µm2) is shown across N post-challenge time points. Different letters indicate a statistically significant difference ($p<0.05$).

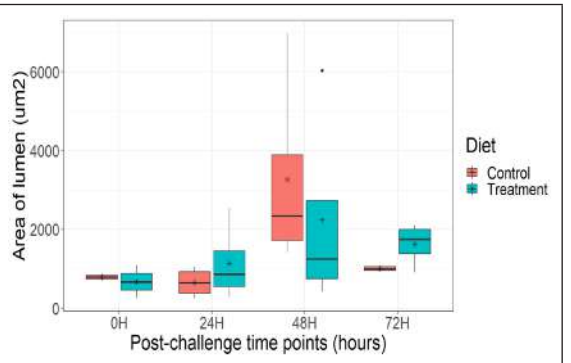

Figure 3. Hepatopancreas measurements of *M. rosenbergii* (n=28) categorized by dietary variable; The computed mean lumen area per tubule (µm²) is shown across N post-challenge time periods.

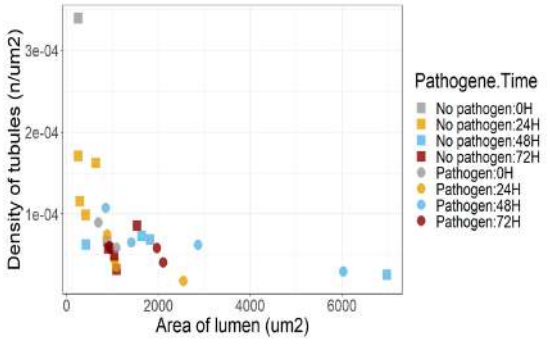

Figure 4. Hepatopancreas measurement of *M. rosenbergii* (n=28) categorized by exposure to pathogens. The calculated mean lumen area (µm²) is presented relative to the calculated density of tubules.

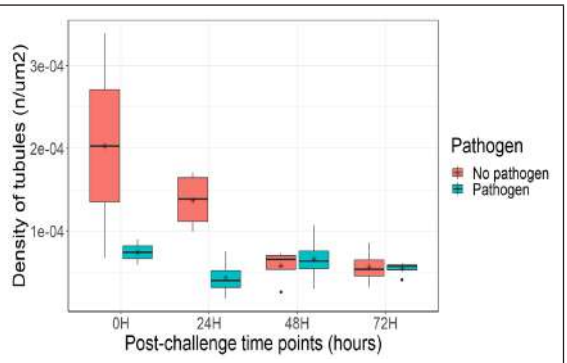

Figure 5. Hepatopancreas measurements of *M. rosenbergii* (n=28), categorized by pathogen exposure; The relative (calculated) density of tubules in the hepatopancreas (n/1000 µm²) at post-challenge time periods. Different letters indicate a statistically significant difference ($p<0.05$).

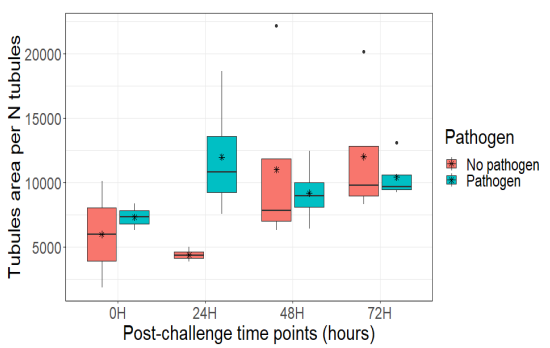

Figure 6. Hepatopancreas measurements of *M. rosenbergii* (n=28) categorized by exposure to pathogen; The Ratio of the Relative (calculated) Total area of tubules to the Number of tubules (the relative area of tubules) in the hepatopancreas at N-post-challenge time-points. Different letters indicate a statistically significant difference ($p<0.05$).

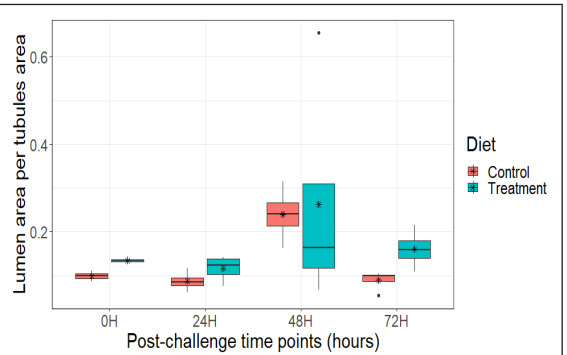

Figure 7. Hepatopancreas measurements of *M. rosenbergii* (n=28) by diet variable; The relative (calculated) area of lumen per relative area of tubules in hepatopancreas.

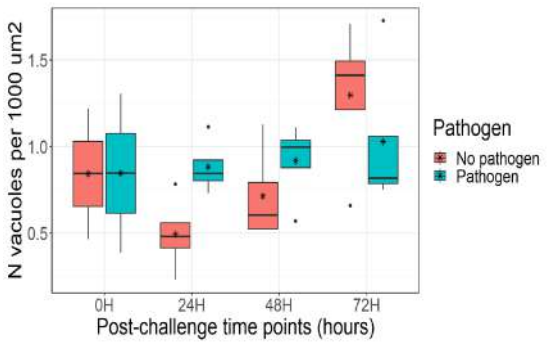

Figure 8. Hepatopancreas measurements of *M. rosenbergii* (n=28); The number of vacuoles per 1000 µm² of hepatopancreas. Letters are used to indicate statistical significance ($p<0.05$).

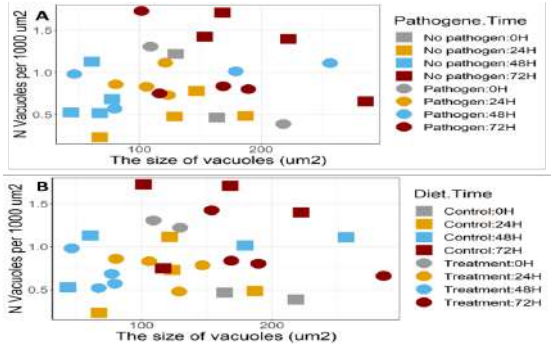

Figure 9. Hepatopancreas measurements of *M. rosenbergii* (n=28); The size (area) of vacuoles vs N vacuoles per 1000 µm².

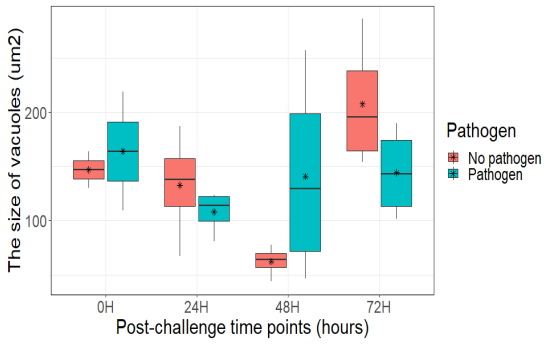

Figure 10. Hepatopancreas measurements of *M. rosenbergii* (n=28), categorized by pathogen exposure, showing the vacuole size within hepatopancreatic tubules (µm²).

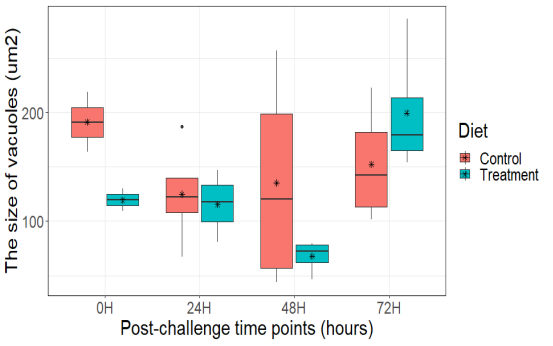

Figure 11. Hepatopancreas measurements of *M. rosenbergii* (n=28), grouped by diet, showing vacuole size in hepatopancreatic tubules (µm²).

Multivariate Analysis

Component Analysis was performed at 24 hours post-challenge. The first two principal components explained 84.6% of the total data variation (Figure 12). The factors that contributed most to this separation were vacuole density, lumen area, and tubule size. However, at the same time point, when specimens were grouped by diet, there was no evident clustering (Figure 13), indicating that the changes in histology are not due to nutritional state but pathogen exposure.

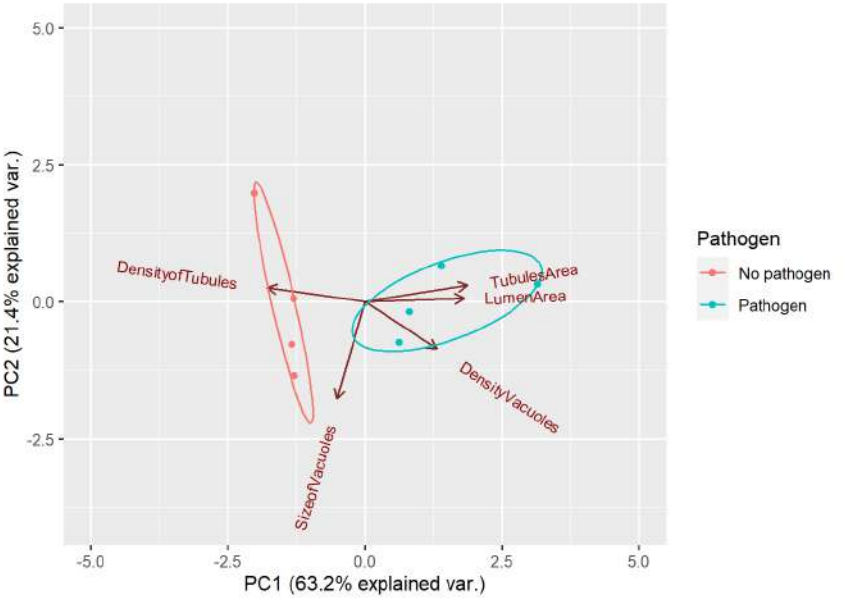

Figure 12. Principal component analysis showing the overview hepatopancreas of *M. rosenbergii* 24 h post challenge (n=8) grouped by exposure to pathogen

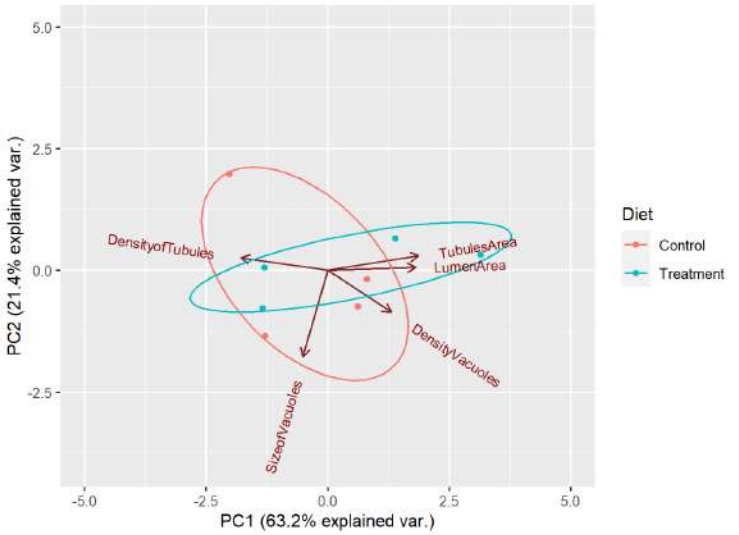

Figure 13. Principal component analysis showing the overview hepatopancreas of *M. rosenbergii* 24 h post challenge (n=8) grouped by control and treatment diet

The identification of significant histological alterations within 24 hours of pathogen exposure is a significant improvement in disease monitoring for crustacean farming. Conventional diagnostic techniques can only detect *V. parahaemolyticus* infection after the onset of clinical symptoms, which occur between 48 and 72 hours after exposure (Tran et al., 2013). At this stage, the infection has already spread to a certain extent, reducing treatment efficacy. The results are in line with those of Seibert and Pinto (2012), who observed early hepatopancreatic responses to bacterial challenges in

Farfantepenaeus brasiliensis. However, they did not provide quantifiable parameters for detecting these responses.

Vacuole density was considered an extremely sensitive and reliable first sign of infection among the measured parameters. The fast development of vacuoles indicates that cells are reorganizing through innate immune activation mechanisms. The presence of vacuoles indicates that cells absorb digested food and store it in vacuoles. The more active the process, the more vacuoles there will be. The more vacuoles there are, the bigger the cells will be. The observed cellular response matches the findings of Soonthornchai et al. (2010), who studied early cellular reactions to *Vibrio harveyi* in *Penaeus monodon* hepatopancreas tissue. The researchers used gene expression analysis instead of histological methods to reach their conclusions. The findings obtained in this study on hepatopancreatic vacuoles that reached sizes up to 300 μm² are consistent with the detailed ultrastructural research of crustacean digestive organs reported by Franceschini-Vicentini et al. (2009).

The observed vacuolization patterns indicate an adaptive response intended to sequester infections or their toxins, which resembles the approach described by Vogt (2019) for decapod hepatopancreas anatomy and function. The observed vacuolization patterns in R-cells suggest an adaptive response to pathogen exposure. R-cells, which contain multiple vacuoles and are responsible for nutrient storage and detoxification processes, show increased vacuole density when challenged with bacterial pathogens (Al-Mohanna and Nott, 1987; Vogt, 2020). This is in contrast to B-cells, which contain a single large vacuole and are primarily involved in enzyme secretion (Caceci et al., 1988). The coordinated changes in tubule structure, together with luminal characteristics and vacuole responses, demonstrate that the hepatopancreas functions as a single system to respond to pathogens and nutritional stimuli. The relationship between tubule density and luminal expansion shows an inverse pattern, which indicates tissue remodelling during the initial stages of infection. The hepatopancreatic reorganization observed by Jiang et al. (2021) in *M. rosenbergii* due to pesticide exposure showed similar patterns to our findings, although they involved different causative agents.

The lumen-to-tubule ratio demonstrates a steady increase across all timepoints in challenged specimens, which demonstrates progressive changes in tissue architecture. The study by Lightner and Redman (1994) described similar pathological changes in shrimp hepatopancreatic diseases, but our quantitative assessment provides better measurement of these changes than their qualitative grading system.

The quantitative histological indicators identified in this work are useful for early disease diagnosis in aquaculture. In their FAO technical study on improving aquatic animal health management, Bondad-Reantaso et al. (2012b) emphasized the need for standardized diagnostic procedures and that early detection is still the most critical barrier in disease control. Our findings close this gap by identifying particular factors that can be evaluated using routine histology samples.

The clear statistical distinction between healthy and challenged specimens at 24 hours post-exposure allows intervention during the crucial initial infection period, potentially before pathogen levels become critical. The study by Flegel (2012) demonstrated that early medical intervention yields

better treatment outcomes for shrimp viral diseases because it reduces mortality rates by 40-60% before tissues endure significant damage. The established markers in our research should yield a similarly positive outcome for detecting bacterial diseases.

The practical application of disease detection based on these promising results faces several challenges. The histological examination process involves tissue sampling and processing along with specialized equipment, which limits its use in field conditions. A small number of indicator animals undergoing targeted sampling can function as an early warning system for detecting disease outbreaks in production systems.

The statistical significance of key findings, despite this limitation, highlights their reliability, despite the relatively small sample size (n=28) of this study. Stentiford et al. (2012) noted in their study of aquaculture disease diagnostics that histopathology is among the most reliable tools for early diagnosis, despite its technical demands, especially when quantitative parameters are defined.

Conclusion

The research confirms that quantitative histological markers in the hepatopancreas of *M. rosenbergii* serve as dependable indicators to detect *V. parahaemolyticus* infection at an early stage. The significant changes in vacuole density, tubule morphology, and luminal characteristics become detectable within 24 hours of challenge, thus providing an early detection period before clinical symptoms appear. The analysis demonstrates that vacuole density reacts strongly to pathogen exposure by showing a continuous increase in size in infected samples but remaining small in uninfected individuals. The relationship between tubule density and luminal expansion shows a negative correlation. This indicates that tissue remodelling occurs as a coordinated process during the initial stages of infection. The first two components from the Principal Component Analysis demonstrated strong diagnostic value because they explained 84.6% of data variation and produced distinct separation between treatment groups at the 24-hour mark. These quantifiable indicators offer enhanced diagnostic capabilities compared to traditional subjective methods, which provide aquaculture experts with standardized health evaluation criteria. Future research should validate these markers across different pathogens and conditions to develop warning systems that reduce disease-related losses in crustacean aquaculture.

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