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OBJECTIVE AND SCOPE

As a multi-disciplinary journal, The Palawan Scientist aims to publish high-quality and original research in the fields of agriculture, fisheries and aquatic sciences, environment, education, engineering, mathematics, sociology, and related disciplines (including arts and humanities).

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ABOUT THE COVER

The *Holothuria scabra*, or kortido, as the locals call it, is one of the most sought-after species of sea cucumbers because of its attractive price. As a result of unregulated harvesting, its dwindling wild populations and shortage of breeding adults call for immediate conservation measures. The paper of Sumbe et al. explores the possibility of rearing sub-adults into mature individuals for breeding and restocking purposes (Photo by AG Sumbe and JBS Jontila).

COVER DESIGN: Jovan A. Gimarangan

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EDITORIAL

Hello Dear Reader, and welcome to the Volume 17, Issue 1 of The Palawan Scientist (TPS).

Are you still there? Or did you just turn the page on me? If you did, I understand: nobody reads forewords or editorials anymore. Worse yet, nobody reads journals anymore. In fact, an Indiana University study found that 50% of journals and scientific papers are never consumed by the public, except their authors, peers, and editors. Hubbard and Dunbar (2017) also cemented the fact that even experienced researchers rely on superficial reading when outside their field, as deep engagement depends more on prior knowledge and conceptual frameworks than on reading experience alone.

That's why I totally get it! To be honest, I can't recall the last time I read a journal cover to cover. Probably like me, most people who actually do consume journals do so per article - probably only reading the articles relevant to one's study. And that's a shame, because **each journal issue, when taken as a whole, holds a story.**

Oh…you're still here? That's amazing! Maybe some people *do* still read journals. For you, Dear Reader, I'll go on.

Each journal holds a story. If you go through the titles in this issue…you will *not* get that sense. At least, not right away. So let me tell you the story of this issue.

The Story of, Nature, Society, and Variety

You might feel like there's no overarching story in this issue with research topics ranging from managing marine protected areas, to fermenting soy-fern, to different sandfish feeds, to evacuation behaviors during volcano eruptions. But that's because The Palawan Scientist is a **multidisciplinary journal**. It's SUPPOSED to capture a wide array of scientific developments from a variety of disciplines. And these developments and multi-disciplines, though seemingly unrelated, are linked through **the story of nature and society**. Nature is also a slurry of matter and forces, existing in infinite forms and constantly changing. Society is a cacophony of infinite variations of people interacting and changing each other minute by minute, second by second. So it makes sense that the approach to science (and its publication) must be multidisciplinary in order to paint a fuller, more accurate picture of our complex world. In this issue, **the variety IS the story.**

The Story of Partnership and Sustainable Development

But if you *must* have an overarching theme to this issue, just think of two words: **partnership** and **sustainable development**. WPU is after all, "Your strong partner for sustainable development.

The study by WPU's very own brilliant team of Malolos et al. explored the **partnership** between Local Government Units and Funding Partners towards the **sustainable development** of Marine Protected Areas in Cagayancillo, Palawan.

The **partnership** of some of the brightest researchers in WPU in Sumbe et al. tested different feeds to grow sandfish in captivity, to maintain the **sustainable** harvest of this valuable but endangered sea cucumber species.

Susada's study connecting mathematical performance and psychological, physiological, and psychosocial factors point to the value of the **partnership** between educators and educational support practitioners towards a more robust educational **development.**

A study **partnered** by researchers (Opena et al.) from 3 Philippine Universities brings us research on soy-fern fermentation that could have implications in **sustainable** and healthy food production.

Lim and Anabo's study on evacuation behaviors of volcano-eruption affected households gives evidence to the importance of close **partnerships** between local communities and governments in the **development** of strategies for effective evacuation.

Doblas et al.'s study shows promise for a **sustainable** antioxidant and anti-inflammatory in a fern used as a traditional remedy in Bukidnon, Philippines.

A study on cooperatives, an important organizational **partner** for the grassroots level in **sustainable development**, were found by Mantig and Deriada to benefit from giving focus to intangible resources like structural, social, and spiritual capital.

The discourse analysis by Noval revealed the complexity of societal and **sustainability** issues we faced **together** as a Filipino community during the COVID-19 Pandemic.

And the 3 articles from Universities outside the Philippines show us that **sustainable development** should not be an isolated effort; it **takes all nations and cultures, partnering** in researches, spreading research results far and wide, and bringing together the local pieces of the puzzle of nature and society from all corners of the globe to build a bigger picture that could fuel local and global sustainable development efforts.

The Story of WPU and TPS

This issue also tells the story of The Palawan Scientist (TPS) itself, and the University it continuously brings pride to, the Western Philippines University (WPU). You see, this particular issue of the TPS comes at the heels of **three achievements of WPU and the TPS.**

First, last March, WPU was awarded as the **sole winner of the Top-Performing MIMAROPA Universities in Research Production in 2024**, for being the only state university in the Region of MIMAROPA with scopus indexed research publications. For context, Scopus is a leading abstract and citation database of peer-reviewed literature used globally by researchers, institutions, and policymakers to assess the quality and impact of scholarly work. It is also one of the specific metrics in many international University Rankings. In fact, our Scopus publications is a major contributor to the second achievement of the University: we are the first and only MIMAROPA University to be **internationally ranked in the Times Higher Education Impact Rankings**. And third, just, last April, 2025, **The Palawan Scientist itself has been accepted for Scopus indexing.**

These recognitions are testament to the 115 years of prolific scientific participation of WPU and the 12 (2014-2025) years of international level quality of the publications of the TPS. And with Scopus indexed research possible in our very own backyard, TPS can only skyrocket WPU in its scientific relevance and international rankings. **That all starts here, in this issue - the first issue of TPS since these 3 achievements.**

***That* grand story** - the story of painstaking research and writing, of rigorous scientific screening and publication practices, of the countless echoes of thoughts and love put into every word, of the story of nature and society and variety, of a proud journal and University just trying to carve out its place in the world as a strong partner for sustainable development - **you would appreciate that story much more profoundly if you read this whole journal issue.**

Oh…Wow! You made it this far! You deserve a treat!

In an effort to encourage you to at least browse through the whole issue, I have hidden **10 numbers** in the title pages of each article in the issue. Putting them in order of appearance and preceding them with +63, this will make up a Philippines cellular number, which you are to message through Viber with the phrase "I am (NAME) and I read the whole Volume 17, Issue 1 of The Palawan Scientist! My email is (EMAIL)". We will then get in touch with you for a **special surprise.**

So as you flip through this Volume 17, Issue 1 of the Palawan Scientist, I hope you enjoy the brilliant individual articles, and the proud story of the issue as a whole.

Happy reading! Happy number hunting! And to God be all the Glory!

Amabel S. Liao, PhD
University President
Western Philippines University

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Implementation of Area-Specific Action Plans (ASAP) for community-based MPAs in Cagayancillo, Palawan, Philippines: funded versus not funded

Cecilia F. Malolos[1], Michael Angelo D. Maga-ao[2], Abraham P. Cea[1], Sagrado C. Magallanes[3], Jennifer T. Diamante[2], Reagan Venturillo[4], Maria Mojena Gonzales-Plasus[5,*], and Benjamin J. Gonzales[6]

[1]*Western Philippines University, College of Public Administration and Management*
[2]*Western Philippines University, College of Arts and Sciences*
[3] *Romblon State University, Sta. Maria Campus, Romblon*
[4]*Western Philippines University, College of Agriculture, Forestry and Environmental Sciences*
[5]*Western Philippines University, College of Fisheries and Natural Sciences*
[6]*Senior Consultant, BGERMC*

[*]*Corresponding Author: mojenagonzales@yahoo.com*

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ABSTRACT

The Area Specific Action Plan (ASAP) for community-based MPAs serves as an outline of the framework for the purpose, design, and implementation of MPAs. The funding from the US Department of the Interior (USDOI) through Tanggol Kalikasan (TK) was used for the planning, training, and implementation of the Area-Specific Action Plan (ASAP) through Tanggol Kalikasan (TK). Two barangays from the Municipality of Cagayancillo were chosen as beneficiaries of the funding. This study aimed to assess the level of implementation of the ASAP on coastal resource management (CRM) and law enforcement in the community-managed MPAs in Cagayancillo Island, Palawan, through a comparison of the results of the perception survey interviews of the respondents from the control sites and funded sites. Four marine protected areas (MPAs) in the Cagayancillo region were selected as study areas: the MPAs in Barangays (Bgy.) Sta. Cruz (C1) and Nusa (C2) which did not receive additional funding from TK served as control sites; while Bgy. Mampio (F1) and Bgy. Talaga (F2) which received funding from TK, served as the funded site. The results showed that all CRM indicators were fully implemented (4.0) in C1, C2, and F2. The F1, on the other hand, had moderate implementation (2.0) of conducting seminar-workshops on MPA and poor implementation (1.0) of posting tarpaulin, signage, and other infographics. In terms of law enforcement, I1 had significantly moderate implementation (2.0) compared to other barangays. The implementation of ASAP in F1 was badly affected by Typhoon Odette in December 2021. Taking all factors into consideration, personal interview results revealed that the key to the effective implementation of MPAs' CRM and law enforcement activities significantly depends on the leaders of a barangay. Well-established leadership can efficiently and responsibly implement projects within their areas.

Keywords: ASAP, Barangay, CRM, law enforcement, MPA

INTRODUCTION

Marine protected areas (MPAs) are considered one of the best coastal management strategies and have gained worldwide recognition as an effective fishery management tool (White et al. 2006; Day et al. 2019). They are also recognized as one of the most powerful and effective methods to rebuild, protect, and sustain fisheries and ocean ecosystems (IUCN 2019).

A study on monitoring MPAs in the Philippines was conducted by Russ and Alcala as early

as 1989, and several studies on the assessment and management of marine resources in Palawan can be found in the works of Gonzales et al. (2014a), Gonzales et al. (2014b), Gonzales et al. (2014c), Palla et al. (2015), Gonzales and Gonzales (2016), and Gonzales et al. (2021).

There are various guides available for the assessment, monitoring, and evaluation of MPAs. These include monitoring water quality, monitoring changes in fish stocks, and measuring the level of impact from tourists as visitor numbers grow, as well as other related socio-economic factors (Russ and Alcala 1999; Pomeroy et al. 2001; Pomeroy et al. 2005). However, it is important to note that these indicators and parameters should be based on the specific objectives of each MPA (Russ and Alcala 1999).

Sustainability should be given due importance, as little to no attention to the sustainability of MPAs can hinder efforts to accomplish the objectives of protective management projects (Russ and Alcala 1999). To determine the success of MPAs, a functional monitoring and evaluation scheme must be in place, and scientific assessment of marine resource development within MPAs is crucial (Gallacher et al. 2016). However, the lack of funds or availability of qualified personnel, which often restricts these efforts (Sanders et al. 2013). Therefore, adaptive monitoring, which integrates science and monitoring, is necessary. This involves identifying new questions, introducing new monitoring methods, continuously developing indicators, and synthesizing information to ensure the integrity of long-term records on MPA management is maintained. In addition, local knowledge should be integrated into the monitoring and evaluation scheme.

Tanggol Kalikasan (TK) is a non-stock, non-profit public interest environmental law office based in the Philippines. Tanggol Kalikasan established the Institute of Environmental Governance Program (IEG) to enhance the capacity of Barangay, municipal, and provincial officials in the Philippines in environmental management, including MPAs (Gonzales et al. 2021; Tanggol Kalikasan, 2024). One of TK's projects is the Environmental Justice Sector Reform Program, which receives support funding from the United States Department of the Interior and Technical Affair Program and the International Narcotics and Law Enforcement Affair Bureau (USDOI-INL). Under this project, TK provides seed funding for the implementation of Area-Specific Action Plans (ASAP) for community-based MPAs. The ASAP serves as a framework outlining the purpose, design, and implementation of MPAs. Currently, in Palawan, TK provides seed funding for implementing ASAP for MPAs in the municipalities of Jose P. Rizal, Culion, and Cagayancillo.

Cagayancillo is an offshore island municipality of the province of Palawan (Dedace 2015). It consists of 30 islets and one main island, with land area of 26.29 km^2 inhabited by a population of 6,348 people. There are 12 barangays in Cagayancillo, with an estimated population of 7,000 (WWF 2020). The common livelihood and sources of income in the municipality are fishing, seaweed farming, livestock, rice and corn farming, cottage industries, and tourism (WWF 2020). Approximately 1,013,340 ha of Cagayancillo waters are protected as a coastal marine area, including 528,000 ha of municipal fisheries and an ecotourism zone, as well as about 485,000 ha of open water that connects Cagayancillo and the Tubbataha Reefs Natural Park (WWF 2020). Cagayancillo is an archipelagic municipality in Palawan, located in the northeastern part of the Sulu Sea, about 130 km from Tubbataha Reefs Natural Park and 300 km from the city of Puerto Princesa.

The control sites (C) for this study are the MPAs in Barangay (Bgy.) Sta. Cruz (C1) and Bgy. Nusa (C2), which have been operating for 14 years and did not receive additional financial assistance from TK for the implementation of their ASAP. On the other hand, the funded sites (F) are the two newly reactivated MPAs in Bgy. Mampio (F1) and Bgy. Talaga (F2), which have been operating for less than 10 years, received financial assistance from TK for the implementation of their ASAP.

This study aimed to assess the implementation of ASAP in local community-based MPAs in Cagayancillo Island, Palawan. Specifically, the objectives of this study are to: (1) evaluate the level of implementation of ASAP activities in four barangays in Cagayancillo; and (2) compare the level of ASAP implementation among barangay MPAs that received financial assistance and those that did not receive financial assistance.

The control sites for this study are the MPAs in Bgy. Sta. Cruz and Bgy. Nusa, which have been operating for 14 years and did not receive any financial assistance for the implementation of their ASAP. The funded sites are the two newly reactivated MPAs in Bgy. Talaga and Bgy. Mampio, which have been operating for less than 10 years and received financial assistance for the implementation of their ASAP.

METHODS

Study Site Description

This study was conducted in the municipality of Cagayancillo, Palawan. Cagayancillo is located at 9'55 latitude and 121'14.5 East longitude, 84 nautical miles from Puerto Princesa City, and approximately 56 nautical miles to Panay Island (Google map 2023). Four marine protected areas (MPAs) in the Cagayancillo region were selected as study areas: MPAs in Bgy. Sta. Cruz (C1) and Bgy. Nusa (C2), served as control sites while Bgy. Mampio (F1) and Bgy. Talaga (F2), served as the funded sites (Figure 1 and Table 1).

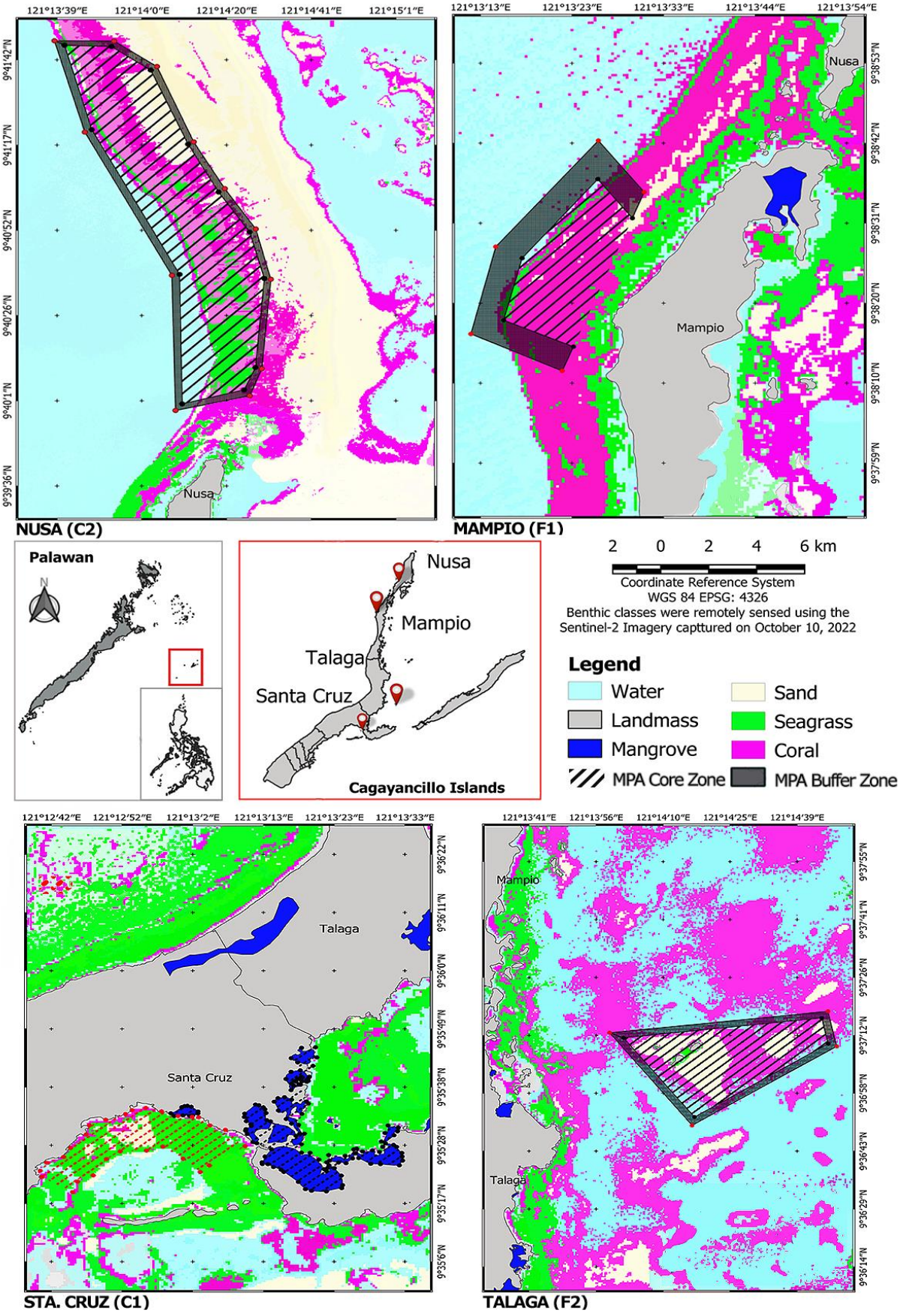

Figure 1. Map of Cagayancillo, Palawan indicating the locations of the four Marine Protected Areas.

Table 1. Information on the MPAs in Cagayancillo.

MPA sites	Total area (ha)	Core (ha)	Buffer (ha)	Year established	Descriptions
Sta. Cruz (C1)	33.51	18.91	14.62	2009	Harbors relatively large seagrass area
Nusa (C2)	237.51	77.25	36.58	2004	Mostly occupied by coral reefs
Mampio (F1)	29.52	16.06	13.46	2009	Abundance of marine life such as turtles, fish, and clams
Talaga (F2)	70.56	52.27	18.29	2004	Presence of two types of habitats, seagrass, and corals.

The C1 MPA covers an area of 33.53 ha and is located in Nipay, Balugo, Kimbang, Tegbengan, and Tiosan. This MPA is the largest seagrass area in the region and was launched in November 2009 (Figure 1 and Table 1). On the other hand, C2, which covers an area of 197 hectares, is located north of Barangay Nusa. The MPA is home to various marine resources, such as corals and oysters. It was launched in May 2004 and is currently carrying out projects for the conservation and preservation of marine resources (Figure 1 and Table 1).

One of the newly reactivated MPAs in the area is F1 MPA, which covers a total area of 29.52 ha in the western coastal part of the Barangay. The MPA is divided into a core zone covering 16.06 ha and a buffer zone covering 13.46 ha. The MPA is home to different types of marine life, such as turtles, fish, and clams. It was established in March 2009 (Figure 1). Lastly, the F2 MPA is located in the Leek Islets and covers an area of 70.56 ha, with 52.87 ha designated as the core zone and 17.69 ha as the buffer zone with a depth of 5 to 8 m. The F2 covers two types of habitats, seagrass and corals, and is home to various marine creatures such as fish and corals. The MPA of F2 was established on June 21, 2004 and expanded in July 2009 (Figure 1 and Table 1). Both the F1 and F2 MPAs have been laid low due to a lack of funding.

Respondents of the Study

The respondents of this study were selected based on their participation in the implementation of the MPAs in Cagayancillo who are mostly members of the community (37%); NGO/ PO/ Association members and officers (23.53%); Barangay officials (21.57%); Bantay Dagat personnel (3.92%); Barangay captains (0.98%); and various locals (12.75%). Most of them are male (57.56%), between 36 and 45 years old (30.84%), married (84.62%), and have finished secondary education (54.78%) (Table 2).

Survey Questionnaire and Data Collection

The developed survey questionnaire has three sections: the socio-demographic profile of the respondents; the assessment of the level of ASAP implementation on coastal resource management and law enforcement; and the open-ended questions on the issues and barriers that the community has encountered in their ASAP implementation.

A 4-point Likert scale was used to assess the level of implementation of ASAP in the two funded sites: 4-fully implemented (FI), 3-strongly implemented (SI), 2-moderately implemented (MI), and 1-poorly implemented (PI). All respondents were asked to assess the implementation of their proposed CRM and LE projects by rating each identified indicator as Poorly Implemented (PI) (if around 0–25%of the proposed activity was implemented); Moderately Implemented (MI) (if around 26%–50%of the proposed activity was implemented); Strongly Implemented (SI) (if around 51%–75% of the proposed activity was implemented); or Fully Implemented (FI) (if around 76%–100%of the proposed activity was implemented). The scores were then tallied and implementation levels were measured using the average of the scores: 3.26-4.0 = fully implemented; 2.51-3.25 = strongly implemented; 1.76-2.50 = moderately implemented; and 1.00-1.75 = poorly implemented.

The survey focused only on the Coastal Resource Management (CRM) and law enforcement projects indicated by the stakeholders and MPA managers in their ASAP. Indicators used to assess their level of implementation include the activities in the approved ASAP, such as their Coastal Resource Management project, that was focused on activities relating to the reactivation of their MPAs. Proposed CRM activities such as the drafting of MPA Management Plan, delineating and marking of MPAs, creating information and education campaign materials, and conducting community meetings were used as indicators for the CRM survey instrument. On the other hand, law enforcement activities such as the conduct of training and orientation of Bantay Dagat personnel, monitoring of MPAs, patrolling activities of Bantay Dagat personnel, and the maintenance of law enforcement facilities were used as indicators for the LE survey instrument. The researchers also added issues/barriers to the implementation as open-ended questions to further evaluate the experiences of the respondents.

Table 2. Socio-demographic profile of the respondents (n=120).

Profiles	Number of respondents (n)	Percentage (%)
Social Status		
Community Member	38	37.25
NGO/PO/Association	24	23.53
Barangay Official	22	21.57
Bantay Dagat	4	3.92
Barangay Captain	1	0.98
Others	13	12.75
Sex		
Male	67	57.76
Female	49	42.24
Age		
18-25	8	7.48
26-35	19	17.76
36-45	33	30.84
46-55	28	26.17
56-65	14	13.08
66-Above	5	4.67
Civil Status		
Married	99	84.62
Single	15	12.82
Co-habiting	2	1.71
Widow	1	0.85
Highest Educational Attainment		
Elementary	39	33.91
Secondary	63	54.78
Vocational	4	3.48
College	9	7.83

Personal interviews were conducted on the island of Cagayancillo in March 2022. Consent from the stakeholders who served as respondents to the study was secured before the data gathering started. The researchers read the instructions and informed the respondents to indicate their answers to the questionnaires. All respondents were given ample time to answer the questionnaire. A total of 120 respondents (30 respondents from each barangay) were composed of the municipality's administrative official, Municipal Planning and Development Officer (MPDO), Planning Officer, Bantay Dagat personnel, Philippines National Police (PNP) personnel, Bgy Captain, Bgy. Kagawads, and community members who were mostly fisherfolks and residents of the four barangays being studied. The municipal and PNP personnel, as well as the barangay chairman and barangay kagawads who served as respondents, were purposefully chosen based on their designations as implementers of the projects. On the other hand, the fisherfolks, both from the funded barangays and the not-funded barangays were randomly chosen to objectively compare their assessment of their MPA activities.

Data analysis

Evaluation of the ASAP implementation used a descriptive design and analysis, and the researcher added qualitative data to enhance the discussions. The current study utilized the Kruskal-Wallis H test and the Dunn-Bonferroni post hoc test to determine variations in the level of implementation of coastal resource management (CRM) and law enforcement (LE) per barangay. The mode was employed as the

measure of central tendency in analyzing the Likert scale data. All the data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.

RESULTS

Level of Implementation of MPAs' ASAP Activities

Based on the findings of this study, the Kruskal-Wallis H test ($P = 0.91$) reveals no significant difference in CRM implementation across Barangays. As shown in Figure 2, the four barangays have the same level of implementation (i.e. 3.26–4, equivalent to fully implemented), despite funded interventions in F1 and F2. However, for law enforcement, a significant difference exists across barangays (Kruskal-Wallis H test: $P = 0.002$), with F1 exhibiting moderate implementation (1.76-2.50) of law enforcement activities despite funding (Figure 3).

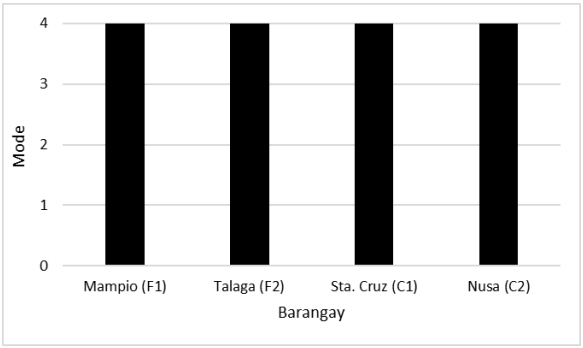

Figure 2. Costal resource management implementation level in each barangay in Cagayancillo.

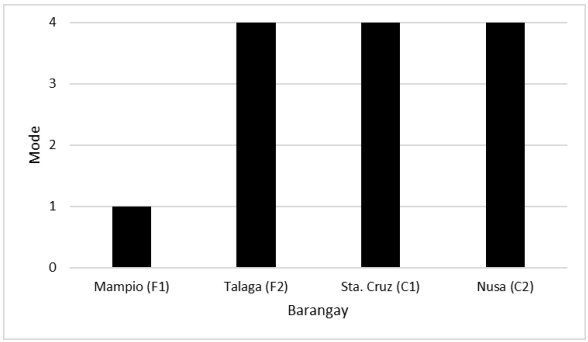

Figure 3. Law enforcement implementation level in each barangay in Cagayancillo.

Most CRM indicators were fully implemented (4.0) in C1 and F2 (Figure 4). The F1 had a low level of implementation of indicators such as conducting seminar-workshops on MPA management (2.0) and conducting information, education, and communication campaigns (3.0) compared to the other three barangays. The C2 and F1 had low scores (1.0) for posted tarpaulins, signage, and infographics.

Regarding the performance of each barangay in terms of law enforcement, C1, C2, and F2 had full implementation (Figure 5). The F1 had poor implementation (1.0) for conducting training/orientation for Bantay Dagat, conducting biophysical monitoring of MPAs (2.0), conducting MPA patrolling by Bantay Dagat (2.0), and enforcing MPA policy/ law (1.0).

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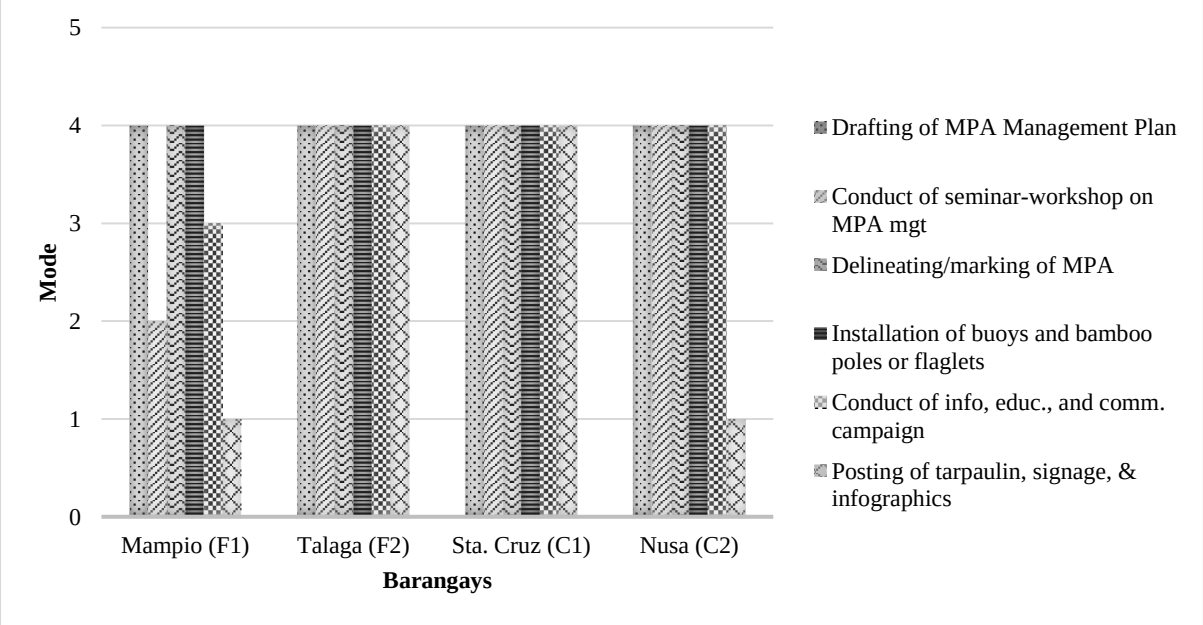

Figure 4. Performance indicator of each barangay for Coastal Resource Management implementation.

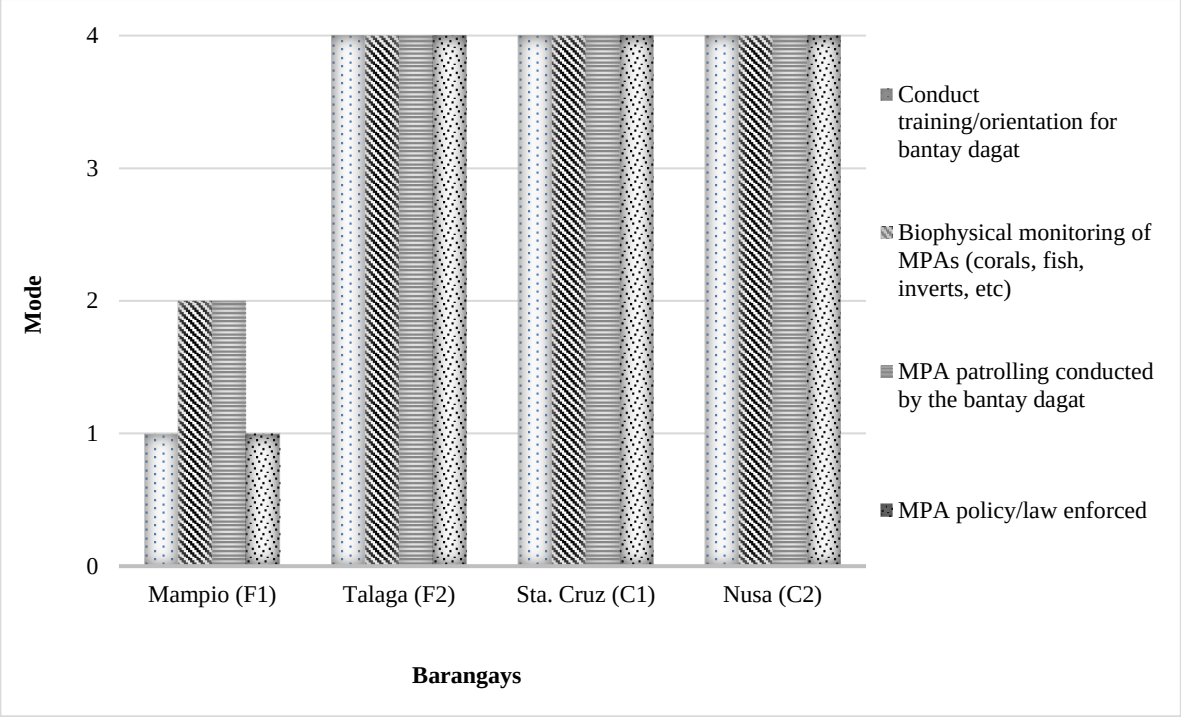

Figure 5. Performance indicator of each barangay for law enforcement.

These findings were supported by the results of personal interviews conducted by the researchers among the LGU officials in the municipality and by photos and videos taken during the ocular inspections conducted at the actual project sites. According to the respondents, most of the signage and markers were installed as part of their ASAP activity implementation. However, when typhoon Odette wreaked havoc on the island, all installations were destroyed (Miranda 2021). Consequently, during the ocular inspection, the researchers personally saw the destruction caused by typhoon Odette. All uninstalled sinkers and markers were still stored in their warehouse.

DISCUSSION

The locally-managed MPAs were primarily co-managed by the community/stakeholders and the local government. The extent of their management roles varied, including areas such as funding, marketing, and research. These differing levels of involvement can have various impacts on the co-management of the MPAs (Berkes 2015). Previous implementations of ASAP in barangays and municipalities were reported to be effectively carried out in Palawan (Gonzales et al. 2021).

In the current study, all study sites were able to implement CRM and law enforcement activities except for F1. Regarding awareness campaigns, the scheduled information dissemination campaign activities were adversely affected by a series of lockdowns related to COVID-19 in the area. Consequently, these circumstances had an impact on the ratings of locals for this indicator. The poor implementation rate related to the posting of tarpaulin, signage, and infographics in C2 and F1 was due to several factors, such as the inaccessibility of the island from the mainland during COVID-19 lockdowns and the effect of Typhoon Odette in December 2021. Furthermore, C2 is an islet near F1 and is only accessible through a pump boat, affecting the procurement process of the materials needed for the CRM ASAP plan of the barangay.

Law enforcement was also fully implemented in C1, C2, and F2. The integration of the kind of leadership that F2's barangay captain has and the support and participation of its constituents played a significant role in why the level of implementation in their barangay was high in both their CRM and law enforcement activities.

According to Ferrer and Nozawa (1997), when policies in the barangay are already in place, collaborative and credible efforts for effective enforcement may just continuously be carried out. On the other hand, factors such as lack of participation and engagement, lack of accountability, low transparency, lack of awareness of active citizens, weak role of civil society, gender inequality, quality of decision making, locals' attitude and participation, political will, legislation, and priority of outsourced funds can greatly affect the success of the implementation of environmental management at the ground level (Gonzales 2011; Taylor 2016; Cameron 2016; PIN 2017). At the barangay level, the establishment of a law enforcement office requires budgetary allocations

and manpower support to have a successful implementation (DENR et al. 2021).

The current study indicates that the key to effective implementation of CRM and law enforcement activities in the MPAs lies significantly in the leaders of a barangay. Well-established leaders can efficiently and responsibly implement projects within their areas because they have the support and participation of their constituents.

FUNDING

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ETHICAL CONSIDERATIONS

Prior informed consent, data privacy, and the confidentiality of the respondents were ensured by the researcher prior to the conduct of the survey.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among any of the authors.

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A structural model of college students' mathematics performance: the role of psychological, physiological, and psychosocial factors

Bryan L. Susada

Cateel Extension Campus, Davao Oriental State University, Cateel, Davao Oriental, 8205 Philippines

Corresponding Author: brylsusada@gmail.com

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ABSTRACT

The study aimed to find the best-fit structural model to describe the mathematics performance of freshmen secondary education students majoring in mathematics concerning psychological, physiological, and psychosocial factors. Psychological factors were measured in terms of self-efficacy and attitudes toward mathematics, while physiological factors were assessed about nutritional status, and wellness and well-being. Psychosocial factors were measured in terms of math anxiety and math interest. A questionnaire was administered to 312 randomly selected mathematics teacher education students who have experienced struggles in their board examination performance. These students came from various higher education institutions in the Davao and the SOCCSKSARGEN (South Cotabato, Cotabato, Sultan Kudarat, Sarangani and General Santos City) regions. The validity and reliability of the questionnaire were established through factor analysis and an internal reliability test, respectively. The findings indicate that students exhibit strong performance in mathematics, possess moderate levels of psychological and psychosocial competencies, and maintain relatively healthy physiological statuses. Additionally, the results reveal a structural model depicting students' mathematics performance with psychological, physiological, and psychosocial factors, which can explain 78% of the data considered in the study. Higher educational institutions may enhance support for students' psychological and psychosocial skills and integrate health and wellness programs to boost their physiological status, given its impact on academic performance. Further research is encouraged to explore additional factors affecting academic success, aiming to develop a more comprehensive understanding of influences on students' performance.

Keywords: bioecological model of development, ecological system theory, structural equation modeling

INTRODUCTION

Students' performance in mathematics has dropped in the Philippines and other parts of the world. This problem is observable from national assessments (Susada 2018) to international examinations (Thomson et al. 2019). According to data from the Programme for International Student Achievement (PISA 2022) and the Trends in International Mathematics and Science Study in the United States of America (TIMSS 2019),

most students around the globe are struggling to learn mathematics (OECD 2016). In the Philippines, this often translates to poor performance in board examinations, particularly in secondary education, for students, including those majoring in mathematics (Amanonce and Maramag 2020).

The difficulty of mathematics education has caught the interest of most professionals, policymakers, and even the media. As a result, the situation has become a topic of various public and political debates (Hauge and Barwell 2017). There was a discussion on what were the causes of the difficulty (Bakker et al. 2021). There were also differint perspectives on approaching mathematics instruction, as a product or a process (Susada and Baquiano 2015). Several investigations were conducted to determine the causes of the problem as well as possible solutions. For instance, students' poor performance may be related to educators' antiquated teaching approaches (Banerjee 2016), students' lack of basic math skills (Acharya 2017), and poor interest in mathematics (Bacsal et al. 2022). Experts researching the issue proposed a variety of solutions to eliminate or mitigate the problem, ranging from government programs (Slaughter et al. 2015) to classroom practices (Kerr et al. 2018).

It should be emphasized that these studies rarely combine characteristics to determine what is optimal for learners but instead profile individuals' learning styles and proceed with the proposed intervention. Most studies focus on a few components but fail to combine them to identify which factor influences mathematics learning most (Acharya 2017). It has to be noted that mathematics performance is significantly influenced by psychological factors such as students' attitudes towards the subject and their self-efficacy, with positive attitudes and high self-efficacy linked to better outcomes (Bandura 1997, Schunk 1995); psychosocial factors, including math interest and math anxiety, also play a crucial role, where interest promotes engagement and achievement, whereas anxiety impedes performance (Renninger and Hidi 2011); and physiological factors like nutritional status and overall well-being are foundational to cognitive function and academic performance, underscoring the importance of physical health in learning mathematics (Florence et al. 2008). While individual factors have been studied extensively in isolation, their collective impact and the interactions among psychological, psychosocial, and physiological factors remain underexplored, particularly among college students, especially those who are majoring in mathematics (Kerr et al. 2018). This study aimed to fill this gap by integrating concepts from ecological systems theory (Bronfenbrenner 1979) and the bioecological model of development (Bronfenbrenner and Ceci 1994), suggesting that individual development is the result of interactions between biological, psychological, and environmental factors to develop a structural model on students' mathematics performance that encompasses these multidimensional influences.

Given the struggle with the poor performance of secondary education graduates in mathematics board examinations in the Philippines (Amanonce and Maramag 2020), and recognizing that their overall mathematics performance often predicts success in these examinations (Gabasa and Raqueño 2021), this study aimed to accomplish the following objectives: to determine the level of mathematics performance among secondary mathematics education students based on their grades in the subjects of trigonometry, and college and advanced algebra; to describe their psychological status (in terms of attitudes towards mathematics and self-efficacy), psychosocial status (in terms of math interest and math anxiety), and physiological status (in terms of nutritional status, wellness, and well-being); and to develop a structural model that effectively predicts the mathematics performance of college mathematics education students considering psychological, physiological, and psychosocial aspects.

METHODS

Data Gathering Procedures

This study began by developing and piloting the research instrument to establish its validity and reliability. Following this initial step, ethical clearance was obtained from the ethics office of Davao Oriental State University. Subsequently, permission to conduct the study was requested from the heads of the selected tertiary educational institutions. Upon receiving the necessary approvals, the heads of the Bachelor of Secondary Education (BSED)-Mathematics programs were requested to arrange a meeting with the first-year students for data collection. During this meeting, the students were informed about the purpose of the study and were asked to provide their consent to participate. Those who chose not to participate were given the option to leave the venue. After obtaining consent, the research questionnaire was distributed to the respondents. The students were allowed to complete the questionnaire at their own pace. Furthermore, the grades of student respondents in college and advanced algebra, as well as trigonometry, were acquired from the registrar's offices of the respective institutions following official requests for this information. Once the data collection process was completed, the responses were tabulated and analyzed.

Research Design

This study employed a descriptive-correlational research design, primarily utilizing a cross-sectional, or one-shot, survey approach. This methodology enabled the researcher to collect data

efficiently by administering a questionnaire to respondents at a single point in time (Lodico et al. 2010). The descriptive aspect of the study focused on assessing the respondents' levels of mathematics performance, psychological status, psychosocial status, and physiological status. Conversely, the correlational aspect was dedicated to exploring the intricate relationships among these variables to develop an optimal model predicting students' mathematics performance based on psychological, psychosocial, and physiological factors.

Respondents and Sampling Procedure

The study targeted first-year college students enrolled in compulsory BSED – Mathematics subjects, including college and advanced algebra, as well as trigonometry (CHED 2017), for the first semester of the 2022-2023 academic year. These participants, aspiring secondary education math teachers from various prestigious institutions across the Davao and the SOCCSKSARGEN (South Cotabato, Cotabato, Sultan Kudarat, Sarangani and General Santos City) regions of Mindanao, are distinguished by their challenging performance in teacher licensure examinations (PRC 2022). The selected institutions were: Davao Oriental State University in Mati City, Davao Oriental; University of Southeastern Philippines in Tagum City, Davao del Norte; Southern Philippines Agribusiness, Marine, and Aquatic School of Technology in Malita, Davao Occidental; Davao del Sur State College in Digos City, Davao del Sur; and King's College of Marbel, Inc. in Koronadal City, South Cotabato.

To accurately reflect the population, the Slovin's formula with a 5% margin of error determined a sample size of 312 out of 373 eligible first-year BSED math students from the aforementioned institutions. Stratified random sampling ensured equitable representation across these institutions, yielding participation as follows: 64 from 77 students at Davao Oriental State University, 72 from 88 at the University of Southeastern Philippines, 70 from 85 at the Southern Philippines Agribusiness and Marine and Aquatic School of Technology, 67 from 80 at Davao del Sur State College, and 39 from 43 at King's College of Marbel, Inc. The assessment focused on their academic performance in key mathematics courses namely college and advanced algebra, and trigonometry.

Research Instrument

This study utilized a self-made survey questionnaire rigorously tested for construct validity and internal reliability, exhibiting strong validity indicated by a Kaiser-Meyer-Olkin measure of 0.851 and a significant Bartlett's Test of Sphericity ($p<0.001$). It also displayed notable reliability with a Cronbach's alpha coefficient of 0.761. The questionnaire was structured into four distinct sections. The second section, consisting of 20 items related to psychological factors, divided evenly between self-efficacy and attitudes towards mathematics. This part requested respondents to indicate their level of agreement or disagreement with each statement. The third section, also comprising 20 items, dealt into physiological factors such as nutritional status, wellness, and well-being, asking respondents to assess their condition in relation to each statement. The final section explored psychosocial factors, with 20 items equally distributed between math anxiety and math interest. Here, respondents were again requested to state their agreement or disagreement with each pertinent statement.

Data Treatment

This study employed mean calculations to assess the mathematics performance and physiological, psychological, and psychosocial statuses of students. To further enrich the discussion on these dimensions, standard deviations were calculated, offering insights into the variability of students' psychological, psychosocial, and physiological states.

Moreover, the research framework incorporated latent variables, each associated with specific observed variables, to capture the multifaceted nature of student performance and well-being. Students' mathematics performance was evaluated based on their grades in college and advanced algebra, and trigonometry—courses that are compulsory for secondary education majoring in mathematics (CHED 2017). The psychological dimension was assessed through students' attitudes towards mathematics and their self-efficacy beliefs. Psychosocial status was gauged by examining math anxiety and interest in mathematics, while physiological status was determined by evaluating nutritional status, and overall wellness and well-being. Thus, in this study, there were three latent variables, each associated with two observed variables, making it suitable for a structural equation modeling (SEM) study (Kline 2015). Accordingly, SEM was employed to identify the most accurate model explaining students' mathematics performance, taking into account psychological, psychosocial, and physiological factors. Table 1 provides comprehensive criteria for model fitness in SEM. The initial survey garnered 312 responses; however, after excluding outliers, the analysis proceeded with 294 responses. These 294 responses satisfied multivariate normality test and non-multicollinearity test of the independent variables. These responses were instrumental in developing the optimal model to describe the intricate relationships between students' academic performance and their psychological, psychosocial, and physiological states. The data analysis and model development were facilitated using SPSS and AMOS Version 22,

providing a robust foundation for understanding the determinants of students' mathematics achievement.

Table 1. Characterization of best fit model.

Types of Model Fit	Parameters and Criteria for Model Fit
Parsimonious Model Fit	CMIN/DF < 2.00
Absolute Model Fit	p-value > 0.05
	RMSEA < 0.05
	GFI > 0.95
Incremental Model Fit	CFI > 0.95
	TLI > 0.95
	NFI > 0.95

RESULTS

Student's Level of Mathematics Performance

Table 2 shows students' mathematics performance regarding their college and advanced algebra, and trigonometry grades. Generally, students have good standing as to their mathematics academic performance. Only 1.9% of the respondents was excellent, and around 7.4% had passing grades in both subjects. There was around 40% of the total respondents who had had a good standing in both subjects.

It further disclosed that students had an average mathematics performance. Students' distribution of their mathematics performance was similar to college and advanced algebra, and trigonometry. It further depicts that respondents of the study were successful in the subject.

Students' Level of Psychological Abilities, Psychosocial Skills, and Physiological Status

Table 3 displays that students have a moderate psychological ability, similar to their attitude towards mathematics. Some agree with statements about their attitudes towards mathematics, while others disagree with some descriptors. They disagree in considering mathematics an unimportant subject, while they agree to consider it useful. Moreover, they varied more in considering mathematics a more difficult subject than believing it is important and unpleasant. On the other hand, students have high self-efficacy. They rated high on their ability to handle whatever comes their way in their pursuit of learning mathematics. On the contrary, they rated lowest on their ability to handle unexpected events during mathematics learning. They also have varied responses, whether they would be calmed during difficulties. At the same time, they are more united to believe that they can find means to achieve their goals in learning the subject despite opposition.

Table 2. Level of student's mathematics performance.

Subject	Grade	Remarks	Frequency	Percentage	Mean
College and Advanced Algebra	1.00 – 1.25	Excellent	6	1.9	2.17, Good
	1.50 – 1.75	Very Good	76	24.4	
	2.00 – 2.25	Good	126	40.4	
	2.50 – 2.75	Satisfactory	81	26.0	
	3.00	Passing	23	7.3	
Trigonometry	1.00 – 1.25	Excellent	6	1.9	2.17, Good
	1.50 – 1.75	Very Good	76	24.4	
	2.00 – 2.25	Good	125	40.0	
	2.50 – 2.75	Satisfactory	82	26.3	
	3.00	Passing	23	7.4	

Table 3. Students' level of psychological abilities, psychosocial skills and physiological status.

Factors	Standard Deviation	Mean	Remarks
I. Psychological Ability	0.32	3.34	Moderate
A. Attitude Towards Mathematics	0.34	3.34	Moderate
B. Self-Efficacy	0.47	3.47	High
II. Psychosocial Skill	0.32	3.08	Moderate
A. Math Anxiety	0.49	3.01	Moderate
B. Math Interest	0.46	3.15	Moderate
III. Physiological Status	0.35	3.28	Somewhat Good
A. Nutritional Status	0.48	3.51	Good
B. Wellness and Well-being	0.40	3.16	Somewhat Good

Additionally, it portrays that students have moderate psychosocial abilities. Some students agree with some descriptors on psychosocial abilities, while others disagree. They even have opposing views about the anxiety they feel toward the subject. The least that they contested is whether or not they have made an effort to avoid mathematics subject. They highly debated their idea that it takes time to solve mathematical problems. They also had more varied responses to consider mathematics their worst subject than to consider it easy. On the other side, it further displayed that students were also divided on their interest in mathematics. Nevertheless, they agreed that mathematics is essential and difficult. However, they had the least contested to consider mathematics class as boring and necessary in a day. They have more varied responses about their like and dislike of the subject while feeling comfortable in mathematics class is the most unified response.

Finally, it depicted that students sometimes focus on their physiological needs. They generally often observed parameters on their nutritional status. They sometimes consumed unsaturated fats but often dark green or deep orange vegetables. Additionally, they were more varied in sugar and caffeine consumption than unsaturated fats. Conversely, it further showed that students sometimes observe descriptors about their wellness and well-being. Among descriptors, they had high-stress management, while maintaining ideal body weight was the least of their concern. This means they are not particular about their ideal weight but have great ability on stress-coping mechanisms. Students are more varied in maintaining their ideal body weight than being more anxious and upset. With this, students are not united in the notion that they must maintain their ideal body weight.

Structural Model of Students' Mathematics Performance

Figure 1 presents a best fit model to describe mathematics performance of respondents in terms of psychological, psychosocial, and physiological factors. This model can explain 78% of the data being considered. Psychological and psychosocial skills of students have a strong relationship, while their psychosocial and physiological attributes have a moderate relationship. The psychosocial abilities of students as well as their physiological status have a direct influence on their mathematics performance. On the contrary, psychological factors have inverse influence students' mathematics performance. Students with a high interest in mathematics and less math anxiety are expected to perform well in the mathematics classroom. Additionally, students who have good nutritional status, wellness, and well-being tend to perform better in mathematics. Lastly, students who have overconfidence and a negative attitude towards mathematics have poor performance in mathematics.

The nutritional status of students has a greater impact on their physiological status than their wellness and well-being do. Further, math interest contributed directly to the psychosocial status of students, while math anxiety has an inverse impact on the psychosocial status of students. Furthermore, the self-efficacy of students has a greater impact on their psychological abilities than their attitudes toward mathematics do. Lastly, mathematics performance is highly attributed to students' algebra grades than their trigonometry grades.

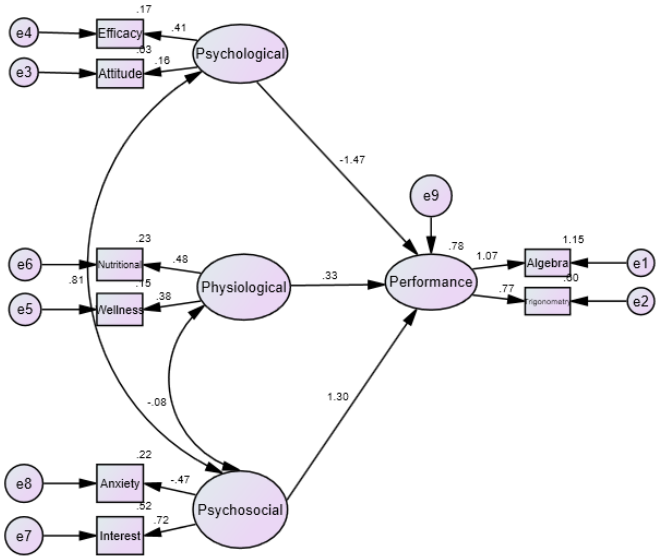

Figure 1. Structural model of students' mathematics performance (CMIN/DF=1.169, p-value=0.288, GFI=0.978, CFI=0.990, TLI=0.982, NFI=0.950, RMSEA=0.030).

DISCUSSION

Student's Level of Mathematics Performance

Mathematics education is vital for success in various fields. Students who have strong mathematical skills have high chance to pass any Philippine licensure examinations (Pantolla et al. 2016). The National Science Foundation revealed that mathematics provides the fundamental knowledge required for comprehending science, engineering, and technology (National Science Foundation 2013). Consequently, individuals who excel in mathematics are more likely to have better prospects for success in mathematics related areas (Slaughter et al. 2015). These findings can also guide teaching approaches and interventions to enhance math education within schools (Breslow et al. 2013).

Improvement in students' mathematics performance is significantly associated with academic self-concept and engagement in math-related activities. Identifying students who fall under the satisfactory and passing categories in Table 2 and providing them with the necessary support to enhance their mathematical abilities is essential. Baker's study further emphasizes the importance of addressing the psychological and psychosocial needs of highly motivated students with a positive attitude towards mathematics, as they tend to achieve higher academic success in this subject (Baker 2004). This observation is consistent with a study by Robertson et al. (2015), that students who excel in mathematics tend to have higher cognitive ability and stronger problem-solving skills.

Students' Level of Psychological Abilities, Psychosocial Skills, and Physiological Status

Table 3 shows students' psychological abilities, psychosocial skills, and physiological status with their math performance. The results showed that students had a moderate level of psychological ability (Mean = 3.34), indicating some proficiency in mathematical concepts and problem-solving (Lubans et al. 2016). However, there is still room for improvement as highlighted by previous literature (Wang et al. 2015). Most mathematics teacher education students need intervention to make the subject of mathematics enjoyable and important.

One crucial element of psychological ability is attitude, and the study found that students also had a moderate attitude toward mathematics (Mean = 3.34). A positive attitude towards math has been linked to increased motivation and performance (Wang 2013), but the moderate level suggests that there may be limitations in students' motivation and ability to succeed in the subject (Kerr et al. 2018). Moreover, the study also measured self-efficacy as another element of psychological ability (Mean = 3.47), suggesting that students believe they can perform well in math (Acharya 2017). High self-efficacy has been associated with improved academic performance (Bernardo 2021), indicating that interventions sustaining and enhancing self-efficacy levels could benefit students throughout their math education (Jett 2019).

The results indicated moderate psychosocial skills (Mean = 3.08). Math anxiety was one such psychosocial skill measured with an average score of 3.01 among students; this anxiety is a significant predictor of math performance (Waller 2014). It highlights the need for programs to manage math anxiety among students (Banerjee 2016).

Regarding physiological status, aspects like nutritional status and wellness and well-being were measured; they had mean scores of 3.51 and 3.16, respectively, indicating good nutritional status but somewhat good wellness/well-being levels among the student population were considered. It is essential to note that healthy living habits have been linked with academic performance (Marsigliante et al. 2023), and interventions promoting exercise and healthy eating could improve both physiological health and academic performance among students (Ghrouz et al. 2019).

Structural Model of Students' Mathematics Performance

Figure 1 portrays the structural equation model of student's mathematics performance in terms of psychological, physiological and psychosocial factors. This shows that students' mathematics performance is directly influenced by psychosocial factors (Waller 2014) and physiological factors (Baker 2004) but inversely affected by psychological factors (Wang 2013). Psychosocial, psychological and physiological elements have been found in studies to impact a student's mathematics ability in a multifaceted way. According to Zientek et al. (2019), psychosocial factors such as math interest and lower math anxiety can directly impact math performance. Moreover, Baker (2004) found that students' well-being influences their school performance. On the other hand, psychological factors can also negatively impact mathematics performance due to overconfidence (Zimmerman 2000) and negative attitudes towards mathematics (Beilock et al. 2010).

The model describes students' mathematics performance as influenced by biological, psychological, and environmental factors offers a holistic framework for enhancing educational outcomes. To improve math performance comprehensively, interventions should address these interconnected areas. Biologically, ensuring that students have access to proper nutrition can bolster cognitive functions crucial for learning mathematics, while incorporating regular physical activity can improve concentration and memory. Ergonomic classrooms also support physical well-being, helping students maintain focus during mathematical problem-

solving. Psychologically, fostering a growth mindset can empower students to view challenges as opportunities for improvement, reducing the fear of failure often associated with math. Techniques to manage math anxiety, such as relaxation, exercises and supportive peer groups can create a more conducive learning environment. Personalized learning approaches that cater to individual learning styles and challenges can also make mathematics more accessible and engaging. Environmentally, creating a supportive learning atmosphere that encourages exploration and values effort over innate ability can significantly enhance student engagement and success in mathematics. Involving parents in educational processes and ensuring equal access to learning resources like textbooks and technology are critical for providing a supportive learning framework. By integrating strategies across these biological, psychological, and environmental dimensions, educators can not only improve math performance but also foster a supportive educational ecosystem that nurtures all aspects of student development. This comprehensive approach not only raises academic achievement but also equips students with the resilience and skills necessary to apply mathematical concepts to real-world situations.

The model also depicts that the psychological and psychosocial skills of students are strongly associated with each other. This implies that students with high psychological skills also have high psychosocial abilities (Bandura 1997; Renninger and Hidi 2011), and both these attributes can enhance students' mathematics performance (Di Martino and Zan 2011). It is reasonable to claim that improving the psychological abilities of students can also enhance their psychosocial capacities (Renninger and Hidi 2011), which can lead to overall improvement in their mathematics performance (Zimmerman 2000). However, the model also reveals that psychosocial and physiological factors were somewhat related to each other. According to Robertson et al. (2015), a significant association exists between psychosocial elements like math interest and math anxiety and physiological factors like nutrition, wellness, and well-being. These factors can impact a person's attitude and interest in mathematics, among other topics. This means that students' psychosocial abilities go along with their physiological status, which is consistent with Waller (2014) who showed that people with strong physiological attributes also have strong psychosocial capacities.

Increasing students' mathematics performance is a central discussion among mathematics researchers and educators. With the declining performances of students in mathematics, which leads to struggling performance in Filipino licensure examinations (Gabasa and Raqueño 2021), this study suggests another approach to addressing the problem. This study offers new insights into how to collectively and holistically address students' mathematics performance. Accordingly, educators must reduce students' math anxiety and increase their interest in the subject because these greatly contribute to their academic performance (Bacsal et al. 2022). Zhang et al. (2019) discovered that math anxiety negatively impacts academic performance, particularly in students with a poor interest in mathematics. The researchers concluded that reducing arithmetic fear and improving students' enthusiasm for mathematics could improve academic performance significantly. This study emphasizes the necessity of addressing math anxiety and interest to improve children's academic performance. Drigas and Pappas (2015) discovered that using math games in the classroom can raise students' interest and motivation in mathematics, leading to higher academic achievement. Enjoyable and engaging activities, such as math games, can help alleviate math anxiety and boost students' enthusiasm (Di Martino and Zan 2011).

Students' mathematics anxiety impacts their wellness and well-being, which has the greater contributory factor of physiological factors. Wang et al. (2015) discovered that math anxiety is connected with greater physiological arousal. These physiological responses can result in stress-related health problems, severely impacting students' wellness and well-being. Another study by Hill et al. (2016) discovered that math anxiety is associated with poorer cognitive flexibility and greater rumination. These cognitive aspects can contribute to negative thoughts and sentiments, such as self-doubt, as well as mental health issues, like sadness and anxiety, which can negatively impact students' wellness and well-being (Renninger and Hidi 2011).

Strengthening students' psychosocial abilities requires good physiological capacity. Marsigliante et al. (2023) discovered a clear association between physiological capability and the development of psychosocial abilities among students. Students with higher levels of physical fitness had stronger psychosocial qualities, such as high math interest and low math anxiety (Robertson et al. 2015). Physical fitness and psychosocial talents were mutually reinforcing because regular exercise improves physiological capacity, which improves psychosocial abilities (Waller 2014).

Several types of research have revealed that physical fitness is favorably related to students' psychosocial ability. Lubans et al. (2016) discovered, for example, that school-based physical exercise interventions improved children's psychosocial qualities such as self-esteem, resilience, and social competence. Ghrouz et al. (2019) discovered that regular exercise was connected with decreased feelings of depression and anxiety in students. Overall, these data indicate a substantial link between physiological

capacity and the development of psychosocial capacities among students. Educators and parents can help students develop crucial psychosocial skills and traits by fostering physical fitness and supporting frequent exercise.

This study found a structural model of students' mathematics performance concerning physiological, psychological and psychosocial factors. The model depicts that students' mathematics performance is directly influenced by physiological factors (nutritional status, wellness, and well-being) and psychosocial abilities (math anxiety and math interest), but is inversely proportional to the psychological attributes of students (self-efficacy and attitudes towards mathematics). These findings suggest that a holistic approach to education reform is necessary—one that considers the physical, emotional, and psychological well-being of students as integral to mathematics success. Educational policies and practices should not only focus on the mathematics curriculum but also integrate support mechanisms that address the broader health and emotional needs of students. Schools could implement programs that integrate mental health services, nutritional counseling, and physical health activities into the regular mathematics curriculum, aimed at optimizing each student's potential for mathematics learning.

Overall, the structural model of this study highlights the multifaceted nature of factors affecting educational outcomes more particularly in mathematics and suggests that effective educational interventions need to be comprehensive, addressing more than just academic skills. This approach could help create a more supportive educational environment that promotes well-being and academic excellence in mathematics to boost the performance of secondary education students majoring in mathematics in their upcoming licensure examination for teachers.

The study considered only three latent variables, namely psychological (self-efficacy and attitudes towards mathematics), physiological (nutritional status and wellness and well-being), and psychosocial (math anxiety and math interest) factors. A study in the future is suggested to consider more latent and measured variables to make it more holistic and encompassing.

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ETHICAL CONSIDERATIONS

This study first secured ethical clearance from the Ethics Office of Davao Oriental State University before data collection. Respondents of the study are all of legal age because they are students of tertiary schools from selected institutions in Mindanao, Philippines. They were asked for consent to become respondents to the study. If anyone refused to become a respondent, they would not be given a survey questionnaire. The research questionnaire was given to them at their convenience, and they were also given the leverage of time to respond to it.

DECLARATION OF COMPETING INTEREST

The author declares no conflict of interest.

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Original Article

Survival and growth performance of sub-adult sandfish *Holothuria scabra* (Jaeger, 1833) in tanks with different supplemental feeds

Alexis G. Sumbe[1,2,*], Jean Beth S. Jontila[1,2], Precious G. Sevidal[1], Lorelie J. Bongabong[1], Ronalyn G. Ramirez[1], and Lota A. Creencia[1]

[1]*College of Fisheries and Natural Sciences, Western Philippines University, Puerto Princesa City, Philippines*
[2]*Capacity Development on Futures Thinking and Strategic Foresight Project, College of Fisheries and Natural Sciences, Western Philippines University, Puerto Princesa City, Philippines*

[*]*Corresponding Author: alexisgervaciosumbe@gmail.com*

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ABSTRACT

Sandfish (*Holothuria scabra* Jaeger, 1833*)* is one of the most valuable sea cucumbers in local and international markets. Although listed as an endangered species, it is among the regularly harvested marine resources in Palawan, Philippines. To continue the trade of sandfish without harvesting from the wild, this study initially ventured into producing seed stocks. However, broodstocks were scarce, only sub-adults were available, and they needed to grow into broodstocks. This study evaluated the survival and growth performance of sub-adult sandfish when fed with soya bean powder (Treatment 1/T_1), rice bran (Treatment 2/T_2), and powdered seagrass leaves (Treatment 3/T_3). Each treatment and control (no supplemental feeding) had three replicates with three sandfish having 59.55 ± 10.24 g mean weight and 9.74 ± 0.98 cm mean length. The samples were cultured in aerated plastic tanks (63.5 cm × 45.72 cm × 35.56 cm) with a 10 cm layer of sandy-muddy sediment filled with 40 L seawater, which was changed twice a day. Results showed 100% survival in T_3 and control, 66% in T_1, and 0% in T_2. The mean weight and length were stable in the first two weeks of culture in all treatments and control, but the latter declined during the 3rd and 4th weeks of culture ($P < 0.05$). Such was attributed to the drop in salinity during the 3rd week. Nevertheless, seagrass powder and soya beans appeared to help maintain the growth and tolerance to stress of sub-adult sandfish while unprocessed rice bran was detrimental to the health of sub-adult sandfish and caused mortality before the end of the culture period.

Keywords: juvenile, mariculture, rice bran, seagrass, soya

INTRODUCTION

Sandfish *Holothuria scabra* (Jaeger, 1833) is considered one of the most valuable sea cucumber species that are processed and dried into “trepang” or “bêche-de-mer”, and used as a main ingredient in Chinese cuisine that commands a high price in local and international markets (Akamine 2002; Brown et al. 2010; Purcell 2010; Jontila 2023). The value of a premium dried sandfish in Palawan, Philippines has steadily increased over the years, from PHP 324.00 kg^{-1} (USD 8.10) (Schoppe 2000) to PHP

6,000.00 kg^{-1} (USD 113.2) (Jontila et al. 2018). The average market price of dried sandfish in Chinese stores in Hong Kong and Guangzhou was USD 369 kg^{-1} and USD 153 kg^{-1} in 2016, respectively (Purcell et al. 2018). Due to this demand, sandfish has been heavily harvested in many areas across its distribution range. In Palawan, Philippines, it is freely collected and traded despite being IUCN-listed as an endangered species (Hamel et al. 2013; Dolorosa et al. 2017). Such has resulted in overharvesting and even depletion of its population in some near-shore areas (Jontila et al. 2018). Overharvesting has already placed the country as a hot spot area for sea cucumbers in Southeast Asia (Choo 2008). With the scarcity of sea cucumbers in near-shore areas, fishers are now venturing to farther sites to gather prime-sized and valuable sea cucumbers, particularly sandfish. In the absence of enforcement, sandfish and sea cucumber fishery will not be sustainable and will likely lead to population depletion in the long run, which would impact the livelihood of coastal communities since sea cucumber gathering is one of their substantial sources of income (Jontila et al. 2018). It would also affect the country's economy because sea cucumber is among its top 10 marine export commodities in terms of value (DA-BFAR 2021).

Sandfish culture is a way to increase the supply to capture the benefits of the high prices and market demand for sea cucumber products (Juinio-Meñez et al. 2016). Given the huge market for sandfish, full-cycle production of this species has been developed but production in the Philippines has not yet scaled up to commercial level, mainly because rearing of sandfish in the hatchery until maturity is not economically viable. To shorten the rearing time in a hatchery, juvenile sandfish can be grown in ocean nurseries using low-cost technology (Juinio-Meñez et al. 2012). In an attempt to demonstrate this, the team sourced out broodstocks from fishers for seed stock production. However, there were not enough broodstocks coming from the same area, which is important in maintaining genetic diversity. The fishers' catch were mainly late juveniles or sub-adults with very few adults. With such available resources, the team thought of growing the sub-adults and seeing if supplemental feeding would improve their growth performance while acclimating them in tanks before rearing them in their natural habitat (Dumalan et al. 2019). The addition of feeds to improve the growth and condition of sandfish in tanks yielded positive outcomes for broodstocks (Agudo 2006) but not for the sub-adults. Also, most of the studies in the Philippines were on diversity, abundance, and population (Schoppe 2000; Olavides et al. 2010; Dolorosa and Jontila 2012; Cabansag and Romero 2014; Jontila et al. 2014; Dolorosa 2015; Jontila et al. 2018) including substrate preference, feeding, and growth both in tanks and natural habitat of sandfish (Juinio-Meñez et al. 2014; Altamirano et al. 2017; Altamirano and Baylon 2020). Considering the different preferences of sandfish at each stage, this study compared the survival and growth of sub-adult sandfish in tanks with natural sediment supplemented with soya bean powder, rice bran, and powdered seagrass leaves and compared with the control (no supplemental feeding). Specifically, this study monitored the weekly survival, length (cm) and weight (g) of sub-adult sandfish. Information on this would be useful in improving the culture techniques for sandfish.

METHODS

Sub-adult Sandfish

The study used sub-adult sandfish bought from fishers of Sitio Silangan, Barangay Tagburos, Puerto Princesa City. A total of 36 sub-adults with a mean weight of 59.55 ± 10.24 g and a mean length of 9.74 ± 0.98 cm was used. The culture was conducted in the Inland Sea Ranching Station (ISRS) of the Department of Agriculture - Bureau of Fisheries and Aquatic Resources (DA-BFAR) situated in Barangay Sta. Lucia, Puerto Princesa City from 06 April 2022 to 12 May 2022.

Supplemental Foods

Three types of supplemental foods were prepared: soya bean powder, rice bran, and pulverized seagrass (*Enhalus acoroides)* leaves. The soya bean was used as a food supplement in sandfish broodstocks (Battaglene 1999) while the rice bran and seagrass powder were used as feed components of sandfish (Agudo 2006). The soya beans were toasted over a medium heat pan, cooled down, and grounded until powdery. The rice bran was purchased from the local market and was no longer processed. As for the seagrass, the leaves were washed, cleaned, cut into small pieces, and roasted in a frying pan until they became dry. It was then pounded until a powdery texture was attained.

Experimental Setup

This study used 40-L plastic crates (63.5 cm × 45.72 cm × 35.56 cm) filled with around 10 cm layer of natural sandy-muddy sediment taken from the natural habitat of sandfish. Three treatments were prepared: soya bean powder (Treatment $1/T_1$), rice bran (Treatment $2/T_2$), and powdered seagrass leaves (Treatment $3/T_3$) including the control with no supplemental food and only sandy-muddy substrate. Treatments and control were aerated continuously for 24 hours and the water was changed twice a day, every seven o'clock in the morning and five o'clock in the afternoon to ensure the continuous supply of dissolved oxygen (DO) and eliminate unwanted chemicals from the waste of sandfish (Tuwo et al. 2019). The amount of food given ranged from 3.5 to 5.0 g based on the

body weight of sandfish (Agudo 2006). The supplemental foods were added every morning after changing the water by thoroughly mixing it with salt water in a dipper before pouring it into each tank. They were allowed to settle by turning off the aeration until the water became clear. Table 1 shows the initial mean weight (± sd) and length (± sd) of 36 sub-adult sandfish with a total stocking biomass of 238.19 g in weight and 38.94 cm in length with three replicates containing three individuals each.

Table 1. Initial mean weight (± sd) and length (± sd) of sub-adult sandfish. T- treatment.

T	Feedings	No. of stock per replicate	No. of stock per treatment	Mean ± sd initial weight (g)	Mean ± sd initial length (cm)	Duration of culture (d)
T_1	Soya bean powder	3	9	59.38 ± 5.76	10.5 ± 1.36	30
T_2	Rice bran	3	9	73.91 ± 6.52	10.61 ± 0.59	21
T_3	Powdered seagrass leaves	3	9	54.39 ±16.64	9.22 ± 1.35	30
Control	No supplemental feedings	3	9	50.51 ± 6.64	8.61 ± 0.75	30

Monitoring of Survival and Growth

The survival of sub-adult sandfish cultured in tanks with natural sediment was determined by counting the number of live individuals every week. As for growth, the weight (g) and length (cm) of each individual were recorded every week for 30 days using a digital weighing scale and transparent plastic ruler, respectively. To minimize the error in measuring the body size, the samples were taken out of the tank by hand and were given 30 seconds to relax and drain the excess water in their bodies before taking the measurements. At the end of the culture period, all sub-adult sandfish were measured for their final weight and length. Water parameters such as salinity (ppt), pH, and temperature (ºC) were also monitored daily at seven o'clock in the morning, noon, and five o'clock in the afternoon using a refractometer (Atago brand), colorimetric method, and mercury thermometer, respectively. Observations on the burrowing behavior of sandfish and the appearance of the natural sediment were also noted.

Data Analysis

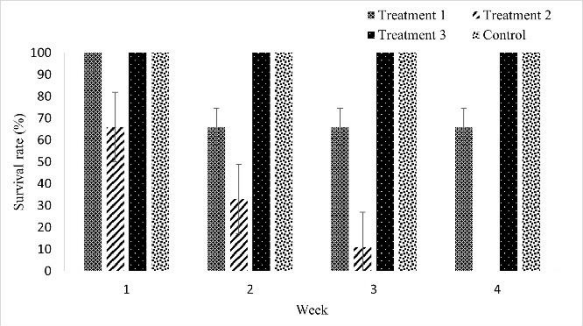
One-way analysis of variance (ANOVA) was used to test the differences in length and weight among the treatments at a 5% level of significance.

RESULTS

Survival

Among treatments, T_3 (powdered seagrass leaves) and the control (no supplemental food) had 100% survival after 30 days of culture (Figure 1). Treatment 1 (soya bean powder) had mortalities during the second week but retained a 66% survival for the rest of the culture period. Treatment 2 (rice bran) had higher mortality during the 2nd week with only 33% survival which eventually went down to 11% until all samples died at the end of the culture period.

Figure 1. Average percentage (± sd) of survival of sub-adult sandfish within a four-week culture period. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.



Growth Performance in Weight

Sandfish displayed a stable mean (± sd) body weight in the first two weeks of culture (Figure 2). Among the treatments, only individuals in T_3 (powdered seagrass leaves) displayed a slight increase in weight while T_2 (rice bran) slightly decreased, though both changes were not significant ($P > 0.05$).

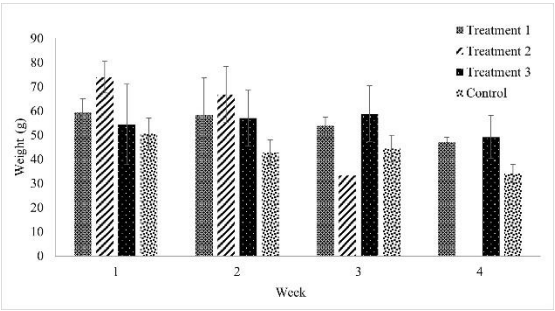


Figure 2. Mean (± sd) body weight of sub-adult sandfish within a four-week culture period. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

On the third week of culture, the weight of sandfish in all treatments remained almost the same, except for T_2 (rice bran), wherein the mean (± sd) weight decreased

significantly from 73.91 ± 6.52 g to a remaining individual weighing 33.27 g ($P < 0.05$). The final mean weight (± sd) of sandfish decreased in all treatments including the control (Table 2), but overall, the decrease was not significant ($P > 0.05$).

Growth Performance in Length

The mean length of sandfish in all treatments was almost the same in the first and second weeks of culture. It was in the third week that a significant decrease in mean (± sd) lengths of T_1 (soya bean powder) and T_2 (rice bran) was observed, from 10.5 ± 1.36 cm to 7.11 ± 6.16 cm and 10.61 ± 0.15 cm to a remaining individual measuring 12.5 cm, respectively (Figure 3). On the fourth week of culture, the length of sandfish in all treatments decreased significantly ($P < 0.05$). The final mean length (± sd) in all treatments including the control showed decrease as shown in Table 2.

Absolute Growth Rate (AGR)

After 30 days of culture, all samples displayed a negative absolute growth rate (AGR) (Figures 4 and 5). All treatments including the control almost had the same AGR (-0.16 cm to -0.36 cm), except for Treatment 2 and the control which had -0.17 g and -2.46 g in weight, respectively.

Water Parameters

The water pH throughout the culture period in all treatments and control was alkaline ranging from 7.8-8.2. The temperature ranged from 26°C to 30°C with the highest mean value at 30.5°C at five o'clock in the afternoon. As for salinity, the value ranged from 31 ppt to 33 ppt, except when there was a Low-Pressure Area (LPA) that brought heavy rain on the 17th day of culture, dropping the salinity in the reservoir to 27 ppt.

Table 2. Final mean weight (± sd) and length (± sd) of sub-adult sandfish.

Treatment	Feedings	Final no. of stock	Mean ± sd final weight (g)	Mean ± sd final length (cm)
T_1	Soya bean powder	7	47.15 ± 1.93	2.77 ± 2.42
T_2	Rice bran	0	-	-
T_3	Powdered seagrass leaves	9	49.17 ± 8.88	4.31 ± 1.49
Control	No supplemental feedings	9	34.1 ± 3.79	2.99 ± 1.81

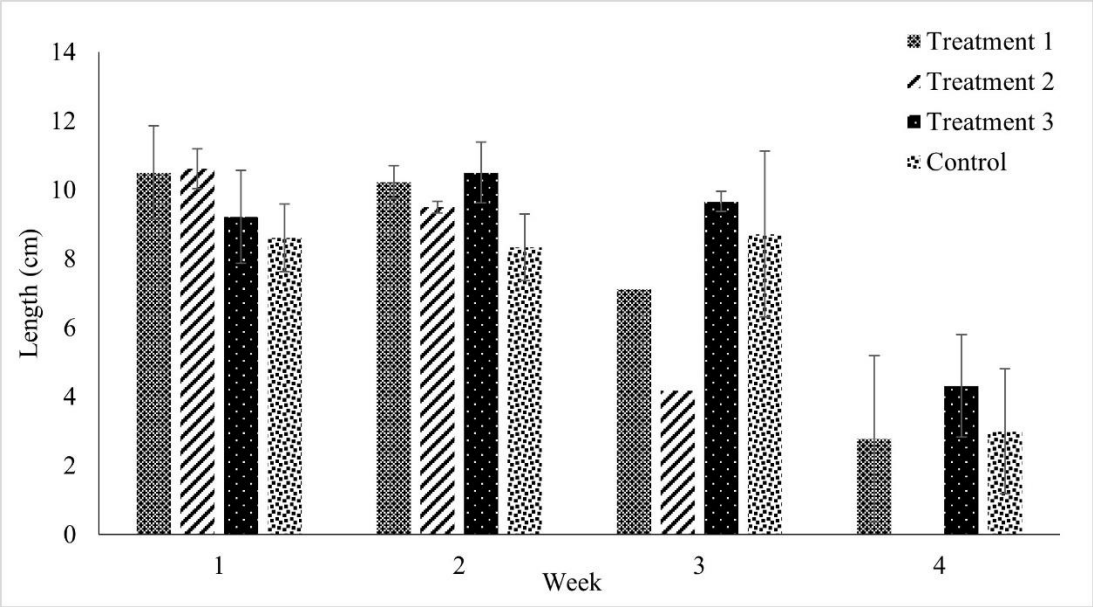


Figure 3. Mean (± sd) body length of sub-adult sandfish within a four-week culture period. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

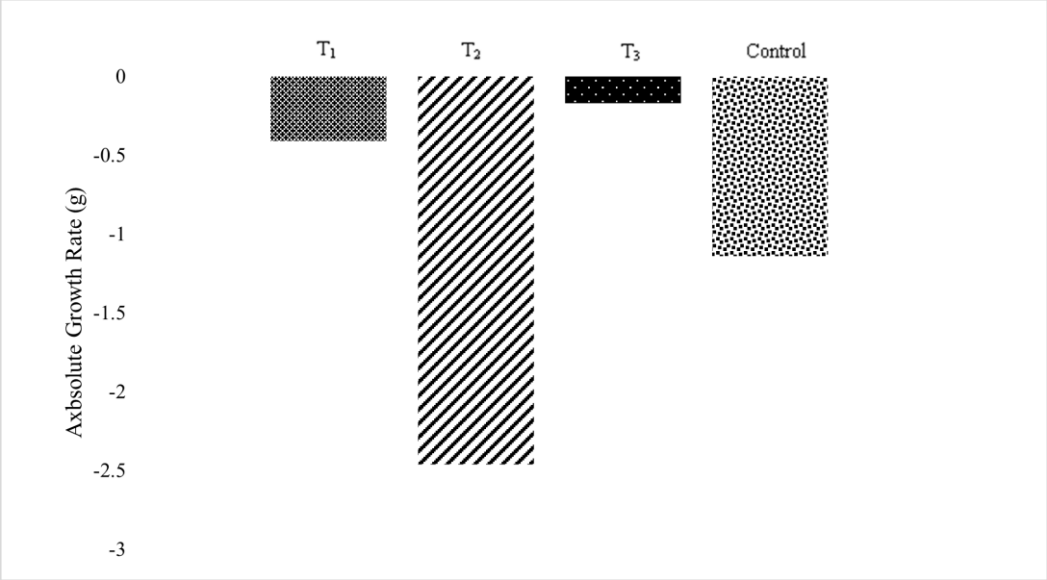


Figure 4. Estimated absolute growth rates (g) of sub-adult sandfish. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

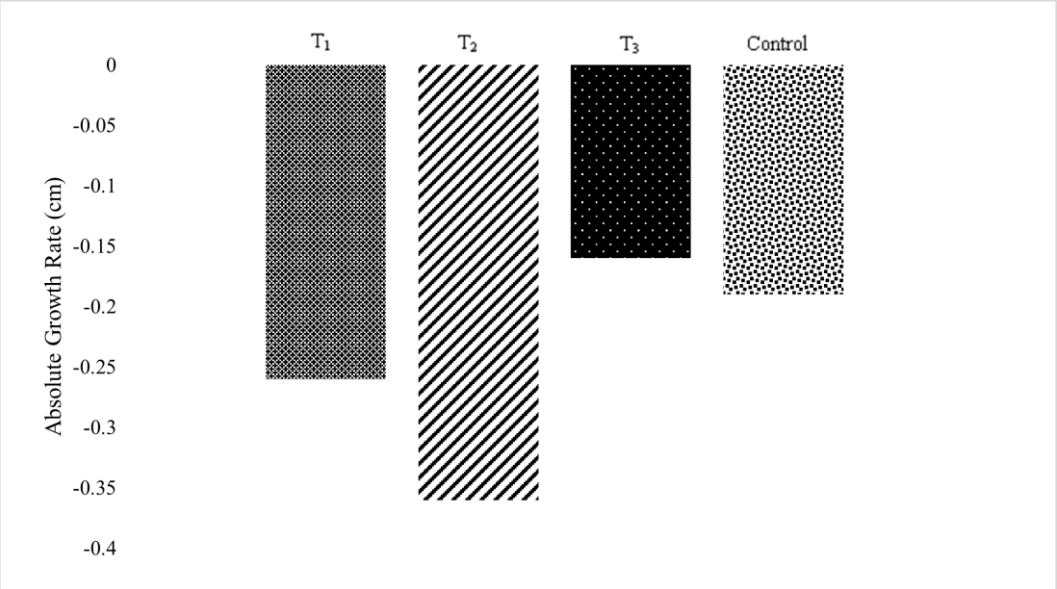


Figure 5. Estimated absolute growth rates (cm) of sub-adult sandfish. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

DISCUSSION

Survival

The survival of sandfish varied among treatments. The highest was noted in T_3 (powdered seagrass leaves) and control (no supplemental food), both with 100% survival after 30 days of culture. This was expected because aside from the natural composition of the substrate taken from the habitat of sandfish in the wild, the addition of powdered seagrass leaves as a supplemental feed in T_3 (powdered seagrass leaves) could have helped achieve good results in survival rate since it naturally occurs on the sandfish's environment and rich in organic matter. Sandfish are generally found in seagrass beds (Purcell et al. 2012, Floren et al. 2021) and seagrasses are known to enrich the organic material in sediments. Seagrasses were also used as an ingredient in feeds of broodstocks (Agudo 2006). Floren et al. (2021) also reported that seagrass contributed 32.0% of the organic matter in the sediment and as much as 70% of the sea cucumber diet was derived from seagrasses. Earlier studies have shown as well that sandfish prefer organically rich sediments (Mercier et al. 1999;

Altamirano and Baylon 2020). Additionally, the distribution of sandfish in the tropical Indo-Pacific region coincides with the distribution of the seagrasses particularly *E. acoroides* and *Thalassia hemprichii* (Short et al. 2007).

A lower survival rate was noted in T_1 (soya bean powder), which was supplemented with soya beans. All individuals in one of its replicates died after one week, yielding a 66% survival that was maintained until the end of the culture period. It is possible that the mortality in T_1 (soya bean powder) during the first week was caused by stress during transport and handling and not really due to the nature of the substrate or the addition of powdered soya beans since all the remaining individuals survived the 30-day culture period. Soya beans are organic in nature and were also added as a food component of sandfish broodstocks (Agudo 2006). However, its use must be studied further since pinkish coloration in the bottom of the tanks was observed during the third week of culture, which is an indication of bacterial build-up that is unfavorable and may cause disease and infection to sandfish.

In contrast, samples fed with rice bran (T_2) displayed a gradual decrease in survival until all were extirpated after 30 days of culture. This shows that unprocessed rice bran is not a suitable supplement for sandfish. As the rice bran was locally sourced and bought straight from the market, it did not undergo fermentation to help improve digestibility which may have also negatively affected the sub-adult sandfish. Nevertheless, rice bran can be used as one of the components of feeds for broodstocks of sandfish (Agudo 2006), but adding it directly to the substrate may have detrimental effects on sub-adult sandfish.

Growth Performance

The growth of sandfish across all treatments including the control (no supplemental food) had minimal variations and was almost stable after two weeks of culture, with a slight increase in the length of individuals in T_3 (powdered seagrass leaves). The same performance was observed during the 3rd week except for T_2 (rice bran), wherein individuals significantly decreased in weight and length from 47.68 g and 6.39 cm down to 11.09 g and 4.17 cm ($P < 0.05$), respectively. Individuals in T_1 (soya bean powder) also decreased in length ($P < 0.05$) but not in weight ($P > 0.05$). However, in the 4th week, there was a significant decrease in length in all treatments including the control (no supplemental food) ($P < 0.05$) with 100% mortality in T_2 (rice bran) ($P < 0.05$). Such could likely be attributed to the sudden drop in salinity (27 ppt) during the 3rd week when heavy rains due to LPA diluted the seawater in the reservoir. It has to be noted that sea cucumbers are stenohaline and osmoconformers with a low level of tolerance to salinity change (Sembiring et al. 2019), thus the abrupt salinity change of 3-5 units at 27 ppt is already detrimental to them. Agudo (2006) also reported that heavy rains resulted in mass mortality of sandfish cultured in ponds while strong typhoons resulted in negative growth increments of sandfish in pens (Juinio-Meñez et al. 2014). In addition, all samples in T_2 (rice bran) were observed eviscerating, which is a sign of stress due to fluctuating salinity but could also be a defense mechanism, to the unfavorable condition before their eventual death. The viscera were removed the moment they were noted, but they already contaminated the water, which could aggravate the condition and lead to mortality (Tuwo et al. 2019). Further, the increasing temperature towards the afternoon reaching up to 30.5°C could have also been stressful to sandfish (Mackey and Hentched 2001), but this does not extend for long as the water is changed constantly in the afternoon and morning.

Overall, supplementing the diet of sandfish with powdered seagrass leaves appeared to have positive results because, among the treatments, individuals supplemented in this treatment (T_3) showed a slight increase in growth after one week of culture and were able to withstand the harsh condition without significant weight change. The same is true for sandfish fed with soya beans, in which only the length decreased considerably but not the weight. Individuals in the control did not show any significant changes in length and weight, only after 3rd week when salinity dropped to 27 ppt. The mean weight and length of individuals decreased significantly at the end of the culture period. In addition, their weight was significantly lower than the individuals in T_1 and T_3, which were fed with soya beans and seagrass leaves, respectively.

It appeared that individuals fed with soya bean and powdered seagrass leaves were able to withstand the stress brought by reduced salinity and fluctuating temperature than those fed with rice bran and in the control. Therefore, the use of seagrass powder is recommended to enhance the growth performance of sub-adult sandfish. The soya beans can also be added but must be studied further as the suspected bacterial build-up in the sediment was noted in this study. Although reported as a feed component of sandfish (Agudo 2006), the use of unprocessed rice bran is not recommended due to potential traces of chemicals and digestibility issues, which were detrimental to sub-adult sandfish. This study also highlights the importance of maintaining the water quality (salinity, pH, and temperature) at the optimum level during the acclimation and culture of sandfish in tanks, even for a short period, as they could be more detrimental to sandfish than the quality of the sediment.

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ETHICAL CONSIDERATIONS

The sandfish used in this study were handled with utmost care particularly during transport to avoid stress and mortality. The surviving individuals after culture were not disposed of but were cultured in pens.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests.

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Quantitation of antioxidant levels of soy-fern fermentation by DPPH assay

Edward Laurence L. Opena[1,2,*], Matilda Cempron[2], Johndel Remon[2], Reggie Dela Cruz[2,3] and Junge Guillena[4]

[1]*Biology Department., College of Computing, Artificial Intelligence and Sciences, Cebu Normal University, Cebu, Philippines*
[2]*Institute of Biological Sciences, Central Mindanao University, Bukidnon, Philippines*
[3]*Tuklas Lunas Development Center, Central Mindanao University, Bukidnon, Philippines*
[4]*Adventist Medical Center Colleges, Iligan City, Lanao del Norte, Philippines*

[*]*Corresponding Author: eopena86@gmail.com*

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ABSTRACT

Fermentation biotechnology is one of the approaches to addressing the issues of food security worldwide, where the demand for healthier and safer foods is becoming mainstream. Even though fermentation has been practiced since ancient times, there are still an infinite number of topics that can serve as subjects for fermentative investigation. Among the popular nutraceutical research is the antioxidant properties of plants. In this study, the radical scavenging activities of soy-fern fermentation by mixed probiotics (13 species) have been quantified using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Four treatments were set up: soy-fern-probiotics, soy-fern, soy-probiotics, and soy. These treatments were fermented (submerged) for up to 72 h. Samples were taken at 0 h, 24 h, 48 h, and 72 h and were analyzed via a 96-well plate microplate photometer. Results showed that the production of antioxidants peaked at 24 h in soy-fern-probiotics and soy-fern (82.82% and 82.77%, respectively), suggesting that the presence of fern molecules could have affected the production of antioxidant molecules. It is also observed that the probiotics have less impact on the antioxidant levels. The analysis of variance (ANOVA) showed that there is a significant difference in this timeframe when compared to other timeframes. On average, the succession of antioxidant levels is as follows (highest to lowest): 24 h, 48 h, 0 h, 72 h. Overall, the level of antioxidants depends on the substrates, fermenting microorganisms, type of fermentation, and fermentation time. More studies on this matter are highly recommended.

Keywords: antioxidants, fiddlehead fern, soybean powder

INTRODUCTION

In recent years, there has been a shift in food research, where the focus has moved from being a source of energy to its role in the total well-being of an individual. This new focus is secondary to the growing interest of consumers in consumables that have something to do with the prolongation of life by preventing the development of chronic diseases such as cancer, obesity, and diabetes to name a few. Functional foods, or those foods that contain a nutritional value for maximal health impact when consumed regularly, are favored over their synthetic counterparts since the latter foods are traced to have certain degrees of negative health impacts (Cencic and Chingwaru 2010; Granato et al. 2010; Lobo et al. 2010; Gul et al. 2016). With this steady interest in functional foods, the agricultural sector has also seen

an increase in its contribution to the economy by supplying and processing raw materials (Bigliardi and Galati 2013), as well as in healthcare, as it is expected to reduce the number of hospitalizations in the coming years (El Sohaimy 2012).

Amid the desire for more efficient production of functional foods, fermentation biotechnology is arguably at the forefront. According to Zhu and Tramper (2013), the innovations in food biotechnology that can be seen today, particularly fermentation, have their roots in traditional food processing techniques; this means that the majority of the foods under investigation was already known since ancient times, only that there have been modifications to how they are prepared to include the use of new additives. The process of fermentation involves the utilization of microorganisms, specifically bacteria, and yeasts, that can improve the quality and safety of the food product, particularly its nutritional values, flavor, aroma, shelf life, and texture, and at the same time decrease the levels of anti-nutrition compounds (Hugenholtz 2013; Mukherjee et al. 2016; Leonard et al. 2021). Due to the realization that fermented products hold potential molecules that can address nutritional concerns, they are now considered more than just a side dish (Shin and Jeong 2015).

One of the foods that have been traditionally fermented is *Glycine max*, commonly known as soy and is known to contain one of the highest level of proteins that benefit both humans and livestock. The crop is known to have originated in East Asia and has been fermented in different ways, thus producing different products. Soymilk, soy sauce, natto, and tempeh are just a few examples of fermented soy products that are now produced worldwide (Cao et al. 2019). According to Jayachandran and Xu (2019), fermented soy unlocks more nutrition than its non-fermented counterparts. This means that microorganisms were able to maximize the transformation of soy components into more molecules that can have a positive nutritional impact, particularly its bioactive peptides, through the hydrolysis of proteins from soybeans (Sanjukta and Rai 2016).

Ferns, on the other hand, are also the subject of fermentation studies. Products from fermented ferns show nutraceutical potential besides having insecticidal and fungicidal properties that help improve livestock and human gut health (Tamang et al. 2016; Mala et al. 2019).

The fern *Diplazium esculentum* Retz. is a common fern found in regions across Asia that have long been one of the food sources of different societies and cultures. Besides its role as an energy source, it is also known to have both pharmaceutical and nutraceutical potentials. It is considered to contain molecules that are antidiabetic, antioxidant, and proteins, can prolong shelf life, and can improve the sensory acceptability of certain food products (Saha and Deka 2017; Junejo et al. 2018; Samad et al. 2022). Thus, the addition of fern to already known food and its respective processes can enhance the overall quality of food in terms of its nutritive value.

As mentioned earlier, fermentation is primarily made possible through the complex metabolic interactions of microorganisms. Yeasts or bacteria act on biomolecules and transform them into other molecules that can enhance the components of a fermented product. Probiotics, a specific group of bacteria that improve human gut health, have already been utilized for fermentative production. For instance, *Lactobacillus plantarum*, a known probiotic, was employed to ferment soy in a study by Xiao et al. (2015). The study concluded that *L. plantarum* was able to elevate the antioxidant potential of soybean.

As the interests in newer non-synthetic functional foods continue to elevate, novel approaches must be utilized both in research and biotechnological designs. Devanthi and Gkatzionis (2019) emphasized that there should be continuous investigations into the fermentative processes that can maximize the nutritive potential of plants, including the search for new approaches to identifying starter cultures (pure and mixed) and the addition of other raw materials. Currently, the fermentation of soy and fern powders by mixed probiotics, particularly in powdered form, is not well reported. This paper therefore seeks to quantify the product of the fermentation of soy and fern powders through known probiotics in terms of their antioxidant properties via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay approach, which is currently one of the most reliable strategies in antioxidant property analysis.

METHODS

Fern Powder Preparation

The protocol used in fern powder preparation follows the work of Ang et al. (2022). Approximately 3 kg of the fern *D. esculentum* were collected from the fernery of Central Mindanao University, Maramag, Bukidnon. The ferns were obtained using pruning scissors and placed inside a plastic container. These samples were then brought to the laboratory of Tuklas Lunas Development Center of the university.

The samples were immediately washed with running tap water twice, and then rinsed with distilled water twice. Water from the samples was then drained for 10 minutes, and then weighed. After weighing, the samples were uniformly distributed inside the laboratory's air dryer and air-dried for 96 h. The samples were then placed in the oven and subjected to a temperature of 50 ºC for 4 h. Before the samples were removed from the oven for milling, they were tested for moisture content and found to have a reading

of less than 10% moisture content. The finely milled fern powder was stored in a clean plastic container.

Soy Powder Preparation

Freshly milled soybean powder (*Glycine max* L.) was purchased from a local grain store in a nearby farmer's market. The soybean powder was then stored in a plastic container and brought to the laboratory.

Probiotics Dilution

Probiotics packed from Atomy Probiotics 10+ served as the fermenting agent for this study. Each pack has 12 known probiotics which include *Bifidobacterium* spp. (*B. breve, B. bifidum, B. longum, B. lactis)*, *Lactobacillus* spp. (*L. rhamnosus, L. casei, L. plantarum, L. helveticus, L. acidophilus, L. paracasei, L. fermentum*), and *Streptococcus thermophiles.* One pack, which contains approximately 3 billion bacterial cells, was dissolved in 999 mL of ultra-pure water, making a dilution of approximately 3 million cells per ml.

Fermentation

Submerged, anaerobic fermentation was employed in this study. Here, four treatments with three replicates per treatment were set up with the following compositions:

Treatment A: Soybean with fern

A_1 (with probiotics): 1 g soybean powder + 1 g fern powder + 1 ml probiotics solution + 37 ml sterile distilled water

A_2 (without probiotics): 1 g soybean powder + 1 g fern powder + 38 ml sterile distilled water

Treatment B: Soy without fern

B_1 (with probiotics): 1 g soybean powder + 1 ml probiotics solution + 38 ml sterile distilled water

B_2 (without probiotics): 1 g soybean powder + 39 ml sterile distilled water

Each treatment was placed in 50 ml conical tube and sealed with a tube cap for submerged fermentation. The samples were centrifuged for 80 s at 1860 rpm, and then incubated at 37 ºC. One milliliter of each sample was taken and stored in a 2.0 ml microfuge tube for DPPH assay at the following time frames: 0 hours, 24 hours, 48 hours, and 72 hours. Seventy-two hours of fermentation is considered by Oyewole et al. (2001) to be one of the peaks of certain fermentation processes.

DPPH Assay

To quantify the antioxidant content of the fermented materials, the DPPH assay was employed (Porquis et al. 2018). The DPPH radical scavenging percentage was obtained following the formula by Shah and Modi (2015), where A_0 is the scavenging value of the ascorbic acid as a positive control (PC), and A_S is the value of the extracts:

$$\%DPPH\ radical\ scavenging\ activity = [\frac{A0 - AS}{A0})]\ x\ 100$$

A_S was generated using the following formula:

$$AS = (APC - ASB)$$

Where: $APC = PC + extract$
and $ASB = solvent + extract$

The solvent used was 95% ethanol, and the values for radical scavenging were generated in a 96-plate Thermo Scientific Multiskan GO version 100.40.

The schematic diagram of the experimental set-up is shown in Figure 1.

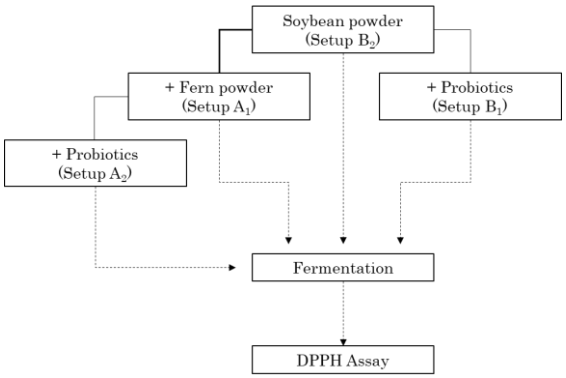

Figure 1. Flow of the experimental approach for setting up the fermentation process of the different treatments (soy-fern-prebiotics (A2), soy-fern (A1), soy-probiotics (B1), soy (B2)).

Statistical Analysis

The analysis of variance (ANOVA) was employed to determine the degree of variability of the radical scavenging properties among different treatments as well as across different timeframes. The degree of variability was also determined in each of the treatments to timeframes. A pairwise comparison was further employed for treatments that showed statistical significance using either $P < 0.05$ or $P < 0.001$. This comparison was used to further compare different timeframes. For data visualization, the program Python was used.

RESULTS

Antioxidant Levels Across Different Treatments on Different Timeframes

Figure 2 shows the pattern of the radical scavenging activities of different treatments across different timeframes based on the DPPH assay. Here, it shows that soy-fern-probiotics and soy-fern treatments have similar patterns where, at 24 h, the

antioxidant properties are at their highest, ranging from 82.82% to 82.77% (soy-fern-probiotics and soy-fern, respectively). From these peaks, both treatments tend to have reduced their antioxidant properties (72.37–37.43%for soy-fern probiotics, and 73.16–48.99%for soy-fern). Furthermore, for these two treatments, it is noteworthy that 0 h has higher antioxidant levels compared to the levels after 72 h of fermentation.

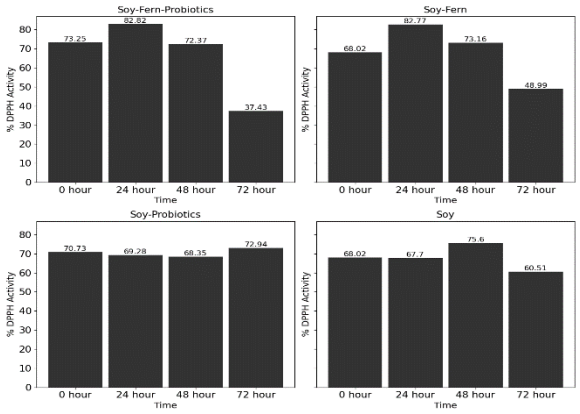

Figure 2. Comparative radical scavenging activities of different treatments across 4 fermentation timelines between the four treatments (soy-fern-prebiotics, soy-fern, soy-probiotics, soy) (in percentage).

Unlike the previous two treatments, soy-probiotics and soy seem to have established patterns in terms of the levels of antioxidant production across the timeframes. For soy-probiotics, the lowest is at 48 h at 68.35% and the highest in 72 h at 72.94%. Meanwhile, in soy, the lowest antioxidant level is during 72 h with 60.51%, and the highest is during 48 h with 75.6%. It can be noted that soy-probiotics and soy display higher antioxidant levels at 72 h compared to the previous two treatments.

Two points can be of interest in this result. First, the presence of ferns in the first two treatments can potentially change in the patterns of the levels of antioxidants in the timeframes, as well as their higher radical scavenging activities compared to treatments without ferns. Secondly, the levels of antioxidants in soy-probiotics, and soy seem to be steady within the timeframe, especially in the first three timeframes. This could mean that if more timeframes are extended (>72 h), more distinct patterns of antioxidant levels could be established. But even with the absence of the aforementioned patterns, the antioxidant levels of soy-probiotics and soy are high, especially going up to 60%.

The heatmap of the average antioxidant levels per replicate is shown in Figure 3. Here, it shows that the highest radical scavenging activities lie inside the 24-h timeframe and are seen to be highest within the replicates of soy-fern-probiotics (SFP) and soy-fern (SF). In contrast, the lowest radical scavenging activities can also be found in these treatments after 72 h of the fermentative process.

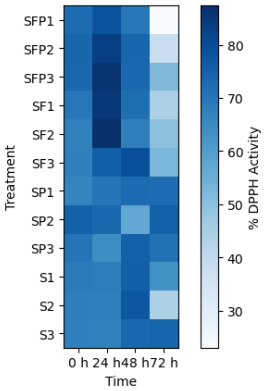

Figure 3. Heatmap which shows the %DPPH radical scavenging activity per replicate in each treatment over the experimental timeframe. Here, 24 h fermentation of soy-fern-probiotics and soy-fern shows the highest antioxidant properties.

To further visualize the data, a contour plot was generated, this time using the average antioxidant levels of each treatment, as shown in Figure 4. This contour plot validates the previous data, as it shows that the highest antioxidant levels are concentrated between the treatments soy-fern probiotics and soy-fern. Furthermore, the figure also reveals that it is in the treatment soy-fern that has the highest radical scavenging activity within the 24-h range.

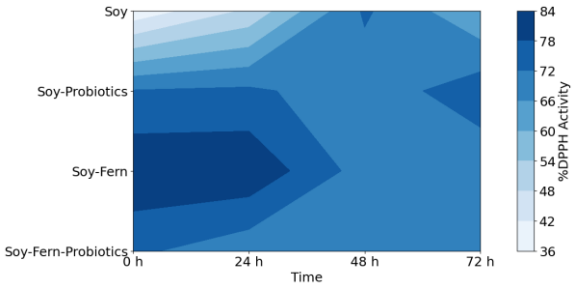

Figure 4. Contour plot showing that soy-fern and soy-fern-probiotics having the highest peaks of radical scavenging activities.

Figure 5 shows the average radical scavenging levels using the DPPH assay of all treatments across different timelines. Here, it shows that on average, the antioxidant level is highest after 24 h of fermentation, followed by 48 h, 0 h, and 72 h. As seen in the graph, the initial antioxidant level (0 h) is higher compared to the level after 72 h of fermentation. It can be inferred that after 24 h, the microorganisms' capacity and efficiency to ferment and release radical-scavenging molecules have diminished over time. Furthermore, it can also imply that for soy-fern fermentation, if there should be any attempts, the isolation of antioxidants should be conducted between 24-48 h.

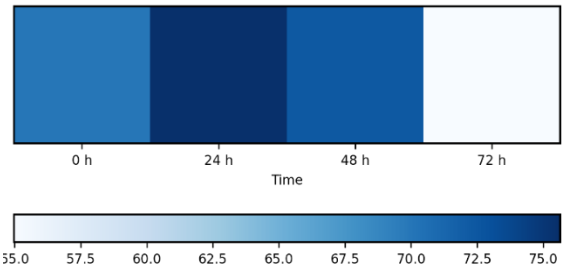

Figure 5. Heatmap showing the average antioxidant levels (in %) of all treatments (soy-fern-prebiotics, soy-fern, soy-probiotics, soy) in different timeframes. Here, 24 h fermentation shows the highest antioxidants produced.

Different Timeframes and Treatments Show Statistical Differences

Statistical analysis for the difference in radical-scavenging activities between different treatments across different timeframes is shown in Table 1. In the 0 h and 48 h timeframes, there is no significant difference in antioxidant rates between the different treatments. This is in contrast to the 24 h and 72 h timeframes, where a significant difference has been observed with *P*-value=0.004 and 0.19 respectively (for $P<0.01$ and $P<0.05$, respectively). These results validate the values reflected in Figure 2.

The Duncan test for pairwise comparison in the 24 h timeframe reveals that soy-fern probiotics and soy-fern treatments are statistically different from soy-probiotics and soy treatments. This means that the antioxidant levels of soy-fern-probiotics and soy-fern are significantly higher compared to soy-probiotics and soy. This statistical difference suggests that the presence of fern may contribute to this difference through its distinct molecules that are absent in soy alone.

For the 72-h timeframe, the Duncan test for pairwise comparison shows varied statistical significance. For instance, soy-fern-probiotics are significant against soy-probiotics and soy. Soy-fern is significant against soy-probiotics and soy. On the other hand, soy-probiotics are significant against soy-fern-probiotics and soy-fern. Lastly, soy is significantly different from soy-fern-probiotics. The varying metabolites that are formed in different treatments at this timeframe could be the potential reason for this varying significance.

Furthermore, since there is no statistical difference between treatments with and without probiotics across different timeframes, it can be concluded that the contribution of probiotics to the radical-scavenging activities is minimal. Therefore, it can be hypothesized that the resident microbial species in fern and soy powders have a significant contribution to the antioxidant properties of the study.

The within-subjects test on the treatment soy-fern probiotics shows a statistically significant *P*-value of 0.000, indicating a difference in antioxidant rates at different time points (Table 2). Due to this significance, the data was subjected to a pairwise comparison which showed in Table 3 that only the 0 h timeframe is not statistically significant when compared to 48 h (*P*-value = 0.435). This lack of significance suggests that both timeframes have similar antioxidant rates.

Table 1. Descriptive and ANOVA statistics result for radical scavenging activities between different treatments across different timeframes (Note: $^{**}P < 0.01$, $^{*}P < 0.05$, ^{ab}Duncan test for pairwise comparison).

Time (h)	Treatment	Mean (antioxidant%)	SD	n	F-value	*P*-value
0	Soy-Fern-Probiotics	73.25	0.85	3	3.233	0.082
	Soy-Fern	68.02	1.44	3		
	Soy-Probiotics	70.73	4.50	3		
	Soy	68.02	0.59	3		
	Total	70.01	3.07	12		
24	Soy-Fern-Probiotics	82.82^{b}	3.98	3	10.668**	0.004
	Soy-Fern	82.77^{b}	6.22	3		
	Soy-Probiotics	69.28^{a}	4.74	3		
	Soy	67.70^{a}	0.45	3		
	Total	75.64	8.37	12		
48	Soy-Fern-Probiotics	72.37	2.37	3	0.756	0.549
	Soy-Fern	73.16	6.11	3		
	Soy-Probiotics	68.35	9.80	3		
	Soy	75.60	2.16	3		
	Total	72.37	5.79	12		
72	Soy-Fern-Probiotics	37.43^{a}	14.40	3	6.075*	0.019
	Soy-Fern	48.99^{ab}	4.19	3		
	Soy-Probiotics	72.94^{c}	2.11	3		
	Soy	60.51^{bc}	15.15	3		
	Total	54.97	16.54	12		

As mentioned earlier, the 24-h timeframe appears to be when antioxidant rates peak due to the release of metabolites as antioxidant molecules. After this time, a decrease in these antioxidant activities is observed until the 72-h timeframe.

A within-subject analysis on soy-fern also showed that radical-scavenging activities are significantly different across different timeframes with a *P*-value of 0.002 (significant at $P < 0.01$) (Table 4). A further analysis employing pairwise comparisons showed that 0 h against 72 h, 24 h against 72 h, and 48 h against 72 h are all significant with *P*-values of 0.027, 0.025, and 0.017, respectively, based on $P < 0.05$ confidence (Table 5). This means that the radical-scavenging rate at the 72-h timeframe has a broader difference compared to other timeframes. Since antioxidant rates are showing a downward trend, this could mean that the antioxidant activity at this timeframe is now significantly lower compared to the other timeframes. Based on the means, the highest radical scavenging activity falls at the 24 h timeframe (82.77%), and the lowest is at 72 h (48.99%).

For the treatments soy-probiotics and soy, there has been no significant difference when antioxidant activities are analyzed in these respective treatments (Tables 6 and 7). And since no statistical difference is detected in these treatments, a pairwise comparison is no longer needed. Within these treatments, levels of antioxidant activities are similar across timeframes; but even then, based on the means of these treatments, radical scavenging activities are considerable, with the least being at least 60.51% (soy, 72 h) and the highest being 75.60% (soy, 48 h).

Table 2. Tests of within-subjects effects of soy-fern-probiotics across time (Note: ***$P < 0.001$).

Treatment	Time	Mean (antioxidant %)	SD	n	F-value	P-value
Soy-Fern-Probiotics	0 h	73.25	0.85	3		
	24 h	82.82	3.98	3		
	48 h	72.37	2.37	3	30.137***	0.000
	72 h	37.43	14.40	3		

Table 3. Pairwise comparison of DPPH activity across time (Soy-Fern-Probiotics) (Note: *$P < 0.05$, [a]Adjustment for multiple comparison: Least Significant Difference).

Comparison	Mean Difference	P-value[a]
0 h vs. 24 h	-9.570*	0.037
0 h vs. 48 h	0.877	0.425
0 h vs. 72 h	35.817*	0.046
24 h vs. 48 h	10.447*	0.013
24 h vs. 72 h	45.387*	0.017
48 h vs. 72 h	34.940*	0.040

Table 4. Tests of within-subjects effects of Soy-Fern across time. (Note: **$P < 0.01$).

Treatment	Time	Mean (antioxidant %)	SD	n	F-value	P-value
Soy-Fern	0 h	68.02	1.44	3		
	24 h	82.77	6.22	3		
	48 h	73.16	6.11	3	19.257**	0.002
	72 h	48.99	4.19	3		

Table 5. Pairwise comparison of DPPH activity across time (Soy-Fern) (Note: *$P < 0.05$, [a]Adjustment for multiple comparison: Least Significant Difference).

Comparison	Mean Difference	P-value[a]
0 h vs. 24 h	-14.747	0.052
0 h vs. 48 h	-5.143	0.296
0 h vs. 72 h	19.025*	0.027
24 h vs. 48 h	9.603	0.308
24 h vs. 72 h	33.772*	0.025
48 h vs. 72 h	24.169*	0.017

Table 6. Tests of within-subjects effects of Soy-Probiotics across time (Note: $P > 0.05$-not significant).

Treatment	Time	Mean (antioxidant %)	SD	n	F-value	P-value
Soy-Fern	0 h	70.73	4.50	3		
	24 h	69.28	4.74	3		
	48 h	68.35	9.80	3	0.251	0.858
	72 h	72.94	2.11	3		

Table 7. Tests of within-subjects effects of Soy across time (Note: $P > 0.05$-not significant).

Treatment	Time	Mean (antioxidant %)	SD	n	F-value	P-value
Soy	0 h	68.02	0.59	3		
	24 h	67.70	0.45	3		
	48 h	75.60	2.16	3	1.759	0.254
	72 h	60.51	15.15	3		

DISCUSSION

The peak of antioxidant properties as shown in Figure 2 at the 24-h timeframe, can be attributed to the capacity of the microorganisms that act on the different substrates. Basically, at this stage, microbial growth enters the stationary phase, where most of the metabolites have already been produced. As fermentation progresses, theoretically, there will be an increase in the population of the microbial species that break down complex molecules via enzymatic reactions. This can result in the consumption of more substrates. As the quantity of substrates decreases, more cells are deprived of nutrients, which prevent further cellular division. This then follows a continuous downward spiral of population decline, coupled with a decline in substrate. Since various probiotics species were initially added at the start of the procedure, it is theoretically possible that, along the timeline, some of these species are already in their death phase. This could be due to either the possible formation and accumulation of toxins as metabolic by-products from the substrates (Doekes et al. 2019), the activation of the antagonistic behavior of different species included in the probiotics additive, or from the resident microbial species from the substrates (soy and fern). Wang et al. (2021) reported in their study that among eight species of lactic acid bacteria, only one strain persisted throughout the fermentation process. They also reported that a plateau of the microbial growth curve was recorded after 20 h. Similar studies on the fermentation peak, as well as the reduction of the number of bacterial species and strains over time, have also been reported (Magala et al. 2015; Pereira et al. 2016). Such results seem to be consistent with what was observed in this study, particularly in the timeframe where antioxidant levels are at their highest (24 h), and might suggest that other probiotics that were initially added were gradually terminated in the progress of the fermentation process.

The peaks at the 24-h timeframe for soy-fern-probiotics and soy-fern are suspected to be primarily due to the fern. It may be that the release of fern antioxidant compounds, or it could also be a result of the synergistic reactions between soy, fern, and the acting microorganisms. Zannah et al. (2017) identified that alkaloids, terpenoids, flavonoids, polyphenols, and saponins are found in the aqueous extracts of *D. esculentum*.

On the other hand, the treatments soy-probiotics and soy showed different patterns of antioxidant levels. As observed, only at 72 h was a significant movement of the antioxidant levels observed. It can be inferred, then, that the reaction time of soy alone (without regard to the probiotics added) is much different compared to the substrates that have ferns. In this case, there are two possible reasons for such behavior. First, Xue et al. (2016) identified that bioactive compounds could reach their maximum availability after 120 h (compared to this study at 72 h). This implies that there could potentially, be increase in the levels of radical scavenging activity if the timeframe is increased. Second, specific antioxidant molecules are already available when soy is dissolved in water, and through the fermentation process, such molecules may have served as the primary substrates of the fermenting microorganisms. This could then account for the decline of specific molecules, as reflected in Figure 2.

Barus et al. (2019) identified that the process of fermentation, the types of microorganisms, and the length of the fermentative process directly affect the antioxidant activities of substrates by employing the DPPH assay. Here they demonstrated that soybeans that underwent fermentation have higher antioxidant activities compared to unfermented soybeans.

Furthermore, the varying antioxidant levels were also recorded when using different microorganisms, specifically *Rhizopus*, *Bacillus*, and *Klebsiella* species.

In a similar study, Cui et al. (2020) also identified the crucial role of selecting microbial species that will ferment substrates, either in a single culture or mixed culture. They stipulated that such microbial strains can vary the outcome of fermentation, specifically in the formation of bioactive compounds like those that are categorized as antioxidants. Based on this study, it is evident that synthesis of antioxidants is the result of the interplay of different substrates (in this case, soy and fern), the length of fermentation, and the types of fermenting microorganisms. Such results agree with the principles of fermentation biotechnology.

In conclusion, fermentation biotechnology is indeed a work in progress, where several methods must be tested to optimize the production of molecules that are of pharmaceutical, nutraceutical, and agricultural significance. In this case, the production of compounds that are capable of scavenging free radicals that can affect cellular health. Hence, fermentation biotechnology should involve various innovative approaches to keep up with the demand of a growing society for healthier, safer, and more affordable food products and derivatives.

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ETHICAL CONSIDERATIONS

This research ethically observed all pertinent methods in meeting the objectives of the study.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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ROLE OF AUTHORS: *ELLO – led the conduct of the experiment as well as on the analysis and interpretation of data; MC- assisted in the collection of samples and experimental setup; JMR – assisted in the collection of samples and experimental setup; RDC – refined the methodology and over-all content of the paper, and allowed the conduct of the experiment in her laboratory; JG – statistical analysis and interpretation of data.*

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Evacuation behavior of 2020 Taal volcano eruption-affected households in Barangay Leynes, Talisay, Batangas, Philippines

Ma. Bernadeth B. Lim[1,*] and Joy Mae L. Anabo[2]

[1,2]*Department of Civil Engineering, College of Engineering and Agro-Industrial Technology, University of the Philippines Los Baños, Laguna, Philippines*

[*]*Corresponding Author: mblim4@up.edu.ph*

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ABSTRACT

The Philippines, which is located in the western Pacific region experiences frequent and destructive disasters. Evacuation is one of the measures to reduce the impacts of disasters. Understanding evacuation behavior and incorporating this into a comprehensive evacuation plan is still needed. This study aimed to understand the evacuation behavior of households from the area at high risk of the impacts of a volcanic eruption. The type of evacuation decision, mode, and accommodation type choice behavior were assessed, and models were developed for each of these evacuation-related behaviors. Discrete choice models were used to identify significant factors to evacuation behavior using actual evacuation data collected from households in Barangay Leynes, Talisay, Batangas, Philippines. Results showed that the calculated *pseudo-*R^2 for the three evacuation-related models were in the range of 0.10-0.33, indicating an acceptable level of data fit in respective models. Additionally, the calculated area under the curve (AUC) for the three models range from 0.72 to 0.85 which means that the models' level of discrimination was acceptable. Also, results of the internal validation calculated likelihood ratio (LR) were 0.83, 0.85 and 0.75, for the type of evacuation decision, mode, and accommodation type choice, respectively. These LR values are less than the critical values, indicating that model validity was established. In terms of significant factors, results showed that evacuation behavior was affected either positively or negatively by some sociodemographic and other variables such as number of household members, source of evacuation warning and vehicle ownership. The significant factors found in this study can be used in developing strategies for future evacuation operations.

Keywords: accommodation type, destination choice, emergency, evacuation decision, evacuation mode choice, evacuation plan

INTRODUCTION

The frequent occurrence of hazards is causing destruction and damage to properties, displacements of millions of people, and worsening poverty (CRED 2020). These can cause natural and anthropogenic disasters. The Philippines is highly vulnerable to natural hazards. The most common natural hazards that affect the country are typhoons, floods, earthquakes, landslides, volcanic eruptions, and fires (IFRC 2018). In January 2020, the Taal volcano, located in the province of Batangas,

Philippines started showing signs of unrest after forty-three (43) years of dormancy. Alert level 4 was then raised leading to a total evacuation of people in high-risk areas within the 14-km radius and 20-km radius from Taal main crater, Taal Volcano Island, and along the Pansipit River Valley. The National Disaster Risk Reduction and Management Council (NDRRMC) in its 6 a.m. situational report of 25 January 2020, reported a total of 90,533 families were affected of which 37,445 families or 137,994 individuals took temporary shelter in 488 evacuation centers while 38,102 families or 148,271 persons stayed outside evacuation centers (NDRRMC 2020).

Evacuation is one of the countermeasures of the Philippine government to minimize loss of lives in case of no-notice disasters such as volcanic eruptions. Evacuation during the Taal volcanic eruption in the past had been completely disorganized (Barangay Official interviewed). Although evacuation plans and designated authorities in charge of evacuation management are put in place, the response of people as well as authorities when faced with sudden eruption can be unpredictable and complex. Therefore, understanding evacuation behavior and incorporating this into a comprehensive evacuation plan is still needed. Despite progress in research on evacuation behavior and modeling in the Philippines (e.g. Lim et al. 2016a; Lim et al. 2019; Lim et al. 2021), limited studies are conducted for no-notice volcanic eruptions. In addition to the risk perception, attitude, and the nature of risk communication, better evacuation compliance, socio-demographic and economic aspects should be well-understood (e.g. Favereau et al. 2018; Lechner and Rouleau 2019).

There are various aspects of household evacuation behavior such as evacuation decision, departure time choice, evacuation mode choice, and destination or specifically accommodation type choice. These decisions can be made simultaneously or sequentially and can vary depending on socio-demographic factors, hazard-related characteristics, and other factors (e.g. Lim et al. 2021; Wang et al. 2021). An evacuation decision, which is useful in estimating and modeling evacuation demand, is defined as a decision to either evacuate or stay in the area at risk of an impending hazard. The type of evacuation decision can include partial and full evacuation. Mode choice is an important logistical factor to consider in the evacuation operation. Lindell and Perry (1992) provided an early review of vehicle use during the evacuation that was later updated by Lindell and Prater (2007). More recent studies recognized and identified personal vehicles as evacuation modes (Huibregtse et al. 2010; Pel et al. 2011). Mass transit and other modes of evacuation have been explored in some evacuation studies for they can transport a considerable number of people to safety.

Another evacuation-related decision that a household should resolve is destination choice. Destination choice is the geographical location a household will go to when leaving their home located in a high-risk area. The destination often contains multiple types of accommodations. Accommodation type choice is the kind of facility where evacuees specifically go to (Bian et al. 2019). Analysis of the destination and accommodation type choice is important to be able to identify the demand for facilities in case of actual evacuations. However, past studies revealed that gaps still exist in the knowledge of the factors that influence these decisions including how these factors differ by population and disaster type (Lim et al. 2021). Another gap found in the literature is that most findings are not focused on no-notice disasters (Lechner and Rouleau 2019). Common disasters from studies are in the context of hurricanes and floods and mostly are in developed countries like the United States (e.g. Huang et al. 2016; Thompson et al. 2017).

This study aimed to understand different decision-making contexts of evacuation behavior, from evacuation decision, evacuation mode, and accommodation type choices. Using a discrete choice modeling framework, data collected from households in Leynes, Talisay Batangas, were used in calibrating and validating behavioral models. Barangay Leynes is one of the areas badly impacted by the eruption of Taal volcano in 2020. With a total population of 1,473 (396 households), it is located within the 14-km radius danger zone from the volcano. It is very near the volcano as it is located along the Taal lake in Talisay area, hence posed with high risks from volcanic eruption. It is frequently visited by tourists and bikers in the area where many business establishments are located. The results of this study can be used as a baseline for developing detailed evacuation plans for no-notice disasters due to volcanic eruptions. This is especially helpful for communities living near active volcanoes. The results in this study contribute to understanding the evacuation behavior in a developing country setting just like the Philippines due to a no-notice disaster. The impacts of disasters are not uniform when comparing disasters in developed and developing countries.

METHODS

Study Area and Data Collection

Leynes is a barangay in the municipality of Talisay, which is a 3rd class municipality in the province of Batangas, Philippines. Leynes is situated at approximately 14˚3′55.44″N, 120˚58′27.48″, on the island of Luzon. Elevation at these coordinates is estimated at 208.8 m or 685.0 ft above mean sea level. Figure 1 shows the location of Leynes, Talisay

Batangas. It shares a common border with the following barangays: Sampaloc, Caloocan, Silang Junction, and San Jose. Leynes is well known for its location on Taal Lake, providing a panoramic view of the Taal Volcano. However, it is also situated within the 14-km radius danger zone making it prone to the risks posed by Taal Volcano.

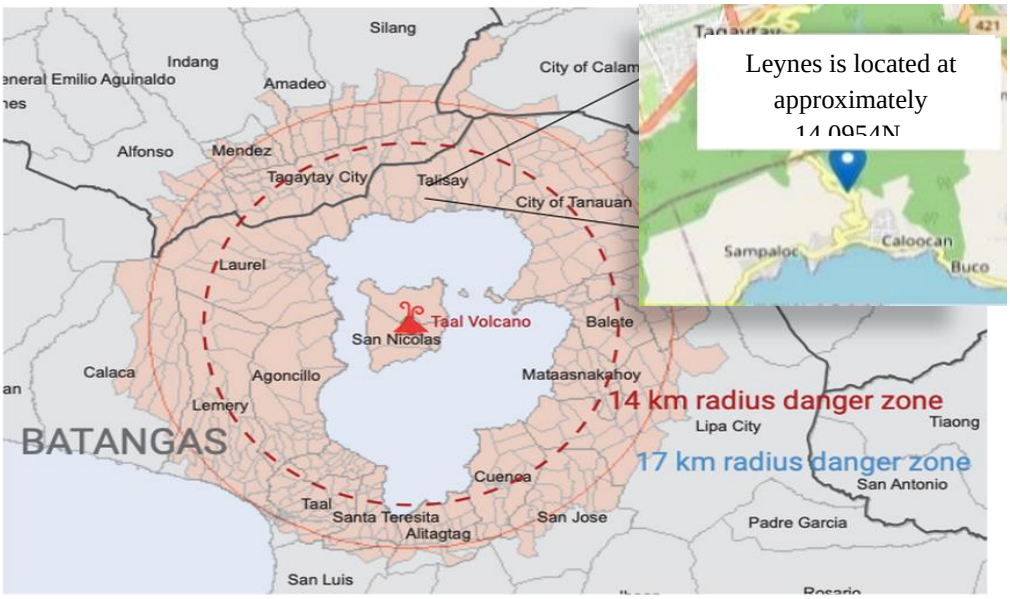

Figure 1. Location of Leynes, Talisay Batangas. Source: Open Street map (2020).

Like the method used for the data collection detailed in Lim et al. (2022a, b), the questionnaires were distributed to the households residing in the barangay through the help of the Barangay Health Workers (BHWs) from 26 June to 14 August 2021. This was done during the COVID-19 pandemic where face-to-face interviews were prohibited. Hence, the BHWs were trained in what to do while conducting limited face-to-face surveys with selected households. The survey questionnaire was designed to solicit evacuation information based on experience during the volcanic eruption in January 2020. The first portion consisted of socio-economic and household characteristics. The second portion was about their knowledge and information regarding the risk posed by the volcano. The third portion covered their evacuation experience, while the fourth section was about their re-entry experience. Lastly, the fifth portion includes households' suggestions on how to improve future evacuations.

A random sampling method was utilized in selecting household respondents. The total number of households in Leynes is 396, the number of questionnaires given was 364, and the answered questionnaires collected were 318. After the questionnaires were completed, data were summarized in a Microsoft Excel sheet. The summarized data was cleaned and checked. The cases with missing information were excluded from further data analyses. The resulting valid number of cases for analysis of evacuation-related decisions including the type of evacuation decision and evacuation mode choice is 296 (n = 296). However, for the analysis of the accommodation type choice, the final valid cases used was 166 (n = 166).

Modeling Framework, Parameter Estimation and Validation

The discrete choice model framework was applied in this study. In literature, discrete choice models have been used extensively in various disciplines such as social sciences, medicine, econometrics, transportation, and evacuation modeling (e.g. Mesa-Arango et al. 2013; Sadri et al. 2014). The recognized decision-makers in this study are the household heads. Equations 1, 2, and 3 show the form of the discrete choice utility function used in this study. The logit model specifies the utility function ($NEDEC_{ih}, DES_{ih}, MODE_{ih}$) for the type of evacuation decision, mode, and accommodation type choice, respectively, with terms ($\beta' X_{ih}$) and (ε_{ih}). β_s are vectors of parameters that were estimated and determined for decision *i*, of household *h*, respectively. Presented variables with Y_{ih} and Z_{ih} are the observed variables, and variables with ε_{ih} estimates variables that are not observed, underlying taste differences, and the use of proxy variables on observed choice.

$$NEDEC_{ih} = \beta_1' Y_{1ih} + \beta_2' Z_{1ih} + \varepsilon_{1ih} \quad (1)$$

$$DES_{ih} = \beta_3' Y_{2ih} + \beta_4' Z_{2ih} + \varepsilon_{2ih} \quad (2)$$

$$MODE_{ih} = \beta_5' Y_{3ih} + \beta_6' t Z_{3ih} + \varepsilon_{3ih} \ldots \quad .(3)$$

Equations 4, 5, and 6 present the probability functions for the type of evacuation decision, mode and accommodation type choice outcomes being chosen, *i*, by households, *h*, where *j* is the outcome decision, while ***e*** is Euler's number. Outcome decisions used for the type of evacuation are partial evacuation or full evacuation. In terms of mode choice, the outcomes include vehicles provided by the government, and owned/rented vehicles. While for accommodation type choice, outcome decisions include evacuation center, schools and government buildings, and friend's/relative's house or rented apartment. The decision where households partially evacuated, households with owned or rented vehicles, and the destination of a friend's/relative's house or rented apartment were the reference categories for parameter estimation of type of evacuation decision, mode choice, and destination choice, respectively.

$$P_{NEDECih} = \frac{e(\beta_1{}'Y_{1ih}+\beta_2{}'Z_{1ih}+\varepsilon_{1ih})}{\sum_i^j e(\beta_1{}'Y_{1ih}+\beta_2{}'Z_{1ih}+\varepsilon_{1ih})} \quad (4)$$

$$P_{DESih} = \frac{e(\beta_3{}'Y_{2ih}+\beta_4{}'Z_{2ih}+\varepsilon_{2ih})}{\sum_i^j e(\beta_3{}'Y_{2ih}+\beta_4{}'Z_{2ih}+\varepsilon_{2ih})} \quad (5)$$

$$P_{MODEih} = \frac{e(\beta_5{}'Y_{3ih}+\beta_6{}'Z_{3ih}+\varepsilon_{3ih})}{\sum_i^j e(\beta_5{}'Y_{3ih}+\beta_6{}'Z_{3ih}+\varepsilon_{3ih})} \quad (6)$$

The coefficients β' in Equations 4, 5, and 6 are determined by the maximum likelihood estimation method with the detailed formula of the log-likelihood functions presented in Equations 7, 8 and 9 for the type of evacuation decision, mode, and accommodation type choice, respectively. In the equation, *H* signifies the number of households and *J* is the outcome type under the choice of the household, *h* being investigated.

$$LL_{NEDEC} = \sum_{i=1}^{J} \sum_{h=1}^{H} \log(P_{NEDECih}) \quad (7)$$

$$LL_{DES} = \sum_{i=1}^{J} \sum_{h=1}^{H} \log(P_{DESih}) \quad \ldots\ldots\ldots\ldots\ldots (8)$$

$$LL_{MODE} = \sum_{i=1}^{J} \sum_{h=1}^{H} \log(P_{MODEih}) \quad \ldots\ldots\ldots\ldots\ldots (9)$$

The null hypothesis, that coefficients in the utility functions in equations 1, 2 and 3 are zero, will be rejected statistically if any relevant model parameter is different from zero at a 0.05 significance level. The listwise deletion was used to identify variables that are included in the models. All variables were first tested for significance, then individual variables were assessed whether they should be included in the model. Insignificant variables were removed one by one. Further, the *pseudo-R^2* was used to test the model fit. The AUC was also utilized to assess outcomes with a 0.5 cut-off point. The AUC with values ranging from 0 to 1, denotes the probability of the desired outcome and base outcome. Moreover, the correct classification rate (CCR) was used to compare the model's prediction performance to the base rate, which reflects the proportion of right classifications anticipated to occur by chance alone (Liu et al. 2014). With the addition of significant factors to the model, the CCR increased in comparison to the base rate, indicating an improvement in prediction accuracy. The sum of the squares of the percentage of outcomes in the data was used to compute the base rate.

An LR test was also used to investigate all model validity. The assumption of the method is a null hypothesis where the parameters of the model estimated using the whole data have no significant difference with that of the divided groups of two from the whole data. When the null hypothesis is not rejected, it means that the specification of the estimated models is established (Hasan et al. 2013). The LR test is as shown in equations 10, 11, and 12 for the type of evacuation decision, mode, and accommodation type choice, respectively.

In the equations shown, $LL\ (\beta_{whole})$ is the complete data model log-likelihood; $LL\ (\beta_{subsample1})$, is the log-probability estimate derived using the divided sub sample 1 of data which was randomly selected from the whole data; then $LL\ (\beta_{subsample2})$ is the log-likelihood at convergence of the model of sub sample group 2 from the whole data corresponding to the evacuation related decision being studied.

$$LR_{NEDEC} = -2[LL(\beta_{Nwhole}) - LL\ (\beta_{Nsubsample1}) - LL(\beta_{Nsubsample2})] \quad (10)$$

$$LR_{DES} = -2[LL(\beta_{Dwhole}) - LL\ (\beta_{Dsubsample1}) - LL(\beta_{Dsubsample2})] \quad (11)$$

$$LR_{MODE} = -2[LL(\beta_{Mwhole}) - LL\ (\beta_{Msubsample1}) - LL(\beta_{Msubsample2})] \quad (12)$$

RESULTS

Descriptive Statistics

Table 1 details the variables and the percentage of data included for the analysis of the three evacuation-related travel behaviors. There were separate numbers of data cases used for the analysis of the evacuation travel behaviors including evacuation decision type and evacuation mode choice, as well as the accommodation type choice. The number of data cases used for the analysis of evacuation decision type and evacuation mode choice was 296 (n = 296). One hundred sixty-six cases (n = 166) were used for the analysis of accommodation type choice. The difference was due to the low *pseudo-R^2* value reported for the latter using n = 296. The data for the

accommodation type choice analysis was reduced to find the model that provided an acceptable *pseudo-R^2* value.

The descriptive summary of the data set used for the analysis of the evacuation-related decisions (evacuation decision type and evacuation mode choice), shows that 219 households (73.99%) have fully evacuated, and 77 household respondents (26.01%) partially evacuated. Most of them (60.47%) evacuated using government vehicles while the rest (39.53%) evacuated using their owned or rented vehicles. One hundred eighty-one respondents (61.15%) evacuated to a house of friends/relatives or an apartment while 115 of them (38.85%) went to an evacuation center. Household respondents evacuated during the eruption (76.35%) and after the eruption (23.65%) since this was a no-notice disaster. Two hundred two of the respondents (68.24%) received evacuation warnings directly from a government official/agency while 94 respondents (31.76%) got their information from media/social media. Further, 57.43% of respondents got risk

Table 1. Descriptive summary of variables used for analysis of evacuation decision type, evacuation mode choice, and accommodation type choice.

Variables	Classifications	Data for NEDEC and MODE analysis		Data for DES analysis	
		N (296)	%	N (166)	%
Evacuation Decision Type (NEDEC)	Households that evacuated partially	77	26.01	44	26.51
	Households that evacuated fully	219	73.99	122	73.49
Evacuation Mode Choice (MODE)	Owned vehicle/rented vehicle	117	39.53	96	57.83
	Government vehicle	179	60.47	70	42.17
Accommodation Choice (DES)	House of friends/relatives or rented apartment	181	61.15	124	74.70
	Evacuation center (including public school, barangay hall, or church)	115	38.85	42	25.30
Departure timing (DEP)	Evacuated after the eruption	70	23.65	64	38.55
	Evacuated during the eruption	226	76.35	102	61.45
Source of Evacuation Warning (SWARN)	Media and social media	94	31.76	45	27.11
	Government official or agency	202	68.24	121	72.89
Distance traveled to the accommodation type (DIST)	≤ 25 km	126	42.57	72	43.37
	> 25 km	170	57.43	94	56.63
Duration of stay in the accommodation type (DUR)	< 1 month	111	37.5	64	38.55
	≥ 1 month	185	62.5	102	61.45
Age of the respondent (AGE)	20-30 years old	62	20.95	23	13.86
	31-40 years old	74	25.00	56	33.73
	41-50 years old	65	21.96	44	26.51
	> 50 years old	95	32.09	43	25.90
Marital status of the respondent (MAR)	Single	63	21.28	20	12.05
	Widow/widower	98	33.11	47	28.31
	Married/live-in	135	45.61	99	59.64
Number of household members (MEM)	≤ 4 members	168	56.76	95	57.23
	> 4 members	128	43.24	71	42.77
Number of senior citizens in the household (NSEN)	No senior citizen present at home	191	64.53	105	63.25
	At least one senior citizen present at home	105	35.47	61	36.75
Monthly income of household (INCOME)	≤ 5,000 pesos	194	65.54	70	42.17
	> 5,000 pesos	102	34.46	96	57.83
Vehicle ownership (OVEH)	Do not own a vehicle	106	35.81	55	33.13
	Owns vehicle	190	64.19	111	66.87
Type of Vehicle owned (TVEH)	None	106	35.81	55	33.13
	Motorcycle/tricycle	94	31.76	43	25.91
	Private car	96	32.43	68	40.96
Source of risk information related to Taal volcano (WINFO)	Media or social media	153	51.69	53	31.93
	Government official/agency	170	57.43	113	68.07
Knowledge of previous Taal eruptions (KNOW)	No knowledge of previous Taal eruption	170	57.43	74	44.58
	Have knowledge of previous Taal eruption	153	51.69	92	55.42

information about the Taal volcano directly from government officials/agencies, while the rest got information from the media/social media. Also, 51.69% of the households knew about previous Taal volcanic eruptions while 48.31% did not.

Further, for the accommodation type choice analysis which consists of 166 cases, it can be observed that most of the households fully evacuated (73.49%) and the rest partially evacuated (26.51%). Ninety-six (96) out of 166 respondents evacuated using their owned or rented vehicle while 70 of them evacuated using the designated government vehicle. Also, 42 respondents went to evacuation centers while 124 respondents stayed in their friend's/relative's house or rented apartment. Most of the respondents evacuated during the eruption (102) while 64 of them evacuated after the eruption. One hundred twenty-one respondents got their source of evacuation warning from government officials/agencies while 45 respondents got it from media outlets or social media. 56.63% of the respondents traveled more than 25 km to reach their accommodation type choice. One-hundred-two (102) household respondents stayed in their accommodation choice for a month or more while 64 of them stayed there for less than a month. For better visualization of evacuation behavior, Figure 2 shows a map of the evacuation movement of the household respondents when evacuating after the Taal volcano erupted in 2020. The map shows the type of transport mode (either government vehicle one or owned/rented vehicle) used when going by the indicated road taken from residential area in Leynes to either their friends/relatives house or rented apartment or to any designated evacuation center.

Figure 2. Evacuation mode and destination choice of household respondents in Barangay Leynes, Talisay, Batangas, Philippines.

Correlation Matrix

The correlation matrix of the variables included in the models is shown in the Appendices. Existing correlations of variables to the type of evacuation decision can be seen in the partial results. Possible determinants of the type of evacuation decision (Table

2) include the departure timing, source of evacuation warning, marital status, and knowledge of previous Taal eruption. All these variables except marital status are positively correlated to the type of evacuation decision. Furthermore, the correlation matrix to the evacuation mode choice (Table 2) indicates that distance traveled, the income of the respondents, vehicle ownership, and type of vehicle owned were significantly correlated to evacuation mode choice. It can be observed however, that the income variable was correlated with the distance traveled, vehicle ownership, and type of vehicle owned. Moreover, focusing on the correlation of accommodation type choice to other variables (Table 3), a positive correlation appeared with the source of evacuation warning, number of household members, and source of risk information about the Taal volcano. Meanwhile, the number of senior citizens in the household was negatively correlated to the accommodation type choice. The correlation matrix gives information on the effect of only one variable at a time on evacuation-related decisions being investigated. Hence, to evaluate the effects of multiple variables on evacuation-related decisions, logit models were estimated. The intercorrelation level among the independent variables implies the selection of variables that are included in the logit model.

Model Parameter Estimates and Validation

The result of the model estimation for households' evacuation behavior is shown in Table 4. The parameter estimates for decisions where households fully evacuated, households that evacuated using government vehicles and the accommodation type choice of evacuation centers are shown in the table. The range of the calculated AUC of the three models is from 0.72 to 0.85. Moreover, the model *pseudo-*R^2 ranges from 0.10 to 0.33. In terms of model validation, the value of *LR* for the type of evacuation decision is 5.12 with degrees of freedom equal to 4 and a critical value equal to 9.49. The mode choice mode has an *LR* value of 6, degrees of freedom equal to 3, and a critical value equal to 7.81. Lastly, the accommodation type choice has an *LR* value of 7.86 with degrees of freedom equal to 4 and a critical value of 9.49.

Table 4 shows that the significant variables for evacuation decision include the departure timing, source of evacuation warning, marital status, and knowledge of previous Taal eruptions. All variables have positive coefficients except for marital status. Meanwhile, the distance traveled to accommodation type choice, vehicle ownership, and the type of vehicle owned are the variables significant to the mode choice. The variables have positive coefficients. Lastly, the determinants for destination choice include source of evacuation warning, number of household members, number of senior citizens in the household, and source of information on dangers and risks of Taal volcano. Positive coefficients were denoted to all variables except the number of senior citizens in the household.

DISCUSSION

The results from this study showed different factors affecting the type of evacuation decision, mode choice, and accommodation type choice. Some of the significant factors are socio-demographic variables and are discussed in this section as presented in Table 4.

Model Parameters and Validation

The results of the logit models developed in this study capture the behavioral complexities of each decision-making covered in this study. The calculated *pseudo-*R^2 for the three evacuation-related models are in the range of 0.1-0.33, indicating acceptable level of data fit in respective models. Additionally, the calculated AUC for the three models range from 0.72 to 0.85 which means that the models' level of discrimination is acceptable. Also, results of the internal validation, *LR* tests show that calculated *LR* for the evacuation related decisions investigated are less than the critical values. These results indicate that model validity for the type of evacuation, mode choice and the accommodation type choice are established.

As shown in Table 4, all variables that determine the type of evacuation decision are significant at 0.05. Departure timing has a positive coefficient (β = 1.54) which means that the households tend to fully evacuate when doing it during the eruption. In addition, the source of evacuation warning has a positive coefficient (β = 1.01), which means that if the warning comes from government officials/agencies, households are more likely to fully evacuate. This shows that households have a high level of trust in the authorities. This complements the findings in the past literature in which the source of warning was identified as an influential predictor of evacuation decisions (Huang et al. 2016; Lim et al. 2016a; Golshani et al. 2019; Roy and Hasan 2021). Further to the results in this study, marital status has a negative coefficient (β = -0.92) which implies that households who are not single are less likely to fully evacuate. Moreover, it is important to note that knowledge of previous Taal eruptions also has a positive coefficient (β = 1.10). The more knowledgeable respondents concerning previous Taal eruptions are, the more likely that they fully evacuate.

Table 2. Correlation matrix of variables used for analysis of the type of evacuation decision and evacuation mode choice. **significant at 99%; *significant at 95%

	NEDEC	MODE	DEP	SWARN	DIST	AGE	MAR	MEM	NSEN	INCOME	OVEH	TVEH	WINFO	KNOW
NEDEC	*1*													
MODE	*-0.05*	*1*												
DEP	*0.32**	*0.09*	*1*											
SWARN	*0.19**	*-0.02*	*0.10*	*1*										
DIST	*0.05*	*0.16**	*-0.11*	*-0.04*	*1*									
AGE	*-0.11*	*0.01*	*-0.15*	*0.11*	*0.07*	*1*								
MAR	*-0.34**	*-0.07*	*-0.16**	*0.04*	*-0.08*	*0.34**	*1*							
MEM	*-0.07*	*-0.09*	*0.04*	*0.02*	*-0.03*	*-0.03*	*0.06*	*1*						
NSEN	*-0.03*	*-0.06*	*-0.04*	*0.01*	*0.07*	*0.32**	*0.02*	*0.15**	*1*					
INCOME	*-0.12*	*0.21**	*-0.13*	*0.02*	*0.22**	*-0.02*	*-0.07*	*0.04*	*-0.02*	*1*				
OVEH	*-0.05*	*0.62**	*0.01*	*0.08*	*0.10*	*0.02*	*-0.05*	*-0.09*	*-0.05*	*0.24**	*1*			
TVEH	*0.08*	*0.60**	*0.08*	*0.05*	*0.14*	*0.03*	*-0.12*	*-0.07*	*-0.01*	*0.25**	*0.85**	*1*		
WINFO	*0.14*	*0.12*	*0.03*	*0.30*	*0.11*	*0.07*	*-0.02*	*0.06*	*0.08*	*0.04*	*0.01*	*-0.02*	*1*	
KNOW	*0.37**	*0.02*	*0.04*	*0.06**	*0.14*	*-0.01*	*-0.29**	*-0.01*	*0.01**	*0.14*	*0.07*	*0.13*	*0.04*	*1*

Table 3. Correlation matrix of data used for analysis of accommodation type choice. **significant at 99%; *significant at 95%

	DES	NEDEC	MODE	DEP	SWARN	DIST	DUR	AGE	MAR	MEM	NSEN	INCOME	OVEH	TVEH	WINFO	KNOW
DES	*1*															
NEDEC	*0.10*	*1*														
MODE	*-0.05*	*-0.10*	*1*													
DEP	*-0.07*	*-0.17*	*-0.03*	*1*												
SWARN	*0.11**	*0.22**	*-0.13*	*-0.06*	*1*											
DIST	*-0.13*	*0.05*	*-0.09*	*0.13*	*-0.07*	*1*										
DUR	*-0.02*	*0.17*	*0.08*	*-0.01*	*0.10*	*0.01*	*1*									
AGE	*0.14*	*0.01*	*-0.15*	*0.10*	*0.13*	*0.10*	*-0.01*	*1*								
MAR	*0.06*	*-0.36**	*-0.02*	*0.15*	*-0.02*	*-0.08*	*-0.10*	*0.01*	*1*							
MEM	*0.16**	*-0.09*	*0.06*	*-0.04*	*-0.08*	*-0.01*	*-0.07*	*-0.09*	*0.02*	*1*						
NSEN	*-0.13**	*-0.02*	*-0.05*	*-0.11*	*-0.01*	*0.01*	*0.08**	*0.15*	*-0.03*	*0.12*	*1*					
INCOME	*-0.07*	*0.12*	*0.05*	*0.03*	*-0.05*	*0.18*	*0.01*	*-0.02*	*-0.05*	*-0.03*	*0.12*	*1*				
OVEH	*-0.06*	*-0.04*	*-0.02*	*0.01*	*0.15*	*0.06*	*-0.10*	*0.01*	*0.17*	*-0.17*	*-0.05*	*0.18*	*1*			
TVEH	*-0.05*	*0.05*	*-0.01*	*-0.02*	*0.10*	*0.15*	*-0.05*	*-0.01*	*0.07*	*-0.15*	*0.10*	*0.16*	*0.87**	*1*		
WINFO	*0.19**	*0.02*	*0.11*	*-0.01*	*0.34*	*0.13*	*-0.09*	*-0.12*	*-0.03*	*0.04*	*0.10*	*0.17*	*0.15*	*0.87*	*1*	
KNOW	*-0.06*	*0.04*	*-0.09*	*0.02*	*0.05*	*0.14*	*0.04*	*0.05*	*-0.11*	*-0.05*	*0.11*	*0.16*	*0.06*	*0.15*	*0.13**	*1*

Table 4. Result of the logit model parameter estimation for households that fully evacuated by government vehicle and went to evacuation centers. **significant at 99%, *significant at 95%

Variables	Type of Evacuation Decision		Mode Choice		Accommodation Type Choice	
	Coefficient (β)	P>|z|	Coefficient (β)	P>|z|	Coefficient (β)	P>|z|
DEP indicator variable for departure timing (1 for evacuation timing during the Taal eruption, 0 otherwise)	1.54**	0.000				
SWARN indicator variable for source of evacuation warning (1 for government official/agency, 0 otherwise)	1.01**	0.002			1.12*	0.028
MAR indicator variable for marital status (1 for married/live-in/widow/widower, 0 for single)	-0.92**	0.000				
KNOW indicator variable for knowledge on previous Taal eruption (1 for the presence of knowledge about Taal, 0 otherwise)	1.10**	0.000				
DIST indicator variable for distance traveled to accommodation type choice (1 for > 25 km, 0 for ≤ 25 km)			0.55	0.081		
OVEH indicator variable for vehicle ownership (1 for households that own a vehicle, 0 otherwise)			1.68**	0.002		
TVEH indicator variable for the type of vehicle owned (1 for private car/motorcycle/tricycle, 0 otherwise)			0.94**	0.008		
MEM indicator variable for the number of household members (1 for more than 4 members, 0 otherwise)					0.81*	0.045
NSEN indicator variable for the number of senior citizens in the household (1 for the presence of senior citizen, 0 for none)					-0.79*	0.047
WINFO indicator variable for source of information on dangers and risks of Taal volcano (1 for government official/agency, 0 otherwise)					1.38**	0.001
Constant	-0.49	0.369	-1.68	0.000	-1.06	0.024
Number of Observations	*296*		*296*		*166*	
LR chi^2	*91.13*		*131.85*		*19.52*	
Prob > chi^2	*0.00*		*0.00*		*0.00*	
Pseudo-R^2	*0.27*		*0.33*		*0.10*	
CCR	*80.41*		*81.76*		*76.51*	
CCR Base Rate	*61.51*		*52.19*		*62.20*	
AUC	*0.83*		*0.85*		*0.72*	

It can be observed from the results in Table 2 that the distance traveled to accommodation type choice has a positive coefficient (β = 0.55) implying that respondents are more likely to use a government vehicle if they are going to travel for more than 25 km. The critical range value of more or less than 25km distance was set because the study area is situated within the 14-km danger zone and designated evacuation centers are within the provinces of Cavite, Laguna, and Quezon. It is also worth noting that although the distance traveled is significant at 90% as shown in Table 2, it is still included in the model due to previous studies and consistent with other findings. Longer distances traveled result in a higher probability of using vehicles compared to walking (e.g. Lim et al. 2016b).

Moreover, vehicle ownership and type of vehicle owned has positive coefficients, β = 1.68, 0.94, respectively. The result of vehicle ownership indicates that households that own a vehicle are more likely to evacuate using a government vehicle. This can be explained by the type of vehicle most households own, which is also a significant variable in the model. Also, from the descriptive data in Table 1, only 32.43% of them owned a private car and the remaining more than 67% either owned a motorcycle or tricycle while others had none. Those respondents who have motorcycles or tricycles have resorted to using government vehicles because their capacity cannot accommodate all evacuating family members. Also, during the context of evacuation, the ashes on the road were so thick with mud causing the need for bigger vehicles for evacuation. This result in the current study is contrary to the results of Chen et al. (2021) where vehicle ownership is a determinant of personal vehicle use. This difference is plausible as in the case of Chen et al. (2021), where there would be no opportunity for government agencies to provide transportation for evacuation in a local tsunami setting.

The source of warning has a positive coefficient (β = 1.12) which means that warnings that came from government officials/agencies are more likely to encourage respondents to go to designated evacuation centers. Similarly, past studies reported that mandatory evacuation notices coming from government officials encouraged respondents to stay in the designated evacuation centers (Golshani et al. 2019; Lim et al. 2021; Nagarajan and Shaw 2021). Further, the number of household members has a positive coefficient (β = 0.81), which means that households with more than 4 family members are more likely to go to the evacuation centers. This is complementary to the findings of Wu et al. (2012) in which they also found out that larger household sizes have a higher probability of staying in public shelters. Moreover, the presence of senior citizens in the household indicated a negative coefficient (β = -0.79) which signifies that those households with elderly members are less likely to go to evacuation centers. This result is according to Golshani et al. (2019) who found that retired household members tend to stay with their family because they typically rely on their family members or relatives for emergency evacuation. Moreover, the source of information related to Taal volcano, and its risks is positively correlated to destination choice (β = 1.38). This indicates that respondents whose source of risk information regarding Taal volcano is the government official/agency will be more likely to go to an evacuation center.

The above findings can lead to several policy and operational recommendations and ramifications. Evacuation planners should think about the entire evacuation process, from the choice to leave to the availability and capacity of evacuation modes, shelter, and return. The term "evacuation" does not merely mean "withdrawal." Furthermore, withdrawal is not the most difficult part of the evacuation process. It is more effective for operational personnel to think of evacuation as a roundtrip process including the four interrelated stages, rather than just a movement away from danger. Indeed, Siebeneck et al. (2020) and Manandhar and Siebeneck (2021) have studied this issue extensively. Also, rather than being homogeneous, the phenomena should be viewed as diverse. The evacuee population is composed of several segments that will not all be present at the same time at any given location. Some could be just beginning to flee, while others have arrived at their chosen accommodations. Further, there are others who may have returned home due to frustration of traffic jams along the way. Another implication is that future message, order language, and timing in communicating warnings and disaster risks to residents should be considered and strengthened. The required evacuation resources, such as evacuation vehicles and rescue automobiles, should always be provided and readily available in the study's community environment. Additionally, agencies should explore boosting the number of public and alternative shelters available to alleviate evacuees' anxieties about locating and paying for accommodation.

Although models have been developed and evaluated as discussed, this study has some limitations. A bigger sample size can be taken from other barangays. Pooled data from different barangays might result in a better understanding of the volcanic travel behavior of Talisay, Batangas. The study area might be expanded for a more robust understanding of the evacuation decision, mode, and accommodation type choices for the city and municipal level. Also, other factors aside from the factors considered in this study can be investigated and contextualized in the research. Utilizing evacuation intentions or a combination of this and actual evacuation intentions for modeling might also be useful in future research.

Lastly, this study only addressed two warning sources, so the effects of other sources such as peers—as well as warning channels (e.g. Lindell and Perry 1987) and message content (e.g. voluntary vs. mandatory evacuation (Baker 1991)— should be addressed as directions for future research.

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ETHICAL CONSIDERATIONS

In the conduct of interviews, participants' informed consent was ensured. Only those who were willing to participate were interviewed. The personal information of participants is kept confidential. Only the results of the data analysis are published in this paper.

DECLARATION OF COMPETING INTEREST

We declare no competing interests in this work.

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Determination of Water Quality Characteristics of Süphan and Hıdırmenteş Lakes (Van-Eastern Türkiye)

Muhammet Demir

Provincial Directorate of Agriculture and Forestry, Van, Türkiye

Corresponding Author: muhammet.demir1453@gmail.com

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ABSTRACT

This study investigated the physical and chemical properties of the waters of Süphan and Hıdırmenteş Lakes in Van province, Eastern Turkey. Water samples were collected between November 2022 and August 2023 covering three seasons: autumn, spring, and summer. A total of 38 parameters from each lake were analyzed. The average parameter values were evaluated according to water quality classes. For Hıdırmenteş Lake, the average water temperature was 12.74°C, salinity was 0.40 mg L^{-1}, electrical conductivity (EC) was 785.12 µS/cm, suspended solids (SS) was 181.01 mg L^{-1}, turbidity was 152.33 NTU, pH was 8.17, dissolved oxygen (DO) was 8.14 mg L^{-1}, nitrite (NO_2^-) was 0.01 mg L^{-1}, nitrate (NO_3^-) was 6.23 mg L^{-1}, ammonia (NH_3) was 0.57 mg L^{-1}, ammonium (NH_4) was 0.60 mg L^{-1}, phosphate (PO_4^{-3}) was 1.22 mg L^{-1}, phosphorus (P) was 0.41 mg L^{-1}, and iron (Fe^{+2}) was 2.877 mg L^{-1}. For Süphan Lake, the average water temperature was 14.57°C, salinity was 0.23 mg L^{-1}, EC was 453.13 µS/cm, SS was 156.00 mg L^{-1}, turbidity was 125.33 NTU, pH was 8.35, DO was 8.19 mg L^{-1}, NO_2^- was 0.12 mg L^{-1}, NO_3^- was 8.03 mg L^{-1}, NH_3 was 0.96 mg L^{-1}, NH_4 was 1.01 mg L^{-1}, PO_4^{-3} was 0.03 mg L^{-1}, P was 0.01 mg L^{-1}, and Fe^{+2} was 0.377 mg L^{-1}. The water parameters, except for suspended solids, were suitable for agricultural use. According to the Regulation on the Protection and Improvement of waters where trout and carp fish species live, the phosphate, ammonia, and SS in Hıdırmenteş Lake, and the ammonia, SS, and nitrite in Süphan Lake, were not suitable for trout and carp production. To prevent water waste, modern irrigation systems should be used instead of traditional flood irrigation methods. Additionally, farmers should be educated about this issue and encouraged to adopt modern irrigation techniques.

Keywords: lakes, ponds, Van province, water quality parameters, water pollution

INTRODUCTION

Water has played a crucial role in the rise and progress of societies, effectively determining where civilizations have been established. With the increase in population, environmental problems have emerged, making water an even more critical resource due to the growing demand and excessive consumption, as well as limitations caused by the current population trends (Şen 2016; Yılmaz and Peker 2013).

In the 1960s, Turkey had a population of 28 million, with an average water use right of 4,000 m³ per person. By the 2000s, with the population reaching 70 million, this figure had decreased to 2,000 m³ per

person. Today, with a population exceeding 85 million, the per capita water use right has further decreased to around 1,500 m³. It is projected that by 2030, when the population is estimated to be approximately 100 million, water use per person will drop to 1,400 m³ (Şen 2016). These calculations do not account for potential drought and typical rainfall scenarios. Currently, there is a decline in water resources due to global warming and other negative factors. If these limited water resources are not managed scientifically and rationally, water scarcity and drought are imminent (Ceylan et al. 2009; Şen 2016).

Drought can lead to fish kills, reduced yields in animal and agricultural production, problems in food supply, decreased water availability, oxygen shortages in water, and high aquaculture stock density (Kabay 2019). For instance, in regions experiencing severe drought, large-scale fish kills have been reported, significantly impacting local ecosystems and economies. Similarly, drought conditions have led to substantial decreased in milk and meat production in livestock, and crop yields have suffered, causing food prices to rise and threatening food security. To mitigate drought impacts on agriculture, advanced planning and good management practices are essential (Kapluhan 2013).

As a result of climate changes, the earth's crust temperature increased by approximately 0.7-0.8°C between the 20th and 21st centuries due to global warming. Without necessary precautions, these temperature values will continue to rise, leading to the melting of glaciers, rising sea levels, and the occurrence of natural disasters (Şen 2016). Consequently, agricultural production will be significantly affected, and many plant and animal species may face extinction due to deteriorating living conditions (Karaman and Gökalp 2010; Şen 2016).

Lakes and ponds are generally formed naturally or through human-made dam constructions. They are named differently based on their formation structures, such as tectonic, volcanic, glacial, karst, landslide set, and coastal delta lakes. Human-made ponds are created for various purposes, including drinking, irrigation, and electricity generation (Duran 2016).

The Van Lake Basin, one of Turkey's basins, contains numerous lakes, ponds, dams, and streams. Key water bodies include Lake Van (607 km³), Lake Erçek (31 km³), Morgedik Dam (102 hm³), Sarımemet Dam (134 hm³), Koçköprü Dam (86 hm³), and Zernek Dam (104 hm³). The water potential of the Van Lake Basin sub-basins is as follows: Ahlat-West (452.96 hm³ year^{-1}), Bendimahi-East (457.16 hm³ year^{-1}), Deliçay-East (275.63 hm³/year), Engil (309.78 hm³ year^{-1}), South (418.36 hm³ year^{-1}), Karasu-East (551.11 hm³ year^{-1}), and Zilan-North (869.83 hm³/year) (TOB 2018). Additionally, there are numerous smaller lakes and ponds such as Arin (1,260 ha), Nazik (4,625 ha), Nemrut (1,280 ha), and many others (Demir 2023a).

In the Van Lake Basin, several fish species both endemic and introduced are found in lakes, ponds, streams, and dams. These include *Alburnus tarichi* Güldenstädt, 1814, *Alburnus timarensis* Kuru, 1980, *Barbus ercisianus* Karaman, 1971, *Capoeta kosswigi* Karaman, 1969, *Oxynoemacheilus ercisianus* Erk'akan & Kuru, 1986, *Cyprinus carpio* Linnaeus, 1758, *Oncorhynchus mykiss* Walbaum, 1792, and *Gambusia holbrooki* Girard, 1859 (Elp et al. 2016; Şen et al. 2018). Pollution, including industrial waste, solid waste from streams and sewers, oils, synthetic detergents, pesticides, artificial organic chemicals, and bilge waste from ships, is a major threat to the life of the pearl mullet (Alburnus tarichi) in Lake Van. Furthermore, hydroelectric power plants (HEPPs) and sand quarries on streams flowing into Lake Van negatively impact the ecosystem, particularly affecting the pearl mullet. Climate change also significantly affects water resources in Van province, leading to reduced water flow in streams, drying up small water sources, and disrupting the migration of pearl mullet. Severe droughts have resulted in the complete drying of some ponds and significant water loss in others, leading to widespread fish deaths (Elaçmaz, Altınboğa, Çubuklu, Define Ponds; Değirmigöl and Dolutaş Ponds) (Demir and Şen 2021).

The Van Lake Basin, covering a total area of 17,964 km², receives an average annual rainfall of 474 mm and has an average annual flow of 95.32 m³ s^{-1}. The basin's annual average yield is 6.25 L s^{-1} km$^{3-1}$, with a flow-to-precipitation ratio of 0.42 and a participation rate of 1.64%. The provinces of Van, Bitlis, and Ağrı are located within the Van Lake Basin (Batur et al. 2009).

This study aimed to determine the water quality characteristics of Hıdırmenteş (Çaldıran) and Süphan (Çaldıran) Lakes, located in the Van Lake Basin and used by local communities for agricultural activities. Water analysis was conducted to evaluate the usability of these lakes for fishing, fish transfer, aquaculture, and irrigation activities. The data obtained were interpreted according to the values specified in relevant regulations to provide a comprehensive evaluation of the lakes' water quality.

METHODS

Study Area

Information about the locations of Hıdırmenteş and Süphan Lakes (Figures 1 and 2) is provided in Table 1. These lakes host mirror carp and scaled carp (*Cyprinus carpio*, L., 1758) fish species. Commercial fishing is prohibited year-round, while amateur fishing is permitted outside the breeding periods.

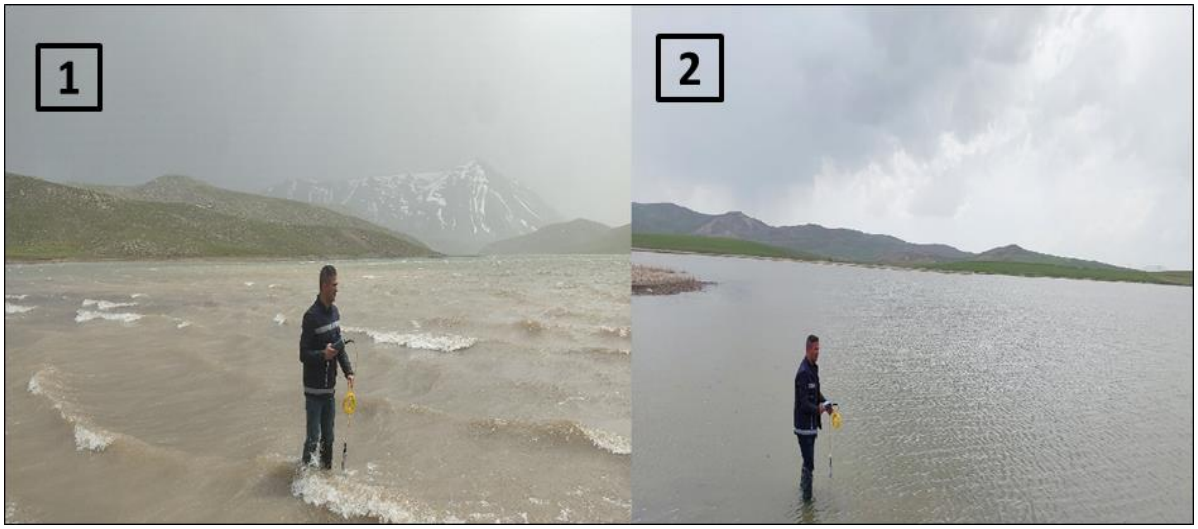

Figure 1. Sampling from the lakes Süphan (1) and Hıdırmenteş (2) in Van Lake Basin (Türkiye).

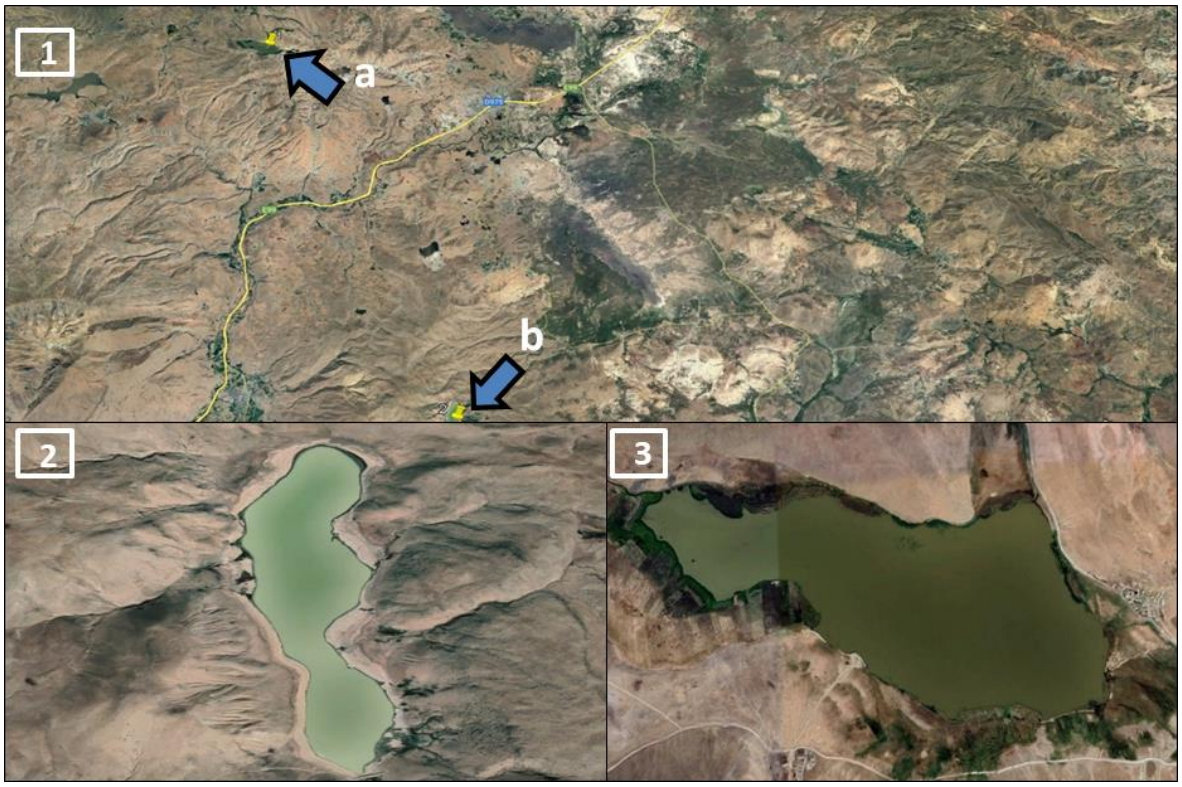

Figure 2. The locations of the Lakes Hıdırmenteş (1a, 3) and Süphan (1b, 2) in Van Lake Basin (Türkiye).

Table 1. Coastal length, surface area, and location information of Lakes.

Lakes	**District**	**Coast length (km^2)**	**Surface area (km^2)**	**Coordinates**	
				Latitude	Longitude
Hıdırmenteş	Çaldıran	2.00	0.96	39°10'50.30"K	43°46'17.43"D
Süphan	Çaldıran	2.95	1.80	38°57'45.79"K	43°52'06.08"D

Sampling and Data Analysis

This study evaluated various physical and chemical parameters of lakes used for agricultural irrigation purposes before, during, and after the irrigation period (May, July, and November). Water sampling methods adhered to Ayyıldız (1983).

Water samples from Hıdırmenteş and Süphan Lakes were collected between November 2022 and July 2023, covering autumn, spring, and summer seasons, as winter access to the lakes was not feasible (Figures 1 and 2). Temperature, dissolved oxygen (DO), total dissolved solids (TDS), salinity (‰), electrical conductivity (EC), and pH measurements from water samples were obtained using an HQ2200 Portable Multimeter device. Turbidity was analyzed with a turbidimeter device (HACH 2100 Q, Germany), and sodium and potassium concentrations were measured using a BWB flame photometer. Analyses of carbonate, bicarbonate, calcium, chloride, magnesium, total hardness, and organic matter (permanganate value) were performed using the titrimetric method (APHA 1995). Zinc, copper, nickel, cobalt, nitrite, nitrate, ammonium, ammonia, and phosphate were analyzed using an ICP-MS device (Agilent Technologies 7700 Series ICP-MS). Sulfate and boron were analyzed using a Shimano 2V spectrophotometer at the Soil, Fertilizer, and Water Resources Central Research Institute Laboratory. Phosphorus and suspended solids (SS) were measured with a spectrophotometer (HACH Lange DR 5000, Germany) at the Van Yüzüncü Yıl University Fisheries Faculty Laboratory (HACH 2005).

Water samples were collected using 1-liter plastic (polyethylene) containers from 40-50 cm below the water surface in areas where the lakes were at least 1-2 meters deep. The sample containers were rinsed 4-5 times with the lake water before being filled to the brim and sealed tightly. Each container was labeled with the sample taker's name, the sample location and date, and the sample number. The samples were then placed in thermoses and stored in a sample cabinet at the Provincial Directorate at +4°C for no more than 24 hours before being sent to the laboratory by courier for analysis. A letter requesting analysis accompanied the samples to the laboratory.

The quality of the obtained water parameters was classified based on the following standards: Regulation on the Protection and Improvement of Waters Where Trout and Carp Type Fish Live (ASSKY 2014), Surface Water Quality Management Regulation (YSKYY 2015), Quality Criteria of Intra-Continental Surface Water Resources According to Classes in Terms of General Chemical and Physicochemical Parameters, Waters for Human Consumption (TSE 2005), Regulation on the Quality and Purification of Water Supply (İSY 2019), (WKY 2008), and World Health Organization (WHO 1993).

RESULTS

The seasonal water quality parameter values (excluding winter) for Hıdırmenteş and Süphan Lakes in Van province are presented in Table 2.

In Hıdırmenteş Lake, the average values for water temperature, salinity, EC, suspended solids, turbidity, and TDS were higher than those in Süphan Lake. However, the average pH value in Hıdırmenteş Lake was lower. Both lakes exhibited similar average readings for DO (Table 2).

Regarding mineral content, Hıdırmenteş Lake had higher average values of calcium and bicarbonate, and lower average values of chloride, magnesium, calcium + magnesium, and total hardness compared to Süphan Lake. The carbonate levels were similar in both lakes (Table 2).

Except for phosphorus, the concentrations of the following compounds were lower in Hıdırmenteş Lake than in Süphan Lake: nitrite, nitrite nitrogen, nitrate, nitrate nitrogen, ammonium, and ammonia, phosphate (Table 2).

Sulfate ang potassium elements had higher concentrations in Hıdırmenteş Lake compared to Süphan Lake. The sodium concentration was lower in Hıdırmenteş Lake, while both lakes had the same concentration of boron. Hıdırmenteş Lake exhibited higher levels of organic matter and sodium absorption rate (Table 2).

Both lakes contained the same levels of chromium and zinc. However, Hıdırmenteş Lake had higher concentrations of iron, manganese, and copper (Table 2).

The classification of water quality parameter values measured seasonally (except winter) in Hıdırmenteş and Süphan Lakes in Van province is given in Table 3.

DISCUSSION

This study was conducted in Süphan and Hıdırmenteş lakes in the Çaldıran district, within the borders of Van province.

The average temperature values measured in Hıdırmenteş and Süphan lakes were classified as 1st class water values according to YSKYY (2015) and WKY (2008), and deemed appropriate according to ASSKY (2014). In other studies, the average water temperature was reported as 13.6±2.5°C in Aygır Lake (Çavuş 2018) and 17.4°C in Koçköprü (Demir 2023b). This variation is likely due to differences in measurement times, climate, and geopolitical conditions.

The average dissolved oxygen (DO) value of Hıdırmenteş and Süphan Lakes was classified as class 1 according to YSKYY (2015) and deemed appropriate according to ASSKY (2014). In other

studies, conducted in the Van Lake basin, the average DO value was reported as 8.15 mg L^{-1} in Yumruklu Pond (Atıcı 2020) and 8.10±0.4 mg L^{-1} in Arin Lake (Çavuş 2018). When water samples are taken, arid climate conditions and decreasing water levels negatively affect the amount of dissolved oxygen (Gümüş and Akgöz, 2020). The resulting values are similar to those reported in declarations.

In some scientific studies, the salinity value was reported as 0.29 mg L^{-1} in Kabaklı Pond (Kaya and Şen, 2022) and 0.21±0.003 mg L^{-1} in Aygır Lake (Çavuş 2018). The average salinity value obtained is similar to the values reported for Süphan Lake, while Hıdırmenteş has the highest value. The salinity of water varies depending on precipitation, evaporation, and soil characteristics. As evaporation increases, so does the salinity, particularly in the summer months (Dorak et al., 2018).

Table 2. Seasonal, average and standard deviation values of water quality parameters of lakes (mg L^{-1}). EC: Electrical Conductivity, TDS: Total Dissolved Solids, SS: Suspended Solid, SAR: Sodium Adsorption Rate. T2-A1: These are moderately saline and low-sodium waters. T1-A1: are common salt and low sodium waters. DLA: Below Detection Limit, SD: Standard Deviation

Parameters		Lake Hıdırmenteş					Lake Süphan				
		Autumn	Spring	Summer	Ave	SD	Autumn	Spring	Summer	Ave	SD
Physical Parameters	Temperature (°C)	4.90	14.30	19.03	12.74	±7.19	5.50	17.10	21.10	14.57	±8.10
	Salinity (mg L^{-1})	0.40	0.40	0.40	0.40	±0.00	0.20	0.20	0.30	0.23	±0.07
	EC (µS/cm)	784.00	781.30	790.07	785.12	±4.49	435.40	449.40	474.60	453.13	±19.86
	TDS (mg L^{-1})	339.50	338.50	342.70	340.23	±2.19	216.53	223.91	235.10	225.18	±9.35
	SS (mg L^{-1})	151.92	78.10	313.00	181.01	±120.12	161.30	59.70	247.00	156.00	±93.76
	Turbidity (NTU)	110.00	60.00	287.00	152.33	±119.27	130.00	50.00	196.00	125.33	±73.11
Chemical Parameters	PH	8.87	7.57	8.08	8.17	±0.66	8.42	7.94	8.68	8.35	±0.38
	DO (mg L^{-1})	10.18	7.39	6.86	8.14	±1.78	9.70	7.52	7.36	8.19	±1.31
	Alkalinity (mg L^{-1})	T1-A1	T1-A1	T1-A1	T1-A1	-	T1-A1	T2-A1	T1-A1	T1-A1	-
	Cl_2 (mg L^{-1})	19.53	28.75	24.14	24.14	±4.61	41.18	31.95	30.53	34.55	±5.78
	Ca^{+2} (mg L^{-1})	16.80	10.60	13.20	13.53	±3.11	14.60	12.00	8.40	11.67	±3.11
	Mg^{+2} (mg L^{-1})	5.76	6.72	1.68	4.72	±2.68	7.80	14.48	3.84	8.71	±5.38
	Ca^{+2}+Mg^{+2} (mg L^{-1})	42.24	34.88	25.60	34.24	±8.34	44.16	48.48	23.68	38.77	±13.25
	CO_3^{2-} (mg L^{-1})	0.00	0.00	0.00	0.00	±0.00	0.00	0.00	0.00	0.00	±0.00
	HCO_3^- (mg L^{-1})	31.11	35.38	38.43	34.97	±3.68	18.30	26.27	50.2	31.59	±10.44
	Total Hardness (mg L^{-1})	6.59	5.46	4.02	5.36	±1.29	6.90	23.18	3.71	11.26	±10.44
	NO_2^- (mg L^{-1})	0.01	0.01	0.01	0.01	±0.00	0.13	0.10	0.14	0.12	±0.02
	NO_2–N (mg L^{-1})	0.01	0.01	0.01	0.01	±0.00	0.03	0.03	0.04	0.03	±0.01
	NO_3^- (mg L^{-1})	5.40	6.20	7.10	6.23	±0.85	6.10	8.00	10.00	8.03	±1.95
	NO_3–N (mg L^{-1})	1.35	1.30	1.60	1.42	±0.16	1.90	2.05	2.30	2.08	±0.20
	NH_3 (mg L^{-1})	0.56	0.55	0.60	0.57	±0.03	0.96	0.98	0.93	0.96	±0.03
	NH_3-N (mg L^{-1})	0.50	0.50	0.55	0.52	±0.03	0.80	0.81	0.79	0.80	±0.01
	NH_4 (mg L^{-1})	0.58	0.58	0.64	0.60	±0.03	1.00	1.01	1.02	1.01	±0.01
	PO_4^{-3} (mg L^{-1})	0.98	1.11	1.68	1.22	±0.40	0.03	0.02	0.05	0.03	±0.02
	P (mg L^{-1})	0.37	0.31	0.55	0.41	±0.12	0.01	0.00	0.02	0.01	±0.01
	SO_4^- (mg L^{-1})	29.28	11.92	10.00	17.07	±10.62	12.40	11.28	5.00	9.56	±3.99
	K (mg L^{-1})	3.51	5.07	4.29	4.29	±0.78	0.39	1.95	2.34	1.56	±1.03
	Na (mg L^{-1})	5.98	4.60	6.21	5.60	±0.87	2.79	9.20	5.52	5.84	±3.22
	Organic matter (mg L^{-1})	7.32	3.56	0.66	3.85	±3.34	1.54	0.82	0.90	1.09	±0.39
	SAR	0.32	0.27	0.43	0.34	±0.08	0.15	0.26	0.40	0.27	±0.13
Heavy Metals	B (mg L^{-1})	0.75	0.05	0.04	0.28	±0.41	0.75	0.10	0.02	0.29	±0.40
	Co (mg L^{-1})	DLA	DLA	DLA	DLA	-	DLA	DLA	DLA	DLA	-
	Cr^{+6} (mg L^{-1})	0.003	0.001	0.002	0.002	0±.001	0.002	0.002	0.002	0.002	±0.000
	Fe^{+2} (mg L^{-1})	3.760	2.800	2.070	2.877	±0.848	0.530	0.270	0.330	0.377	±0.136
	Mn^{+2} (mg L^{-1})	0.039	0.030	0.010	0.026	±0.015	0.001	0.005	0.020	0.009	±0.010
	Cu (mg L^{-1})	0.003	0.001	0.003	0.002	0±.001	0.001	0.001	0.001	0.001	0±.000
	Ni (mg L^{-1})	DLA	DLA	DLA	DLA	-	DLA	DLA	DLA	DLA	-
	Zn (mg L^{-1})	0.017	0.008	0.003	0.009	0±.007	DLA	DLA	DLA	DLA	-

Table 3. Classification of lakes according to average parameter values. A1: Water becomes drinkable after simple physical purification and disinfection. A2: Water becomes drinkable after physical treatment, chemical treatment and disinfection. A3: Water becomes drinkable after physical treatment, chemical treatment, advanced treatment and disinfection. DLA: Below Detection Limit

Parameters	Average Values	Lake Hıdırmenteş						Parameters	Lake Süphan					
		YSKYY	WKY	ASSKY	İSY	TSE	WHO	Ave	YSKYY	WKY	ASSKY	İSY	TSE	WHO
Temperature (°C)	12.74	1	1	Suitable	-	-	-	14.57	1	1	Suitable	-	-	-
EC (µS/cm)	785.12	2	-	-	A1	Low	-	453.13	2	-	-	A1	Low	-
SS (mg L^{-1})	181.01	-	-	High	-	-	-	156.00	-	-	High	-	-	-
Turbidity (NTU)	152.33	-	-	-	A3	-	-	125.33	-	-	-	A3	-	-
PH	8.17	1	1	Suitable	A1	Suitable	-	8.35	1	1	Suitable	A1	-	-
DO (mg L^{-1})	8.14	1	1	Suitable	-	-	-	8.19	1	1	Suitable	-	-	-
Cl_2 (mg L^{-1})	24.14	-	1	-	A1	Low	-	34.55	-	1	-	A1	Low	-
NO_2^- (mg L^{-1})	0.01	-	-	Low	A1	-	Low	0.12	-	-	High	A1	-	Low
NO_2–N (mg L^{-1})	0.01	-	2	-	-	-	-	0.03	-	3	-	-	-	-
NO_3^- (mg L^{-1})	6.23	-	-	-	A1	Low	Low	8.03	-	-	-	A1	Low	Low
NO_3–N (mg L^{-1})	1.42	1	1	-	-	-	-	2.08	1	1	-	-	-	-
NH_3 (mg L^{-1})	0.57	-	-	High	-	-	-	0.96	-	-	High	-	-	-
NH_4 (mg L^{-1})	0.60	-	-	Suitable	A2	High	-	1.01	-	-	Suitable	A2	High	-
PO_4^{-3} (mg L^{-1})	1.22	-	-	High	-	-	-	0.03	-	-	Suitable	-	-	-
P (mg L^{-1})	0.41	3	3	-	-	-	-	0.01	1	1	-	-	-	-
SO_4^- (mg L^{-1})	17.07	-	1	-	A1	Low	Low	9.56	-	1	-	A1	Low	-
Na (mg L^{-1})	5.60	-	1	-	A1	Low	Low	5.84	-	1	-	A1	Low	-
B (mg L^{-1})	0.28	1	1	-	A1	Low	Low	0.29	1	1	-	A1	Low	Low
Co (mg L^{-1})	DLA	1	1	-	A1	-	-	DLA	1	1	-	A1	-	-
Cr^{+6} (mg L^{-1})	0.002	2	1	-	A1	Low	Low	0.002	2	1	-	A1	Low	Low
Fe^{+2} (mg L^{-1})	2.877	3	3	-	A3	High	-	0.377	2	2	-	A1	High	-
Mn^{+2} (mg L^{-1})	0.026	1	1	-	A1	-	-	0.009	1	1	-	A1	-	-
Cu (mg L^{-1})	0.002	1	1	Low	A1	Low	Low	0.001	1	1	Low	A1	Low	Low
Ni (mg L^{-1})	DLA	1	1	-	A1	Low	Low	DLA	1	1	-	A1	Low	Low
Zn (mg L^{-1})	0.009	1	1	Low	A1	-	Low	DLA	1	1	Low	A1	-	Low

The average EC values of Süphan and Hıdırmenteş Lakes are classified as 2nd class waters according to YSKYY (2015), A1 class waters according to ISY, and low value waters according to TSE (2005). In previous studies, the EC value was reported as 662 µS/cm in Dolutaş, 515 µS/cm in Değirmigöl, 579 µS/cm in Yumruklu, 488 µS/cm in Dönerdere (Atıcı 2020), 434.20 µS/cm in Nemrut Crater Lake (Sepil 2020), and 578.0 µS/cm in Kabaklı Pond (Kaya and Şen 2022). The EC value obtained in this study is higher than the reported values for Hıdırmenteş but similar to the reported values for Süphan.

The average pH values of Hıdırmenteş and Süphan Lakes are lower than the parametric value according to TSE (2005), but classified as 1st class quality waters according to WKY (2008), YSKYY (2015), and ISY (2019), and within mandatory values according to ASSKY (2014). In different limnological studies conducted in Turkey (Çavuş 2018) and in this study, it was observed that these lakes have a slightly alkaline structure. In some scientific studies, the pH value was reported to be between 7.50-8.20 in Bendimahi Stream (Bulum 2015), 7.73 in Koçköprü, 7.79 in Sarımemet, and 7.17 in Zernek (Demir 2023b). The pH values obtained were found to be higher than the reported values.

The average SS values of Hıdırmenteş and Süphan lakes were determined as inappropriate according to ASSKY (2014). According to YSKYY (2015), the SS value should be less than 5 mg L^{-1} for eutrophication monitoring in dam lakes. In some scientific studies, the SS value was reported as 110.0 mg L^{-1} in Dolutaş (Atıcı 2020) and 46.2 mg L^{-1} in Kabaklı Pond (Kaya and Şen 2022). The obtained values are higher than the reported values.

The average turbidity values of Hıdırmenteş and Süphan Lakes are classified as A3 waters

according to ISY (2019). In some scientific studies conducted in Turkey, the turbidity value was reported as 106 NTU in Dolutaş (Atıcı 2020), an average of 180 NTU in Karasu Stream (Atıcı 2020), and 71.3 NTU in Kabaklı Pond (Kaya and Şen 2022). SS values are directly proportional to turbidity. On-site water measurements revealed that the waters of Hıdırmenteş and Süphan lakes were quite turbid due to soil particles carried by rain.

High SS values cause organisms to sink into the sediment, prevent adequate respiration, and can cause death, reduce the amount of light entering the water, impair fish feeding efficiency, alter behavior, and change species distribution by altering the substrate (Donahue and Irvine 2003). The obtained turbidity values are close to those reported.

The nitrite values of Hıdırmenteş and Süphan Lakes were low according to WHO (1993), classified as A1 waters according to ISY (2019), while Hıdırmenteş was low and Süphan was high according to ASSKY (2014). In some scientific studies, the nitrite value in Aygır Lake was determined as 0.018 mg L^{-1} (Çavuş 2018), 0.070 mg L^{-1} in Akköprü Stream, 0.021 mg L^{-1} in Güzelkonak Stream (Bayram 2016), and 0.024 mg L^{-1} in Karasu Stream (Atıcı 2017), 0.004 mg L^{-1} in Değirmigöl, 0.009 mg L^{-1} in Yumruklu, and 0.081 mg L^{-1} in Dönerdere (Atıcı 2020). The higher nitrite amount in Süphan compared to Hıdırmenteş is likely due to fertilizers used in agriculture.

According to YKYY (2015) the average nitrite nitrogen values of Hıdırmenteş and Süphan lakes are classified as 2nd class and 3rd class quality waters, respectively, according to YSKYY (2015). In some scientific studies, the nitrite nitrogen value has been reported as 0.006 mg L^{-1} in Aygır Lake (Çavuş 2018), 0.000 mg L^{-1} in Dolutaş, 0.003 mg L^{-1} in Yumruklu, and 0.025 mg L^{-1} in Dönerdere (Atıcı 2020). Based on the obtained nitrite and nitrite nitrogen values, Hıdırmenteş is lower than the values stated in the reports, while Süphan is higher. The high nitrite value in Süphan is likely due to the shrinking water surface areas from recent droughts.

According to ISY (2019) the nitrate values of Hıdırmenteş and Süphan Lakes are classified as A1 class waters but WHO (1993) and TSE (2005) classified the low value as low. In some scientific studies, the nitrate value is reported as 4.98 mg L^{-1} in Kabaklı Pond (Kaya and Şen 2022), 4.5 mg L^{-1} in Değirmigöl, and 7.9 mg L^{-1} in Yumruklu (Atıcı 2020). In non-agricultural areas, nitrate levels are between 0-10 mg L^{-1} in surface and groundwater (Olhan and Ataseven 2009). The obtained values are similar to those reported.

According to WKY (2008) and YSKYY (2015), the nitrate nitrogen value of the lakes is classified as 1st class quality waters. In some scientific studies, the nitrate nitrogen value is reported as 1.14 mg L^{-1} in Kabaklı Pond (Kaya and Şen 2022), 1.1 mg L^{-1} in Değirmigöl, 1.9 mg L^{-1} in Yumruklu, and 3.7 mg L^{-1} in Dönerdere (Atıcı 2020).

The ammonium value of the lakes is classified as A2 waters according to ISY (2019), mandatory values according to ASSKY (2014), and low values according to TSE (2005). The ammonium value is reported as 1.85 mg L^{-1} in Kabaklı Pond (Kaya and Şen 2022), 0.063±0.001 mg L^{-1} in Aygır Lake (Çavuş 2018), and 0.25-0.88 mg L^{-1} in Murat River (Elazığ) (Çağlar 2011). The values obtained from the lakes differ from the reported values.

According to ASSKY (2014), the ammonia value of the lakes was above mandatory values. The ammonia value was reported as 1.76 mg L^{-1} in Kabaklı Pond (Kaya and Şen 2022) and 0.059 mg L^{-1} in Aygır Lake (Çavuş 2018). The resulting values are different from those reported.

According to ASSKY (2014), phosphate levels were found to be elevated in Hıdırmenteş Lake and low in Süphan Lake. Specific phosphate readings include Yumruklu at 0.03 L^{-1}, Dönerdere at 0.01 L^{-1} (Atıcı 2020), and Hazar (Elazığ) Lake ranging from 0.05 to 1.99 L^{-1} (Çoban 2007). Based on the acquired data, it was concluded that Hıdırmendeş exhibited higher levels while Süphan displayed lower readings compared to the notified values.

The phosphorus metrics for the lakes indicate that, based on YSKYY (2015) and WKY (2008), Hıdırmenteş falls under the 3rd grade whereas Süphan is classified in the 1st grade. The phosphorus content was documented as 0.01 mg L^{-1} in Yumruklu (Atıcı 2020) and varying from 0.15 to 2.21 mg L^{-1} in Hazar (Elazığ) Lake (Çoban 2007). These outcomes differ from the ones in the initial report, suggesting that agricultural activities predominantly contribute to the augmented phosphorus levels in Hıdırmenteş.

Regarding iron content in the lake waters, Hıdırmenteş was classified as 3rd class and Süphan as 2nd class as per WKY (2008) and YSKYY (2015), while Hıdırmenteş got an A3 and Süphan an A1 grade in accordance with İSY (2019). Notably, the lakes exhibit high iron content according to TSE (2005). Various scientific examinations have recorded the iron values as 0.018 mg L-1 in Kabaklı Pond (Kaya and Şen 2022), 0.025 mg L-1 in Bendimahi Stream (Van Province) (Bulum 2015), and Dolutaş at 0.12 mg/L, Değirmigöl at 0.065 mg/L, Yumruklu at 0.02 mg/L, Dönerdere at 0.02 mg/L (Atıcı 2020). The proximity of Hıdırmenteş Lake to the volcanic Tendürek Mountain is believed to account for its elevated iron levels.

In summary, upon evaluating the average parameter values of the lakes in focus based on water quality classes, it was established that, with the exception of suspended solids, parameters other than those mentioned are deemed suitable for agricultural irrigation. On the contrary, phosphate, ammonia, and suspended solids parameters in Hıdırmenteş, as well as

ammonia, suspended solids, and nitrite parameters in Süphan, were found unsuitable for the production of trout and carp, as indicated by various sources. However, ammonia levels in Hıdırmenteş and Süphan lakes were not lethal for carp but reached dangerous levels for trout according to different studies. Carp fish introduction by the Provincial Directorate of Agriculture and Forestry for fishing and agricultural purposes was found to be a recurring practice.

The narrative also addresses concerns like water loss due to intensive agricultural irrigation as well as the pressing need for modern irrigation techniques over traditional methods to curtail water wastage. Furthermore, raising awareness amongst farmers and implementing measures to control evaporation in water bodies to sustain aquatic life are emphasized. It is crucial to uphold sufficient water levels in these resources to perpetuate fish and aquatic biodiversity. Furthermore, despite being distant from urban centers and exposed to animal waste, Süphan and Hıdırmenteş lakes were noted as suitable for carp farming but posed risks for trout aquaculture.

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ETHICAL CONSIDERATIONS

The study was carried out following permission Republic of Turkey Ministry of Agriculture and Forestry grand number E-67852565-140.03.03-7215205

DECLARATION OF COMPETING INTERESTS

The author declares no conflict of interest.

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Toxicity, anti-inflammatory, and phytochemical properties of *Christella parasitica* (L.) H.Lev. ex Y.H.Chang in Bukidnon, Philippines

Glenda Z. Doblas[1,2], Ivy Lou R. Catane[1], Victor B. Amoroso[2], Aileen May G. Ang[3], Heidi C. Porquis[2], Diana Rose Y. Jacalan[1], Ellen Joy P. Pandan[3], and Reggie Y. Dela Cruz[1,2,*]

[1]*Tuklas Lunas Development Center, Central Mindanao University, 8710 Bukidnon*
[2]*Institute of Biological Sciences, Central Mindanao University, 8710 Bukidnon*
[3]*Department of Chemistry, Central Mindanao University, 8710 Bukidnon*
[*]*Corresponding Author: reggiecmu@gmail.com*

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ABSTRACT

Christella parasitica (L.) H.Lev. ex Y.H.Chang is a terrestrial fern traditionally used to treat gout and rheumatism, conditions caused by intense inflammation. Since inflammation is linked to many health problems in humans, investigation on the toxicity and anti-inflammatory potential of *C. parasitica* is of current relevance for drug discovery potential. Crude methanolic extracts of *C. parasitica* fronds and rhizomes were tested for total phenolic content (TPC), total flavonoid content (TFC), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, cyclooxygenase-2 (COX2) inhibition and toxicity tests against neonatal human epidermal keratinocytes (HEKn) and lung adenocarcinoma (A549). Plant habit, morphological characteristics, and the ribulose-bisphosphate carboxylase (rbcL) region confirmed the plant's identity. Alkaloids and tannins were present only in the fronds, and anthraquinones only in the rhizome while phenolics, saponins, and terpenoids were found in both fronds and rhizomes. Total phenolic content was significantly higher ($P < 0.05$) in the rhizomes compared to fronds. Flavonoids are present in both fronds and rhizomes. Fronds and rhizomes exhibited antioxidant activity based on DPPH radical-scavenging activity relative to ascorbic acid. They also exhibited high anti-inflammatory activity based on the inhibition of COX2. Both frond and rhizome extracts were nontoxic to HEKn and LA A549. These findings indicate that *C. parasitica* is nontoxic and has anti-oxidant and anti-inflammatory activities, which make it a promising natural source of anti-oxidant and anti-inflammatory compounds.

Keywords: anti-oxidants, cyclooxygenase, drug discovery, medicinal ferns, rbcL

INTRODUCTION

Drug development is a highly dynamic process in the history of civilization that has evolved from the knowledge and methods of various indigenous cultures to highly technical processes in many industrial laboratories of pharmaceutical companies. Aside from traditional sources, drug development could also be contingent, as in the case of how Alexander Fleming developed Penicillin.

Many of these development efforts source raw materials from plants. Central Mindanao University (CMU) has been designated as one of Tuklas Lunas Development Center (TLDC) since 2014 and pioneers drug development in the country using pteridophytes. Pteridophytes are a large class of non-flowering plants represented by ferns and lycopods that grow abundantly in many wild areas of Mindanao.

The medicinal importance of pteridophytes has long been established, particularly by the Chinese, who have used these plants in traditional medicine for over 2000 years (Ma et al. 2010). This traditional claim has prompted numerous researchers to investigate their pharmacological values, including their phytochemical composition and bioactivities (Shin and Lee 2010). *Christella parasitica* (L.) H.Lev. ex Y.H.Chang, with synonyms *Cyclosorus parasiticus* (L.) Farw and basionym *Polypodium parasiticum* L. (Evenhuis and Eldredge 2011; Kuo et al. 2019), is one of the 117 reported Philippine fern species listed in the family Thelypteridaceae worldwide (Delos Angeles and Buot 2012). This species is generally a terrestrial fern, found at lower elevations and widespread in tropical areas (Lin et al. 2013). Taxonomically, the genus *Christella* was established by Leveille in 1915 without designating a type species (Li et al. 2013). Holttum (1976) described this genus with *C. parasitica* (L.) Lev. as the type species. Its barcode is also found in the BOLD and NCBI databases using the rbcL gene in the former and trnL-trnF intergenic spacer, matK and psbA-trnH genes in the latter (Ratnasingham and Hebert 2007; NCBI 2020).

In traditional medicine, *C. parasitica* is recognized for treating gout and rheumatism (Benjamin and Manickam 2007; Singh and Upadhyay 2014), conditions caused by intense inflammation (Dalbeth and Haskard 2005). Reports on its phytochemicals (Paul et al. 2011; Mithraja et al. 2012) support its traditional medicinal use. Pursuing the anti-inflammatory potential of *C. parasitica* is of current relevance because inflammation is linked to many health problems in humans. Inflammation is classically viewed as an acute response to tissue injury, but contemporary revelations show it can be chronic and a major factor in developing diseases such as arthritis, atherosclerosis, cancer, heart valve dysfunction, obesity, diabetes, congestive heart failure, digestive system diseases, and Alzheimer's disease (Karin et al. 2006). A total of 410,244 studies on inflammation are indexed on PUBMED of the National Coalition for Biotechnology Information (NCBI). Most of these studies are very recent (Cervellati et al. 2020; Woolbright 2020; Nunes et al. 2020), suggesting that anti-inflammatory therapeutics have primary importance in the drug discovery process. The high volume and recency of these studies underscore the urgent need for new and effective anti-inflammatory agents.

The ongoing search for new therapeutic agents is crucial due to the rising prevalence of various health conditions and the need for effective treatments with fewer side effects. In inflammation, the goal is to achieve anti-inflammatory efficacy with few side effects. Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are used as anti-inflammatory drugs; however, several harmful side effects are recorded (Buchman 2001; Jones and Tait 1995). The former reduces inflammation by suppressing the immune system, while the latter inhibits cyclooxygenase, thereby preventing the production of prostaglandins, which is a key molecule in the inflammatory response. NSAIDs selective to COX2 inhibitors are preferred since they produce fewer digestive problems as side effects (Green 2001).

Recognizing the potential of *C. parasitica* for drug development, this study undertook a comprehensive examination of the species' frond and rhizome parts. The first objective was to establish its biological identity through traditional taxonomy and modern barcoding methods, ensuring accurate validation of field samples for subsequent laboratory analysis. The second objective was to verify the presence of phytochemicals in the frond and rhizome, particularly for Philippine species, where such data are currently insufficient. Additionally, the study aimed to gather empirical evidence on the efficacy and safety of *C. parasitica* using standardized methods and a tiered approach in testing (McKim and James 2010; Bacskay et al. 2018). These steps are critical in the drug discovery process. The resulting highlights are discussed in this report.

METHODS

Collection, Identification, and Preparation of Plant Material

Whole plant samples of *C. parasitica* (L.) H. Lev. ex Y.H. Chang (Figure 1) were collected from Mt. Musuan, Maramag, Bukidnon. These were identified through morphology by keying out using the Fern Flora of the Philippines (Copeland 1958), confirmed by the taxonomist in the research team, and deposited at the University Herbarium (CMUH). Deoxyribonucleic acid (DNA) barcoding using the rbcL gene was employed to confirm the species identity of the plant sample at the genetic level. The DNA extraction and amplification were conducted at the CMU Tuklas Lunas Development Center. Young fronds of *C. parasitica* were silica-dried and processed for total genomic DNA extraction using a modified cetyltrimethylammonium bromide (CTAB) method of Rogers and Bendich (1994). The amplification of the rbcL region was done using the primer pair rbcLaF and rbcLaR. This was amplified through a polymerase chain reaction (PCR) machine, Veriti® thermal cycler

(P/N 4375786, Life Technologies). The amplicons from thermal cycling were resolved using agarose gel electrophoresis, stained with Gel Red®, and visualized in GelDoc™ EZ documentation (Bio-Rad Technologies, Inc.). The PCR amplicons were sent to Macrogen, South Korea for bidirectional capillary electrophoresis sequencing (Sanger). BioEdit™ was used to edit the sequences. Edited sequences were submitted for homology to the Basic Local Alignment System Tool (BLAST) and Barcode of Life Database (BOLD) Identification System then deposited to Genbank.

The mature frond and rhizome parts were washed thoroughly with distilled water before air drying for 3-6 days at room temperature. Fresh and dry weights of the collected samples were recorded and the percent moisture loss was also calculated using the following formula adapted from Jin et al. (2017):

$$Mn = ((Ww - Wd)/Ww)\ x\ 100$$

Wherein, M_n = moisture content (%) of material, W_W = wet weight of the sample, and W_d = weight of the sample after drying.

The dried frond and rhizome parts of *C. parasitica* (L.) H.Lev. ex Y.H.Chang, below 10% moisture content, were ground separately using a heavy-duty miller and then sieved to produce a more homogeneous product.
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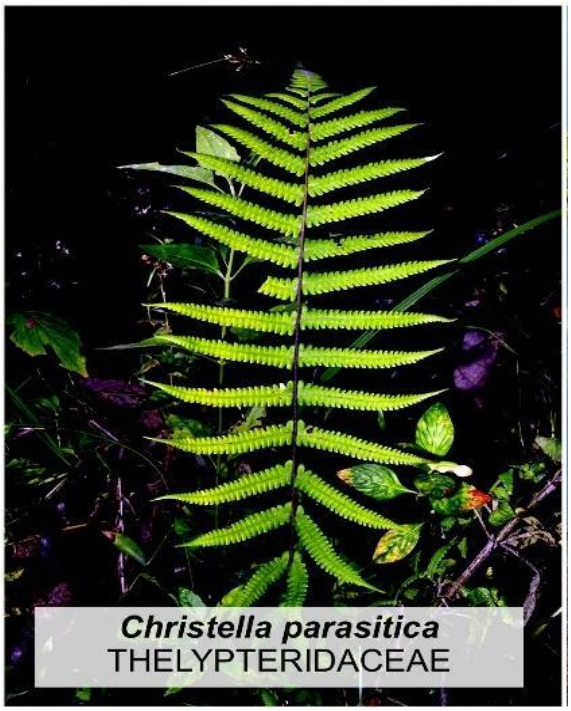

Figure 1. Frond of *Christella parasitica* (L.) H.Lev. ex Y.H.Chang (Thelypteridaceae) located at Mt. Musuan, Bukidnon.

Extraction and Phytochemical Studies

Methanolic extraction. The frond and rhizome samples were dried and powdered. Then, they were soaked in 99.99% HPLC grade methanol at room temperature for 72 hours (100 g sample / 500 mL solvent). The methanolic mixtures were then filtered through Whatman No. 1 filter paper and the filtrates were dried in a vacuum at 40°C using a rotary evaporator (Porquis et al. 2018). The extracted concentrates from the frond and rhizome samples were stored separately in an air-tight container at 4°C until further use.

Qualitative phytochemical analysis. To perform phytochemical screening of the crude methanolic extracts, a bioautographic assay via thin-layer chromatography (TLC) was used. This method was adapted from Brinda et al. (1981) and Gracelin et al. (2013) to detect the presence of important phytochemical constituents such as alkaloids, anthraquinones, phenolics, saponins, tannins, and terpenoids. Ten (10) µL of crude extracts were applied to the TLC plates using capillary tubes and air-dried before placing the TLC plates in a chamber. The plates were developed using chloroform: methanol (5:1) as the mobile phase and observed under UV light (254 nm).

Quantitative Phytochemical Analysis

Determination of total phenolic content (TPC). The total phenolic content was analyzed using the Folin–Ciocalteu colorimetric method in a 96-well microtiter configuration (Ainsworth and Gillespie 2007). The concentrated extracts of the frond and rhizome parts were dissolved in a DMSO (dimethyl sulfoxide): Methanol: Water (15:5:2) solution at 2 mg/mL (Amoroso et al. 2014). Then, 20 µL aliquot of the solution was mixed with Folin-Ciocalteau (1:10) reagent (Sigma) and incubated for 30 minutes. To modify the procedure, a five percent (5%) sodium carbonate solution was added to the solution as described in Bayili et al. (2011). After 2 hours of incubation at room temperature, the absorbance value was measured at 750 nm using a MultiSkan Go (ThermoScientific) UV/VIS spectrophotometer. A standard calibration curve was obtained using Gallic acid (R^2 = 0.9998).

Determination of total flavonoid content (TFC). The total flavonoid content of the samples was determined using a 96-well microtiter configuration (Sahu and Saxena 2013). Thirty (30) µL of fern extract at a concentration of 2 mg/mL in DMSO: methanol: water (15:5:2) was mixed with 30 µL of 10% aluminum chloride and 30 µL of 1M sodium acetate. Then, 110 µL of ultrapure water was added (Porquis et al., 2018). After 30 minutes of incubation at room temperature, the absorbance value of the samples was

read at 415 nm in the MultiSkan Go (ThermoScientific) UV/VIS spectrophotometer. The total TFC was expressed as µg quercetin equivalents per gram samples (µg QE/g).

Determination of antioxidant activity. The antioxidant activity of the extracts was initially determined through the DPPH radical scavenging assay. In a 96-well microtiter plate, 50 µL of concentrated extracts dissolved in a solvent consisting of 15 DMSO: 5 methanol: 2 water with a final concentration of 0.33 mg/ml were added with 150 µL DPPH (2,2,1-diphenyl-1-picrylhydrazyl). A 0.4 mg/mL ascorbic acid (AA) and the solvent (15 DMSO: 5 methanol: 2 water) were used as positive and negative controls, respectively. The plate was incubated at room temperature for 30 min, then absorbance was read at 517 nm. Percent DPPH radical scavenging activities (%DPPH) of the sample extracts and percent DPPH radical scavenging activities relative to ascorbic acid (%DPPH relative to AA) were computed using Equations 1 and 2, respectively (Amoroso et al. 2014):

$$\%\ DPPH\ \ Radical\ Scavenging\ Activity = \ [(A0 - A1)/A0]\ x\ 100 \quad (1)$$

$$\%\ DPPH\ relative\ to\ AA\ = \ (\%DPPHsample\ /\ \%DPPHAA)\ x\ 100 \quad (2)$$

where: A0 and A1 are the absorbance of the solvent and sample extract/ascorbic acid, respectively.

Anti-inflammatory assay (COX-Inhibition Assay). The inhibition activity of the extract on the cyclooxygenase 2 (COX-2) enzyme was determined using a COX (Ovine/Human) Inhibitor Screening Assay kit (Cayman Chemicals, Inc., USA) following the manufacturer's instructions. The extracts were assayed via enzyme-linked immunosorbent assay (ELISA) at a final concentration of 100 ppm. Two trials, each with 4 replicates were done for every extract. Celecoxib (Celebrex) at a 100-ppm concentration was used as a positive control.

Cell Viability Assay

MTT cell proliferation assay. The proliferative activity on neonatal Human Epidermal Keratinocytes (HEKn) was determined using the MTT assay 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide kit (Vybrant, Invitrogen). Cells at passage 3 were plated in a 96-well plate with a concentration of 5,000 cells/well, then incubated for 24 hours. The cells were cultured in EpiLife® basal media (M-EPI-500-CA) supplemented with Human Keratinocyte Growth Supplement (HKGS). The basal media were subsequently changed before adding the plant extract at final concentrations of 20 ppm and 200 ppm. The plated cells with extracts were incubated again at 37˚C for 48 hrs. Ten (10) µL of 12 mM MTT stock solution was added to label the cells, then incubated at 37ºC. After 4 hours of incubation, the media were removed, leaving only 25 µL in the wells. Then, the cells were added with 50 µL of dimethyl sulfoxide (DMSO) and incubated at 37ºC for 10 minutes. Absorbance values per well were read at 540 nm using a microplate reader. Cell viability was calculated as a percentage with untreated cells (Calderón-Montaño et al. 2021). The assay was carried out in two trials with three replicates per extract.

Anti-cancer assay. The following procedure for MTT cytotoxicity assay was adapted from Mosmann (1983). Human lung adenocarcinoma (A549) cell lines were seeded into sterile 96-well microtiter plates using a seeding density of 6 x 10 cells/mL then incubated overnight at 37°C and 5% CO_2. Serial dilutions of the samples at 4mg/mL DMSO were performed to which four different concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL) in a master dilution plate (MDP) were used. From the MDP, 10 µL of each concentration was dispensed onto the plated cells to obtain the final screening concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL. Three replicate wells were used per concentration. Doxorubicin (17nM) and DMSO were used as the positive control and negative control, respectively. The treated cells were then incubated in 5% CO_2 at 37°C for 72 hours. After incubation, the media were removed from the 96-well microtiter plate and 20 µL of 3-(4,5-dimethylethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 5 mg/mL in Phosphate Buffer Saline (PBS) was added. The treated cells were incubated again at 37°C and 5% CO_2 for 4 hours. Then, DMSO was added to each well to dissolve the formazan crystals formed by the reduction of the dye by the live cells. Absorbance was read at 570 nm. Cell viability was calculated as a percentage in relation to untreated cells, while IC50 was obtained through linear regression analysis (Calderón-Montaño et al. 2021; Norberg-King 1993). Three trials with 3 replicates for each concentration were done for each sample. The US National Cancer Institute Plant Screening Program sets a standard of IC 50 ≤ 20 ppm for plant extracts having an active cytotoxic effect (Kaewpiboon et al. 2012). Samples with an IC50 value less than 30 µg/mL are considered active (Jokhadze et al. 2007).

RESULTS

Morphological and Molecular Identification

Based on plant habit and morphological characteristics such as sori shape and distribution, frond type, rhizome characteristics, and the presence or absence of scales or hair, the collected plant specimens were identified as *C. parasitica* (L.) H.Lev. ex Y.H.Chang. Furthermore, the rbcL region with an average length of 561 base pairs was successfully sequenced and confirmed the initial identification with 100% identity when compared to GenBank and BOLD databases. The GenBank accession number for the sequence of this species is MZ501574.

Qualitative Phytochemical Analysis of Crude Methanolic Extracts

Thin-layer chromatography showed the preliminary detection of phytochemicals in crude methanolic extracts of *C. parasitica* through positive color reactions of the spots. This revealed the presence of phenolics, saponins, and terpenoids in both the frond and rhizome parts. Alkaloids and tannins were observed only in the frond while anthraquinones were present only in the rhizome (Table 1).

Quantitative Phytochemical Analysis of Crude Methanolic Extracts

Both the frond and rhizome parts of *C. parasitica* contained phenolic and flavonoid compounds as indicated by the total phenolic content (TPC) and TFC of the crude methanolic extracts. The TPC found to be higher in the rhizome compared to the frond (Table 2).

Antioxidant and Anti-inflammatory Assays

The methanolic extracts of *C. parasitica* frond and rhizome exhibited antioxidant activity by scavenging DPPH radicals, although the activity was lower than that of ascorbic acid (Table 3). On the other hand, both frond and rhizome extracts exhibited high anti-inflammatory activity (≥50%) by inhibitingcyclooxygenase-2 (Table 3). Moreover, the rhizome showed higher antioxidant activity compared to the frond.

Table 1. Phytochemicals in crude methanolic extracts of *Christella parasitica* (L.) H.Lev. ex Y.H.Chang using thin-layer chromatography (TLC). Legend: +/- (presence/absence of the phytochemical).

Phytochemicals	Plant Part	
	Frond	Rhizome
Alkaloids	+	-
Anthraquinones	-	+
Phenolics	+	+
Saponins	+	+
Tannins	+	-
Terpenoids	+	+

Cell Viability Assay

The effects of *C. parasitica* frond and rhizome crude methanolic extracts on the viability of human epidermal keratinocytes (HEKn) were determined using the MTT cell proliferation assay. The extracts were tested at two concentrations: 20 and 200 ppm (Table 4). The frond methanolic extract showed high cell viability at both 20 ppm (88.98 ± 4.81) and 200 ppm (85.95 ± 10.53). The rhizome methanolic extract also showed relatively high cell viability at both 20 ppm (129.14 ± 21.54) and 200 ppm (99.17 ± 3.54).

Based on the total phenolic content, antioxidant activity, and cell viability, the rhizome methanolic extract performed better than the frond extract. Therefore, the rhizome extract was prioritized for further testing in the MTT cell viability assay against lung adenocarcinoma A549. In comparison to the chemotherapeutic agent doxorubicin, which only allowed 29-32% growth of cells at concentrations of 6.25 - 50 μg/ml, the rhizome extract did not hinder the proliferation of LA A549 cells (Table 5). Moreover, while the mean IC50 of Doxorubicin is 2.19 μg/ml, no linear interpretation can be achieved with the rhizome extract.

Table 2. Phenolic and flavonoid content of crude methanolic extracts of *Christella parasitica* (L) H.Lev. ex Y.H.Chang using the Folin-Ciocalteau and Aluminum Chloride method, respectively. Values are presented as mean ± SD (n=3); *** p-value < 0.001; ns - differences between the means not significant at p-value > 0.05.

Plant Part	Total Phenolic Content*** (mg GAE/ g sample)	Total Flavonoid Content^{ns} (μg QE/ g sample)
Frond	41.88 ± 2.22	14.17 ± 2.83
Rhizome	149.37 ± 0.59	2.01 ± 0.44

Table 3. DPPH radical scavenging activity (%) and percent inhibition on cyclooxygenase-2 in crude methanolic extracts of *C. parasitica* (L) H.Lev. ex Y.H.Chang. Values are mean ± SE (n=3); Value for ascorbic acid is 75.6%.; ***p-value < 0.001; ns – no significant differences between the means at p-value > 0.05.

Plant Part	Antioxidant Activity*** (% DPPH Radical Scavenging Activity Relative to Ascorbic Acid; P=0.000)	Anti-inflammatory Activity^{ns} (Cyclooxygenase or COX-2)
Frond	16.90 ± 0.58	60.15 ± 4.82
Rhizome	29.77 ± 0.89	74.97 ± 2.49

Table 4. Percent cell viability in human epidermal keratinocytes (HEKn) treated with *C. parasitica* methanolic extracts at 200 and 20 ppm extract concentration.

Plant part	Concn. (ppm)	Trial 1	Trial 2	Mean ± SD
Frond	20	92.38	85.58	88.98 ± 4.81
	200	93.39	78.51	85.95 ± 10.53
Rhizome	20	113.91	144.37	129.14 ± 21.54
	200	101.67	96.67	99.17 ± 3.54

Table 5. Mean cell viability (%) of the rhizome methanolic extract subjected to anticancer preliminary assay using MTT *in vitro* cell proliferation assay. *p-value (treatment) ≤ 0.001; p-value (concentration) ≤0.05; NLI - no linear interpretation.

Concentration (µg/mL)	% Cell Viability*	
	Doxorubicin	Rhizome
50	29 ± 9.17	105 ± 13.56
25	32 ± 9.76	104 ± 30.26
12.5	29 ± 9.17	108 ± 25.37
6.25	30 ± 10.85	106 ± 21.07
0	100 ± 0.00	100 ± 0.00
IC50 (ug/ml)	2.19	NLI

DISCUSSION

Morphological and Molecular Identification

Drug discovery from plants and plant identification are inseparable, especially when a potential drug is being studied and analyzed. Traditionally, plant identification relies solely on the plant's morphology. However, relying solely on morphology can be challenging, especially for an untrained eye, due to the wide range of plant forms. This challenge is particularly amplified with ferns and their allies, as they lack flowers and fruits that could facilitate easy identification. In this study, the traditional identification method for *C. parasitica*, using morphological characteristics, is supplemented with DNA barcoding. This involves using a short section of DNA from a standardized region of the genome, specifically the rbcl gene (Kress and Erickson 2007), which codes for the large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase (RUBISCO). The rbcl gene is considered a benchmark locus in phylogenetic investigations (Kress and Erickson 2007).

Qualitative Phytochemical Analysis of Crude Methanolic Extracts

Numerous secondary metabolites, primarily from the phenolic, flavonoid, terpenoid, and alkaloid classes, have been identified as active components in fern species. Among these, terpenoids are the most abundant chemical group present in ferns (Ho et al. 2010). Terpenoids have a variety of biological uses, with triterpenoids acting as antioxidants (Garcia et al. 2006), diterpenoids as anti-inflammatory agents (Kim et al. 2016), and sesquiterpenoids as cytotoxic compounds (Ge et al. 2008).

The presence of phenolics, saponins, and terpenoids in both fronds and rhizomes, alkaloids and tannins in fronds, and anthraquinones in rhizomes may confirm the medicinal potential of these ferns. These phytochemical compounds are known to support various biological activities in medicinal plants and contribute to their antioxidant properties. Tannins, saponins, and triterpenes have all been reported to have antitumor, mutagenic, anti-inflammatory, and anti-ulcer activities (Chung et al. 1998; Ferguson et al. 2006; Lemeshko et al. 2006; Roy et al. 2007; Ye et al. 2007; Mohammed 2014). Similarly, naturally occurring anthraquinones exhibit a broad spectrum of bioactivities such as cathartic, anticancer, anti-

inflammatory, antimicrobial, diuretic, vasorelaxant, and phytoextrogent activities (Chien et al. 2015).

In this study, the localization of phytochemicals to a specific plant organ is observed. Alkaloids and tannins were found exclusively in the frond, while anthraquinone was localized in the rhizome. Dela Cruz et al. (2017) also documented the localization of these phytochemicals in certain fern species. Alkaloids and tannins were observed only in the frond of *Drynaria quercifolia* and *Pyrrosia adnascens*, while anthraquinones were found only in the rhizome of *Drynaria quercifolia*. However, *Microsorum punctatum* (L.) and *Pyrrosia adnascens* contained anthraquinones in both the frond and rhizome (Dela Cruz et al. 2017). At the cellular level, secondary metabolites were localized in the parenchymal cells such as secretory tissues, vacuoles, and cytosol of the frond and rhizome in *Pteris* species (Sulisetijono et al. 2020). Tannins are particularly abundant in the xylem of leaves in many plants (Badria and Aboelmaaty 2019). The presence of phytochemicals in multiple plant organs can result in varying amounts and rates of bioactivity. For example, leaves exhibited higher total phytochemical content, total flavonoid content, and DPPH radical scavenging activities compared to stems (Raya et al. 2015). In this study, the rhizome exhibited a high amount of total phenolic content, antioxidant activity, and anti-inflammatory activity but these properties were limited to the frond. These findings imply that the nature of the plant part should be considered in pharmacological studies, as it can influence the production of secondary metabolites. The strategic localization of these compounds optimizes chromatographic explorations and facilitates the cost-effective isolation of natural products for drug development (El Babili et al. 2021).

Phenolic compounds, particularly those derived from plants, have been found to possess anti-inflammatory and anti-cancer properties. They achieve this by inhibiting oxidative stress and conditions associated with inflammation (Tatipamula and Kukavica 2021). Reports have shown that phenolic compounds have significant value in preventing the development and progression of various human diseases (Rahman et al. 2021). In this study, a high TPC of 149.37 ± 0.59 mg GAE/g sample was observed in the *C. parasitica* rhizome methanolic extract. Total phenolic content values greater than 10 mg gallic acid/g are considered high (Zakaria et al. 2010). The flavonoid content varies among different fern species. In this particular study, the frond and rhizome samples had relatively lower observed TFC values of 14.17 and 2.01 µg QE/g, respectively, when compared to other fern species. For example, *Lycopodium cernua* had 11.46 µg QE/g in the fronds and 5.22 µg QE/g in the rhizomes (Porquis et al. 2018). The *M. punctatum* contains 6.69 µg QE/g in the fronds and 17.38 µg QE/g rhizomes, while *D. quercifolia* had 36.74 µg QE/g in the frond samples (Dela Cruz et al. 2017). Flavonoids, which are polyphenols, exhibit several biological effects such as anti-inflammatory and anticancer potential. They achieve this by inhibiting pro-inflammatory cytokines and activating antioxidant transcription factors (Ferraz et al. 2020). Hence, the fern species under investigation in this study may possess anticancer, anti-inflammatory, and antioxidant activities that are correlated to their total flavonoid contents.

Antioxidant and Anti-inflammatory Assays

The DPPH radical scavenging assay was used to evaluate the antioxidative properties of the crude methanolic extracts. The DPPH is commonly used to evaluate the antioxidant potential of herbal extracts. Antioxidant compounds help reduce oxidative damage caused by reactive oxygen species, which are free radicals that can harm nucleic acids and proteins and potentially leading to cell death (Gulcin and Alwasel 2023). In this study, DPPH data aided in prioritizing the extracts for the COX-2 inhibition assay as well as gave a glimpse into the mechanism underlying the bioactivity of the extracts. The anti-inflammatory activities of drugs are mediated by the inhibition of cyclooxygenases which catalyze the bioconversion of arachidonic acid to prostaglandins (Badieyan et al. 2012). The COX-2 isozyme is involved in the anti-inflammatory response, as it is induced by mitogenic and proinflammatory stimuli. Moreover, COX-2 expression is triggered by inflammation and carcinogenesis. The COX-2 is overexpressed in many solid tumors such as colon, breast, prostate, liver, and lung cancers (Badieyan et al. 2012).

Cell Viability Assay

This study showed that the crude methanolic extracts of *C. parasitica* did not exhibit cytotoxic activity, as they failed to inhibit the proliferation of HEKn cell lines (normal cells) and LA A549 (cancer cells). The MTT assay is a cytotoxicity assay used to assess the metabolic activity of cells. Viable cells can produce mitochondrial enzymes that convert tetrazolium into formazan, making the MTT assay an effective method for evaluating the toxicity of materials on cell growth (Tolosa et al. 2015). Also, the MTT in vitro proliferation assay is widely used to preliminarily evaluate the anticancer activity of natural product extracts (McCauley et al. 2013). In this study, the methanolic extracts were assessed for preliminary anticancer activity based on the viability of lung adenocarcinoma A549 cells. Compared to doxorubicin, a chemotherapeutic drug used to treat several cancer types, the rhizome extract did not prevent the proliferation of the cells. Unlike

doxorubicin which prevents or slows down the growth of cancer cells by blocking the enzyme topoisomerase (Kciuk et al. 2023), the rhizome extract may be devoid of metabolites that may damage the DNA of cancer cells.

The presence of phenolic and flavonoids as well as the antioxidant and anti-inflammatory activities of *C. parasitica* extracts may imply potential of this plant for future product development, although confirmatory assays are needed to ensure safety. The present investigation revealed the presence of medicinally important constituents of *C. parasitica* and provided useful information on its cytotoxicity and anti-inflammatory potential. It is nontoxic and has anti-inflammatory activities which make it a promising natural source of anti-inflammatory compounds.

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ETHICAL CONSIDERATIONS

Gratuitous permit for plant collection was approved by the Department of Environment and Natural Resources (DENR) Region 10 (Wildlife Gratuitous Permit No. R10 2019-57) after complying necessary requirements. Prior arrangement and courtesy visits with the municipal and barangay officials were observed.

DECLARATION OF COMPETING INTEST

The authors declare that there are no conflicting interests to any authors.

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***ROLE OF AUTHORS*:** GZD – conception and design, data analysis and interpretation, and wrote the manuscript; ILRC – data collection, analysis, manuscript writing; VBA – manuscript editing, verified plant identification, data collection and interpretation of results; AMGA –designing methodologies for extraction, phytochemical analysis, and bioactivity assessment, data collection, result interpretation, and manuscript editing; HCP – study conception, design, result interpretation, and manuscript revision; DRYJ – data collection, data analysis and manuscript revision; EJPP – manuscript editing, data collection and analysis; RYD – study conception, design, interpretation of results and manuscript revision.

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Embryogenesis and early larval development in rosy barb (*Pethia conchonius*, Hamilton 1822)

Ahmet Sepil[1,*], Burcu Ergoz Azizoglu[2], Zehra Alkan Çekiç[2], and Ahmet Regaib Oguz[2]

[1]*Van Yüzüncü Yıl University, Faculty of Aquaculture, Zeve Campus, 65090, Van, Turkey*
[2]*Van Yuzuncu Yıl University, Faculty of Science, Department of Biology, 65080, Van, Turkey*

[*]*Corresponding Author: ahmetsepil@yyu.edu.tr*

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ABSTRACT

This study aimed to investigate the larval ontogeny of rosy barb *Pethia conchonius* (Hamilton 1822). Embryonic and larval development stages were studied using the microscopy technique. Trials were carried out on 60 broodstock of rosy barb. Round and transparent fertilized eggs with a diameter of 867.6 ± 21.7 µm have started to hatch approximately 27 h after spawning at 26.3°C. The newly hatched larvae (n:30) have a mean total length of 4227 ± 265 µm and started exogenous feeding 4 days after hatching (DAH) and started taking particulate and powder feed at the end of 18 DAH. The early development growth formula of rosy barb has an exponential relationship model of $y = 3.8346e^{0.0313x}$ ($R^2 = 0.9112$, n = 140). Digestive tract differentiation, hepatopancreas, digestive tubes, and gill arches formations were examined by taking histological sections in the early life stages of the rosy barb. Rosy barb is a species whose production protocol is not difficult due to its short egg hatching period and early larval stage zooplankton feeding period.

Keywords: Cyprinidae, fish larvae, microscopy, ontogeny, ornamental fishes

INTRODUCTION

Ornamental aquaculture, once a hobby, has now evolved into a significant agricultural sector with a global appeal, attracting millions of enthusiasts and generating substantial economic returns. This growth has led to the emergence of major large-scale producers of aquarium fish in many countries (Hekimoğlu 2006). The increasing species diversity in the aquarium sector, coupled with the demand for equipment and maintenance materials, has created a new commercial field, further expanding the sector's trade volume on a global scale (Reid et al. 2013; Hunt and Koca 2014).

In aquarium fisheries, many species belonging to different families are used in aquariums prepared with various concepts. Increasing the diversity of aquarium fish species and introducing new

species by determining breeding procedures is very important for the sector. Expanding the breeding of ornamental fish species instead of hunting them from the natural environment is crucial in reducing hunting pressure in natural populations. Ontogeny studies are essential in determining rearing and larval feeding procedures. Therefore, this study aimed to reveal the embryogenic and larval development of an ornamental fish belonging to the Cyprinidae family. Cyprinidae is one of the most important families, and it includes many economical and popular species used as ornamental fish, represented by 161 genera and 1727 species worldwide (Fricke et al. 2024). Although they are generally freshwater species, they are also distributed in brackish waters and marine coastal areas (FishBase 2022). A member of the Cyprinidae family, the rosy barb, was used in this study. The rosy barbs are popular among aquarium fishes due to their visual beauty and compatibility with other small fishes.

Rosy barbs are typical in Asia, specifically in Afghanistan, Pakistan, India, Nepal, Bangladesh (Talwar and Jhingran 1991), and Myanmar (Oo 2002). Individuals of this species can reach a maximum length of 14 cm, and the female is larger than the male (Talwar and Jhingran 1991). Male of this species, typically silver, take on a striking claret-red color, especially during the breeding season (Mills and Vevers 1989; Allen et al. 2002). Their food comprises worms, crustaceans, insects, and plant matter (Allen et al. 2002).

The highest losses in the culture of ornamental fishes occur in the early larval stages. For this reason, knowing the larval development stages is imperative. Fish larvae are generally transparent in their developmental processes up to the postlarval stage, allowing microscopes to be used in ontogeny studies (Sepil et al. 2022). Examination of various tissues and organs formed due to the development of the larvae generally necessitates the use of histological methods. It is essential to use microscopy methods to determine the early larval feeding procedures, monitor metamorphosis, and determine the stages during mouth opening, swim bladder formation, differentiation of the digestive tract, and formation of a functional stomach.

In this study, embryonic and early larval development and allometric growth measurements of rosy barbs were examined microscopically. In this regard, it is aimed to reveal important metamorphosis stages such as the structure of eggs, hatching time, yolk sac absorption time of larvae, exogenous feeding time, determination of larval feeding protocol, development of tissues and organs and their transformation into functional structure.

METHODS

The broodstocks used in this study were obtained from adult rosy barbs (from Van Yüzüncü Yıl University, Faculty of Fisheries, Aquatic Creatures Experimental Unit, Türkiye) with a total length of >4 cm. A set of broodstocks (n: 6) were placed into separate aquariums with 50 L volumes according to identified sexes, where each aquarium was stocked with 5 female and 5 male broodstocks. Commercial flake and granule feeds were used at certain rates (10% Tetra discus granule and 90% Tetra pro-energy flake) in feeding approximately 4% of body weight twice a day (Gosh et al. 2008). Newly hatched Artemia sp. nauplii and bloodworms were fed to adapt the fishes to granular and flake feeds. The average water temperature in aquariums was 26.11 ± 1.16°C, pH at 8.12 ± 0.27, electrical conductivity (EC) at 851 ± 114.6 µS/cm, salinity at 0.41 ± 0.13 mg/L, and dissolved oxygen (DO) at 7.22 ± 0.15 mg/L during the acclimation process. Eggs and larvae of rosy barbs, produced after a certain conditioning period, were examined under the microscope. Then, histological examination was performed after the samples were prepared using appropriate fixative and alcohol series, and sections were taken from paraffin blocks.

Spawning of Broodstock Rosy Barbs

Breeding was carried out in 40 L glass aquariums with a water temperature of 26.3 ± 0.4°C, EC at 49.1 ± 3.2 µS/cm, and pH at 6.90 ± 0.10 after 2 weeks of conditioning. The broodstocks were placed into 1 cm mesh cages with 1 female and 1 male fish to prevent egg predation. After the eggs were observed on the aquarium floor, the broodstocks were removed from the tank, and egg sampling was carried out.

Egg and Larva Sampling and Preparations

The day after hatching (DAH) occurred was considered the first day of the larvae. Egg samples taken from the same broodstock rosy barb in a single batch were sampled at 6, 12, and 24 hours and just before egg hatching. Furthermore, larvae (n: 30) were sampled every day in the first 10 days and once every 2 days from the 10th day until the 50th day. Ten randomly selected eggs and larvae were fixed with Bouin's solution (Sigma-Aldrich, USA) during each sampling time. Eggs and larvae were first soaked in 250 mg L^{-1} of Tricaine Methanesulfonate (MS-222) for euthanasia for about a few minutes (AVMA 2007; Topic-Popovic et al. 2012) and then fixed in Bouin's solution (Moore et al. 2002; Ünal 2010) for 24 hours at room temperature. Following the dehydration process in the alcohol series, the samples taken into base molds were embedded in paraffin. Sections with

a thickness of 5 µm were obtained from paraffin blocks using the rotary microtome device (MICROM HM 315, Walldorf-Germany) (Onal et al. 2008; Santos et al. 2016). Thin sections (5 µ) with Haematoxylin-Eosin and Trichrome staining were examined under a light microscope to determine embryonic and larval developmental stages (Onal et al. 2008 Santos et al. 2016; Aminaghaie and Esmaeili 2017). Morphometric measurements of samples were performed using ImageJ 1.46r software (Onalan and Sepil 2024). The first egg diameter, the number of oil droplets, the pigmentation pattern seen in the egg and larva, the egg hatching period and developmental stages, the first larva size, the absorption time of the yolk sac, the opening of the mouth and anus and the length of the first mouth opening were studied. Mouth gap sizes were examined in fish larvae when the first exogenous feeding begins; the vertical distance between the jaws and the distance between the horizontal joints were measured at the point where the mouth was fully opened (Ramezani Fard et al. 2011; Riar et al. 2018). Allometric growth parameters for total length (TL), head length (HL), tail length (TLL), eye diameter (ED), Pre-anal myomere length (PrAM) and Post-anal myomere length (PoAM) changes were determined for the samples (Figure 1).

Allometric growth models are defined by linear regression formulas, which are determined by associating related body regions with total length (TL) (Gisbert et al. 2002; Çelik et al. 2011; Sepil et al. 2022). According to total height ratios, meristic growth characters such as HL, TLL, ED, PrAM, and PoAM were estimated using the allometric equation below;

$$Y = a \times (W^b)$$

Y = Measured character, W = Independent variable (TL), a = Intersection point, and b = Growth coefficient (Gisbert et al. 2002).

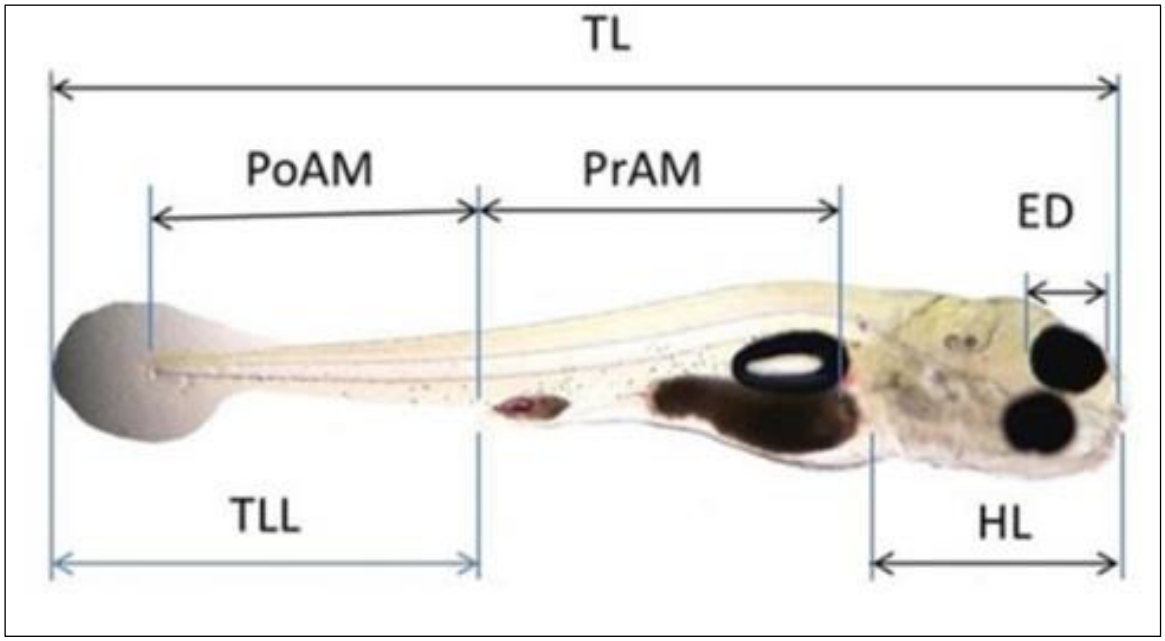

Figure 1. Some meristic growth characters on larvae (TL: total length, PoAM: Post-anal myomere length, PrAM: Pre-anal myomere length, ED: eye diameter, TLL: tail length, HL: head length).

RESULTS

Spawning and Egg Characteristics

It has been observed that the movements of female slow down considerably during reproduction and before laying eggs, while male is more active throughout the entire water column. However, it was observed that the colors of the female were brighter and darker red before breeding, while there was no change in the coloration of the male.

The experimental broodstocks were observed to spawn 12 times, and spawning was carried out at different times from each group during the trial. Different female individuals were used in each breeding process. The female broodstocks with the fullest abdomen from the stock aquariums were taken

into the breeding cage. Eggs were observed on the ground 12-15 hours after the broodstock were placed in the production cages. For the eggs to be fully fertilized, the distance between the production cage floor and the aquarium bottom was kept short, and after spawning was completed, the broodstock was kept in the cage for another 2 hours. In all breeding trials, fecundity was determined to be 224 ± 13 eggs/female. The eggs were observed to be round in shape and transparent (Figure 2). The samples examined within the first 3-6 hours from the release of eggs showed that the egg diameter was in the range of 646-1457 µm, and the average diameter was 867.6 ± 21.7 µm (n: 120). It was found that the water temperature values directly affect the egg hatching time in the decapsulation of eggs. In trials carried out at different temperatures, the relationship between temperature and egg hatching time is given in the graph in Figure 3.

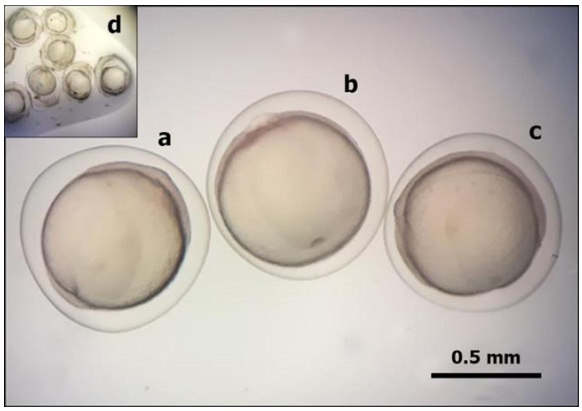

Figure 2. Shape and structure of eggs in different stages (a: 2 somite formation, b: Sphere stage, blastodisc flattened and multi-cell formation, c: embryo formation, d: embryo formation, pre-hatching).

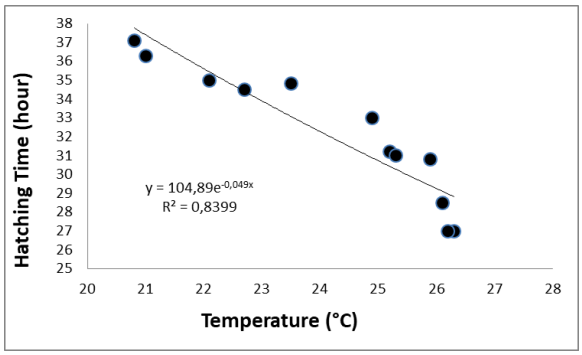

Figure 3. Relationship between temperature and egg hatching time in rosy barb.

Characteristics of Embryogenesis

In this study, all eggs were collected from a single batch. It has been observed that in fertilized eggs, blastodisc formation begins within the first 2 hours and the early morula stage occurs in the 3rd hour. It was determined that the formation of the 16-somite stage started at the 12th hour, and the 24-somite phase started at the end of the 24th hour. Within the 27th hour, the appearance of the embryo became completely clear, and at the earliest 28th hour, decapsulation of the eggs was observed. Figure 4 shows microscopic images of the stages of embryological development from fertilized egg to hatching stage and Table 1 details each stage of embryogenesis.

Characteristics of Larval Development

The total length of newly hatched larvae with yolk sacs was 3245 ± 23 µm. It was observed that the larvae fed endogenously with the vitellus until the 4th day. It was determined that the initial mouth gap size of the larvae was small (304 ± 33 µm). Therefore, the first feeding should be made with small particles such as rotifer or egg yolk, and it was observed that the mouth size became suitable for feeding with Artemia from the 8th day. The post-larval stage started on the 17th day and the transition to artificial powder feed occurred was observed.

Figures 5 and 6 show microscopic images of the sampled larvae. Morphometric values such as the pre-larvae's first total length, the yolk sac's diameter, and the first mouth gap size were measured throughout the development from the first hatching to the post-larval stage (Table 2).

Water quality during egg hatching and larval development was measured, with pH at 6.93 ± 0.4, EC at 49.1 ± 07 µS/cm, salinity at 0.04 ± 0.01 mg/L, DO at 7.34 ± 0.12 mg/L, and temperature at 26.30 ± 1.20°C.

It was determined that the larvae completely absorbed the yolk sac at the 68-71th hour, and mouth opening occurred at 4 DAH. At the opening of the mouth and anus, the yolk was entirely or almost exhausted. Larvae with newly opened mouth gaps are unsuitable for feeding on newly hatched *Artemia*, so crushed egg yolk was the first food. Since egg yolk was observed in the stomach and digestive tract of fed larvae, they started exogenous feeding at the end of the 4 DAH. In addition, it was observed that the larvae were generally motionless on the aquarium floor until 4 DAH, and they started to swim freely after exogenous feeding. The larvae used the entire water column from the 5 DAH. At the end of the 8 DAH, the last segments of the notochord have started to turn upwards, and the caudal fin is turned down, as in the veil tail appearance. The larvae reached the mouth gap

size (412 ± 16µm) that could consume *Artemia* at the end of the 8 DAH, and they fed on brine shrimp from the 9th day. It was observed that the swim bladder became constricted from the 11 DAH and took an utterly two-lobed appearance on the 13th day. The important ontogenic stages and feeding pattern observed during larval development are schematized in Figure 7.

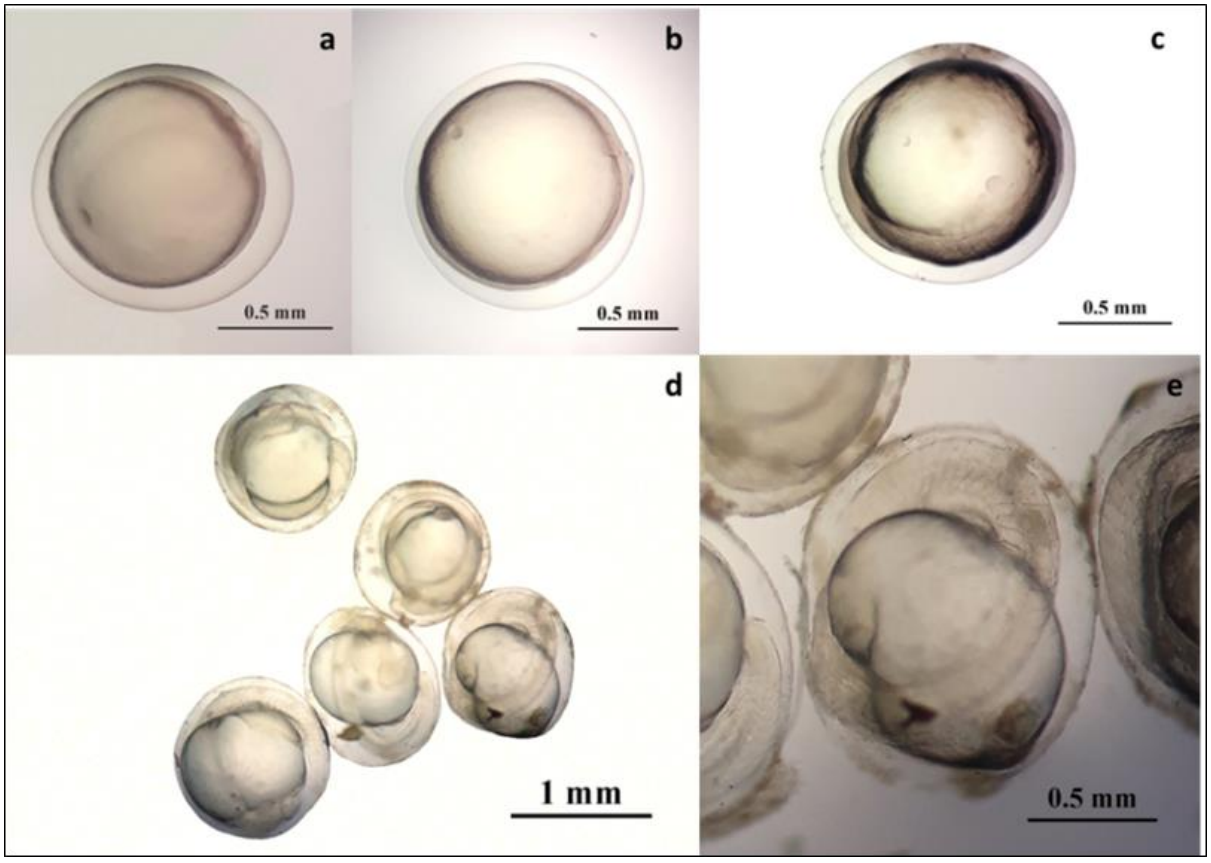

Figure 4. Eggs developmental stages from newly laid eggs to 27th hour. (a: 2nd hour, b: 6th hour, c: 12th hour, d: 24th hour, e: 27th hour).

Table 1. Descriptions of the embryonic development stages (VD: Vitellus diameter, CD: Chorion diameter, ED: Egg diameter. Eggs given embryonic development stages were incubated at 26.3°C and hatched in 27 hours).

Figures	Descriptions/Measurements
a	2nd hour, VD 712 µm, multi-cell (> 64) formation-early morula stage, CD 891 µm, PS 98.7 µm
b	6th hour, 2 somite formation, VD 1067 µm, CD 1213 µm, PS 87.2 µm
c	12th hour, embryo formation, VD 1127 µm, CD 1232 µm, PS 53.1 µm
d	24th hour, embryo formation, ED_1 1608 µm, ED_2 1227 µm, ED_3 1187 µm, ED_4 1197 µm, ED_5 1166 µm
e	27th hour, embryo formation ED 1247 µm

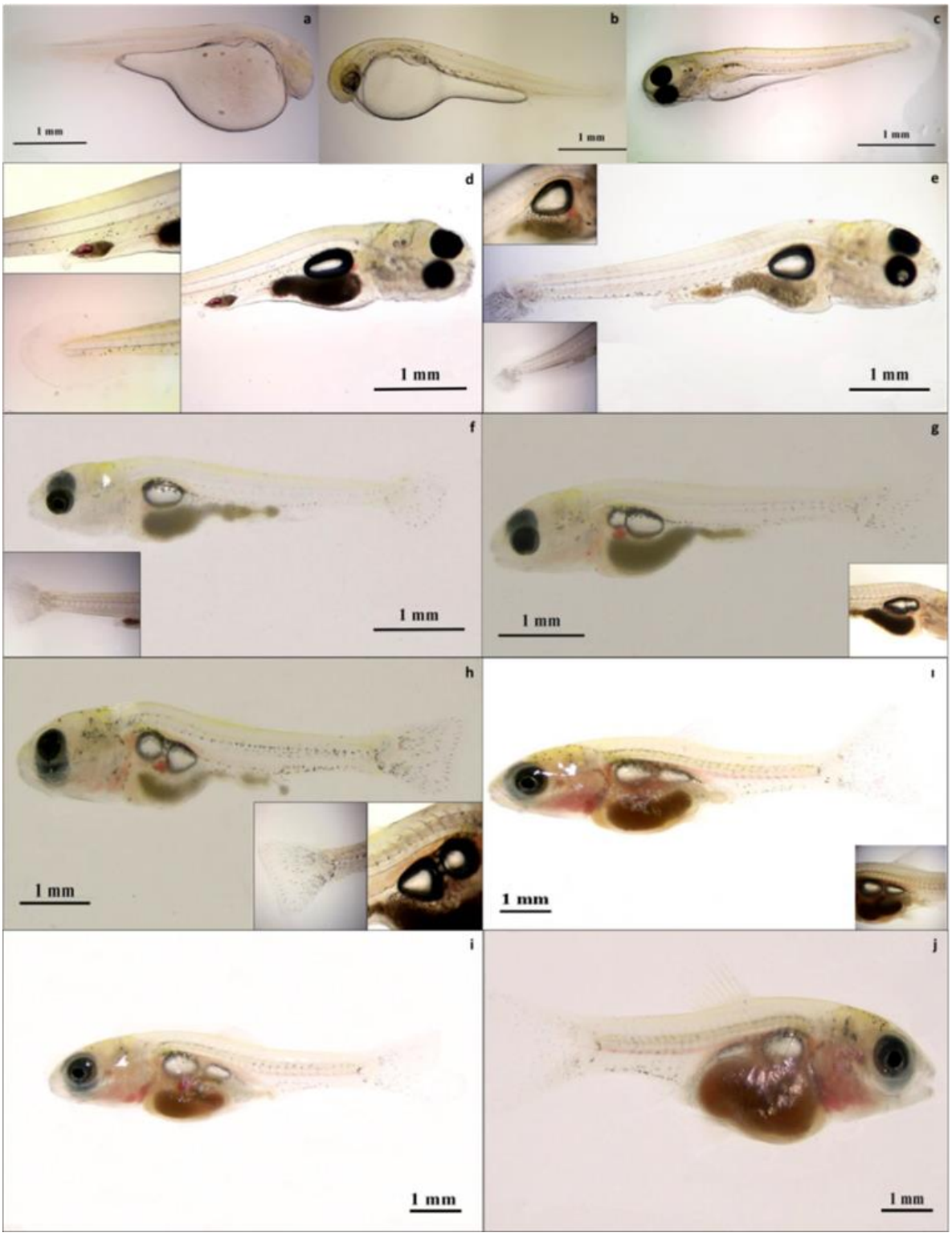

Figure 5. Developmental stages from newly hatched prelarvae to 25 days after hatching (DAH). (a: 1st hour, b: 24th hour, c: 3 DAH, d: 4 DAH, e: 8 DAH, f: 9 DAH, g: 11 DAH, h: 13 DAH, ı: 18 DAH, i: 21 DAH, j: 25 DAH, Scale bar: 1 mm).

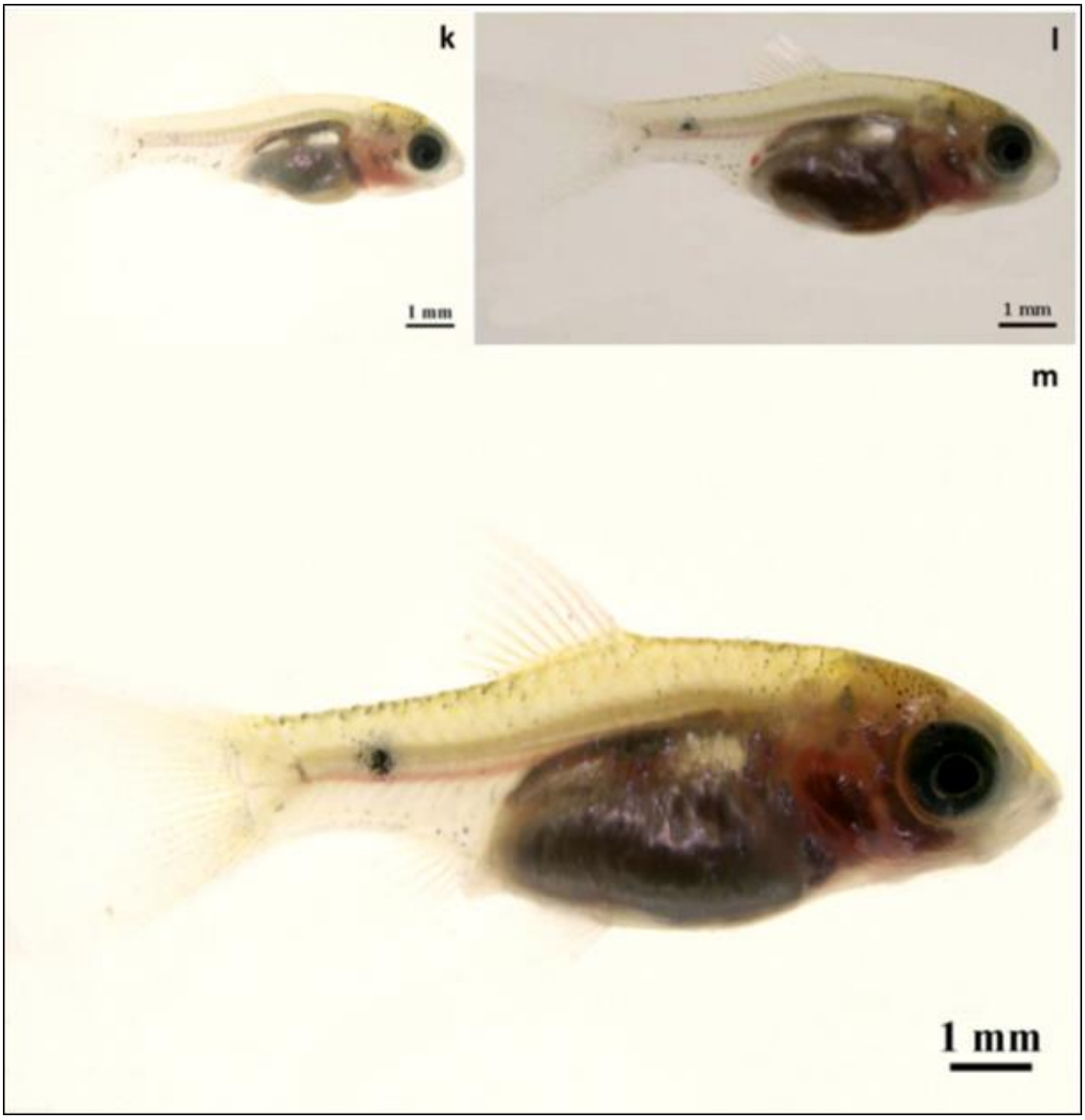

Figure 6. Developmental stages of post-larvae from 29 to 50 DAH. (k: 29 DAH, l: 35 DAH, m: 50 DAH, Scale bar: 1 mm).

Table 2. Descriptions of images about morphometric measurements (DAH: days after hatching, TL: Total Length, YD: Yolk Sac Diameter, MS: Mouth Gap Size. SA: Short axis, LA: Long axis. The larvae in the pictures were kept at 25.4-26.3°C throughout the sampling).

Figures	Descriptions	Figures	Descriptions
a	1th hour, TL 3842 µm, YD: SA 1264 µm, LA 2346 µm	**h**	13 DAH, TL 6400 µm, MS 598 µm
b	24th hour, TL 3977 µm, YD: SA 970 µm, LA 2513 µm	**ı**	18 DAH, TL 9205 µm, MS 801 µm
c	3 DAH, TL 3999 µm, YD: LA 1153 µm, SA 206 µm	**i**	21 DAH, TL 9227 µm
d	4 DAH, TL 4826 µm, MS 279 µm	**j**	25 DAH, TL 9338 µm
e	8 DAH, TL 5673 µm, MS 394 µm	**k**	29 DAH, TL 9570 µm
f	9 DAH, TL 5734 µm, MS 411 µm	**l**	35 DAH, TL 9896 µm

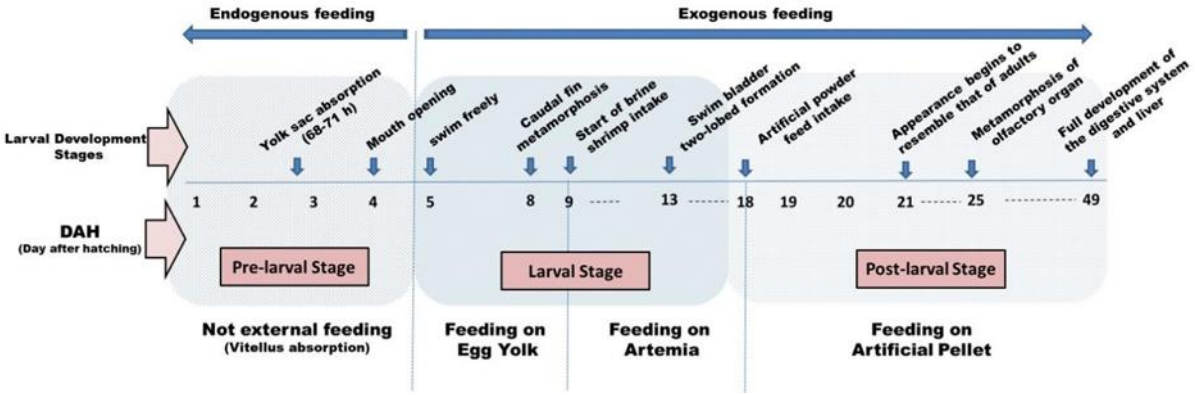

Figure 7. Significant ontogenic changes that occur from the pre-larval stage to the post-larval stage.

Larvae started taking particulate pellets at the end of the 18 DAH. Species-specific black dot pigmentation on both sides of the caudal peduncle was first observed on the 21 DAH. In line with the data obtained, the larvae's early-stage measurement and feeding procedures are summarized in Table 3.

When the larval development stages were evaluated histologically, the digestive tract was not differentiated and segmented on the 5th day, and the liver and the other organs became prominent at the beginning of the 6th day.

The swim bladder became constricted from the 7 DAH, and the digestive tract was differentiated into three regions: the esophagus, stomach, and intestine, which were separated from the 8 DAH. Furthermore, the esophagus became distinguishable at the end of the 8th day. From the 25 DAH onwards, it was observed that the metamorphosis of all nasal system structures was completed. Primarily, this system structure, such as the olfactory organ and olfactory bulb, became fully evident for the first time on the 25th - 27th day. The digestive tract, liver and heart were developed entirely on the 49th – 52th DAH (Figure 8).

The early stage growth formula of rosy barb calculated with the exponential relationship model is $y = 3.8346e^{0.0313x}$ ($R^2 = 0.9112$, n=140). Growth rates of body characters according to total length were estimated according to the allometric equation ($Y = a \times (W^b)$) (Figure 9). From the prelarval stage, HL and ED showed isometric growth, while PrAM and PoAM parameters showed positive allometric growth. From the postlarval stage, PrAM and PoAM showed negative allometric growth.

Table 3. Early larval morphometric measurements and feeding protocol up to postlarval stage (DAH: days after hatching, TL: Total Length, MS: Mouth Gap Size).

DAH	Morphological Measurements/Descriptions (n:120)	Feeding Procedures
1	TL min 3201, max 3271, 3245±23 µm	*not exogenous feeding*
2	TL min 3303, max 3324, 3317±7 µm	*not exogenous feeding*
3	The yolk sac absorption (Almost all of the larvae)	*not exogenous feeding*
4	Mouth opening, MS 304±33 µm	*Egg yolk*
5	MS 369±14 µm, TL min 4373, max 4411, 4379±11 µm	*Egg yolk*
7	MS 401±13µm, TL min 4703, max 4891, 4806±53 µm	*Egg yolk*
9	MS 423±11 µm, TL min 5656, max 5701, 5679±15 µm	*Egg yolk +Artemia*
18	MS 793±19 µm, TL min 7231, max 7506, 7368±95 µm	*Artemia +Particulate pellets*
20	MS 803±21 µm, TL min 8722, max 8889, 8814±53 µm	*Particulate pellets*

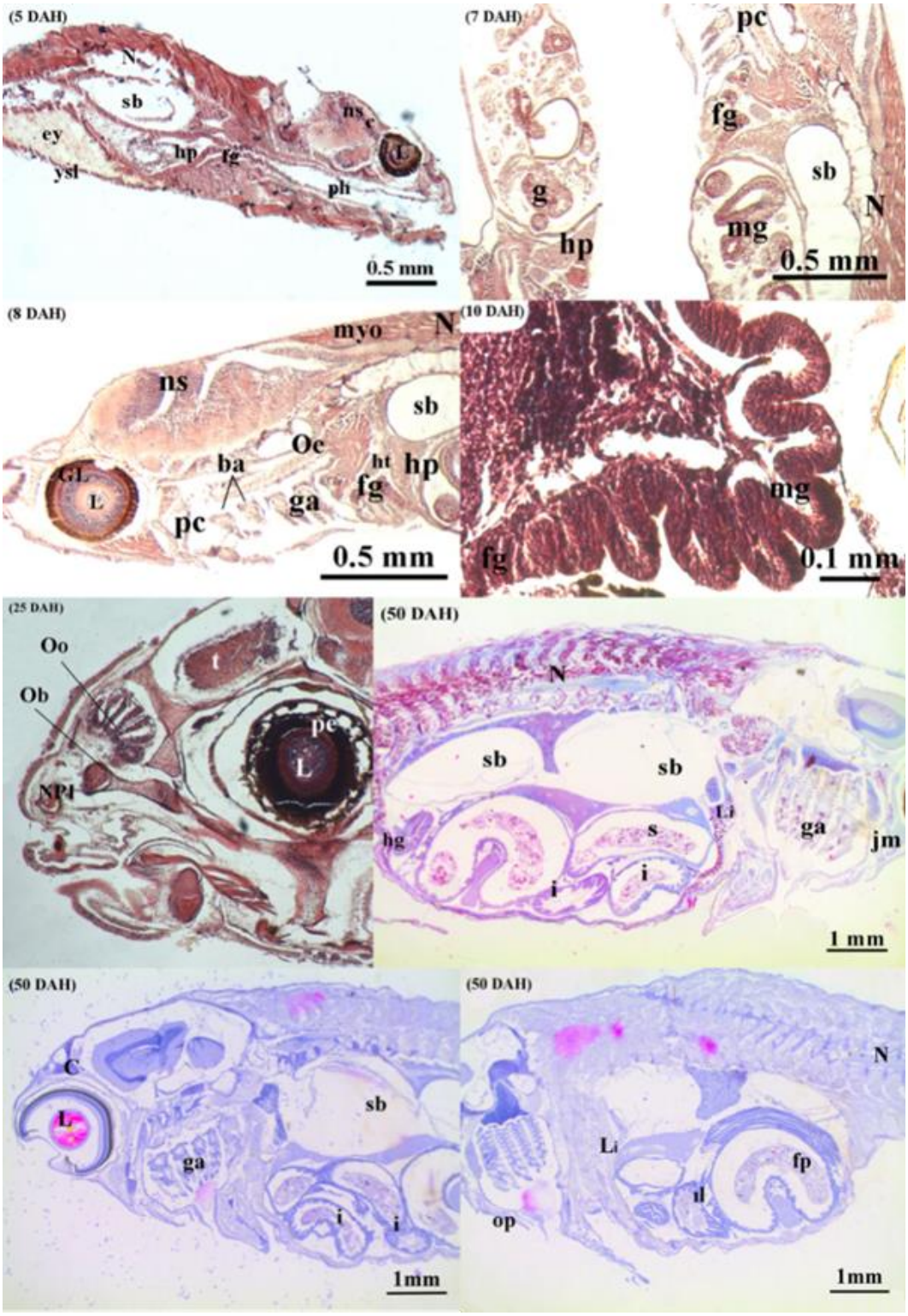

Figure 8. Histological images of larval development stages. (*sb: swim bladder, N: notochord, fg: foregut, hg: hindgut, mg: midgut, hp: hepatopancreas, pc: pharyngeal cavity, ga: gill arch, ey: egg yolk, ns: nervous system, myo: myotome, pe: pigment, ysl: yolk syncytial layer, L: lens, C: cartilages, ht: heart, g: ganglionic layer, ba: branchial arches, NPI: nasal pit, fp: food particles, Li: liver, ıl: intestinal loops, op: operculum, i: intestine, Oo: olfactory organ, Ob: olfactory bulb, t: telencephalon, jm: jaw muscle, s: stomach, GL: ganglionic layer, ba: branchial arches, Oe: esophagus, ph: pharynx).

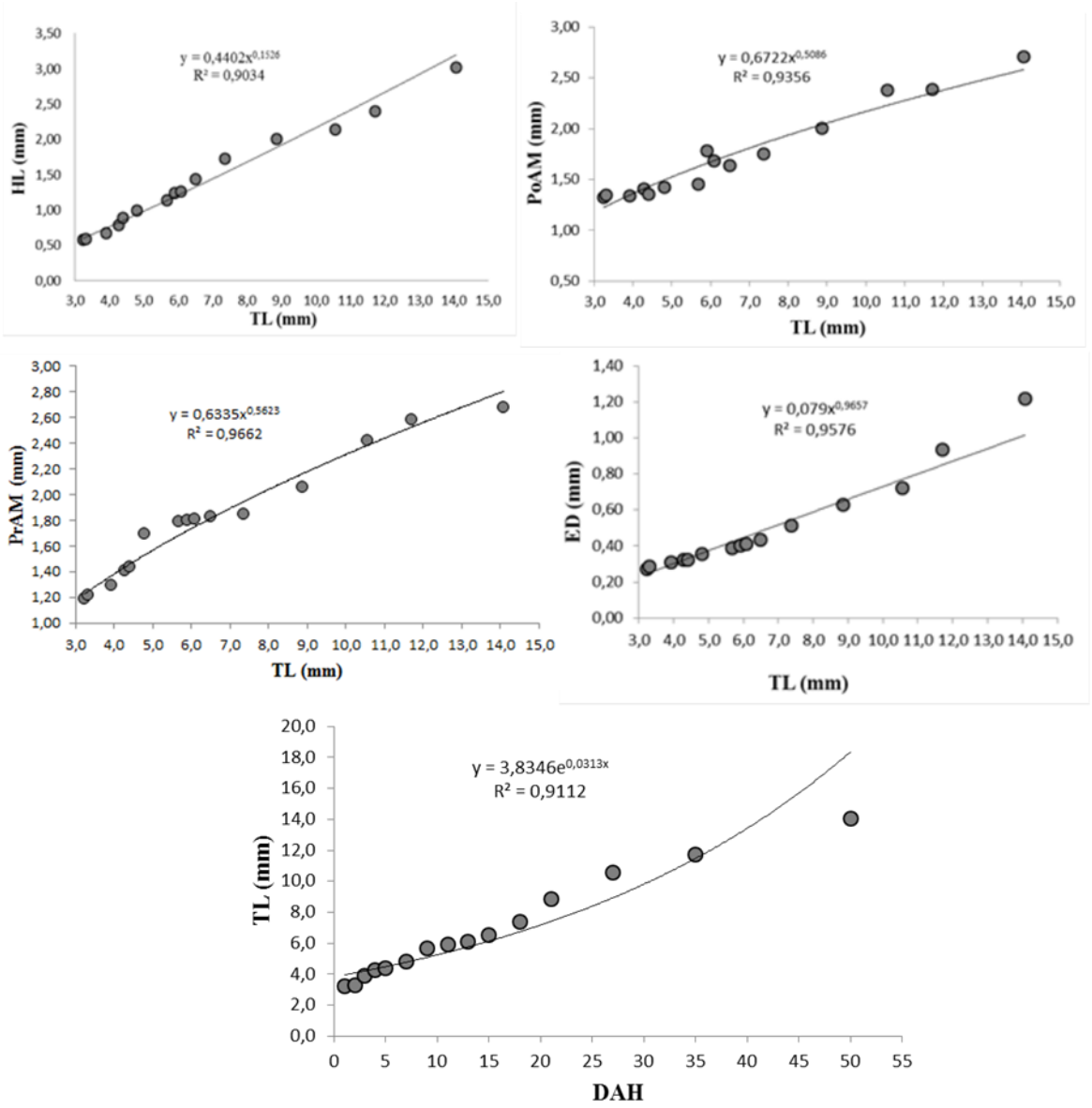

Figure 9. Allometric development curves of morphometric characters during the larval developmental stage and their relationship graphs according to total length (TL: Total Length, DAH: days after hatching).

DISCUSSION

Breeding and Egg Characteristics

In ornamental fish production, some manipulations in a positive way on water quality parameters (EC, temperature and pH that will positively trigger some reproductions are required in line with the requirements of the fish species. There are many studies on breeding ornamental fishes and larval development to determine the optimum water quality (Sezen and Olmez 2012; Sepil et al. 2022). In Amazonian discus species (*Sypmhysodon* spp.), EC at 50-200 µS/cm, pH at 3.9 - 5.7, and temperature at 28.6 - 30.2°C were found to have a reproductive-triggering effect (Çelik et al. 2008). The pH value is between 5.5 - 6.5, the EC value is in the range of 28 - 30 µS/cm and the using humic acid (0.04%) is essential in the breeding of Neon Tetra *Paracheirodon innesi* (Kucharczyk et al. 2010). Optimum breeding values for Tiger Barb *Puntius tetrazona*, which has very similar characteristics with rosy barb, water temperature, pH, and dissolved oxygen, have been reported at 28 ± 0.7ºC,

7.2 ± 0.12 and 6.4 ± 0.34 mg/L, respectively (Abolhasani et al. 2014). When rosy barb is evaluated (26.3 ± 0.4°C, EC 49.1 ± 3.2 µS/cm and pH 6.90 ± 0.10), it is seen that it does not need different special conditions for reproduction with very low pH and EC values, and it lays eggs under conditions similar to other barb species.

Embryonic and Larval Ontogeny

Following the absorption of the yolk sac, mouth opening occurs and exogenous feeding begins in larvae. When the larval metamorphosis of *P. conchonius* is examined, it is seen that the yolk sac is absorbed in a relatively short time (68th - 71st hour), and exogenous feeding begins (4 DAH) in the newly hatched pre-larva compared to other ornamental fish species. In the black neon tetra *Hyphessobrycon herbertaxelrodi*, the yolk sac is completely consumed on the 5th DAH and the average total length of the larva is around 3.70 mm (Çelik, et al. 2011), in the goldfish *Carassius* sp., the yolk sac is completely absorbed at the 81st hour (Savaş and Timur 2006), in the jaguar cichlid *Parachromis managuensis*) the vitellus absorption that occurs on the 4th day (Arık 2013) has been reported. The table in which some aquarium fish species critical early development stages are schematized and compared to the rosy barb is given in Figure 10.

The larva's newly opening mouth gap size (304 ± 33 µm, n: 30) is quite large compared to other species. This situation allows the egg yolk or rotifer-based feeding period to be short and the larvae to get *Artemia* in a shorter time (9 - 10 DAH). These features are considered important in the species' low early larval losses. However, the fact that it can be fed directly with *Artemia* after consuming egg yolk for only a short time makes the maintenance and production of the fish easy for amateur hobbyists.

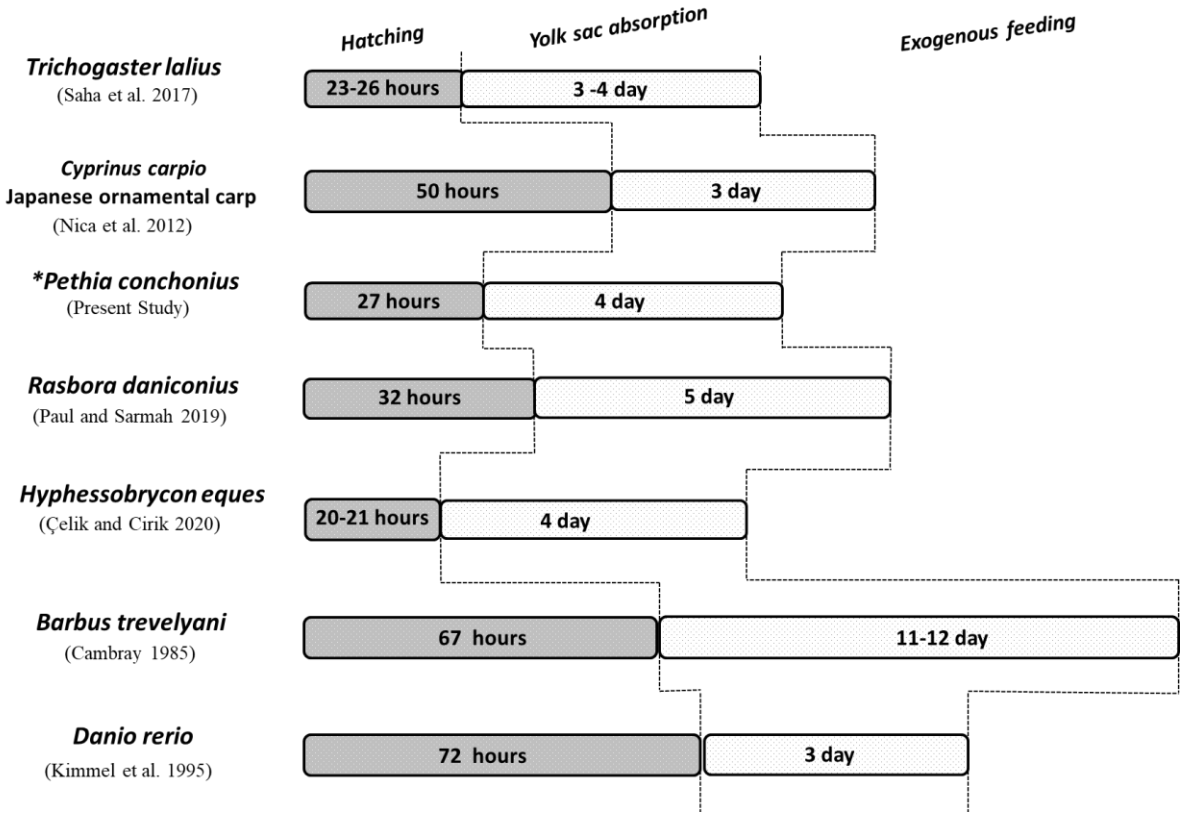

Figure 10. Egg hatching, endogenous and exogenous feeding periods in some important aquarium fish species.

In addition to microscopic examination of the development of fish larvae, there are histological methods to determine the development of tissues and organs in ontogeny studies. There are studies in which microscopic examinations are supported by histological methods in revealing larval ontogeny (Sarasquete et al. 1995; Monsefi et al. 2010; Santos et al. 2016). In the work, the stages of various tissue and

organ differentiation that cannot be visualized microscopically were determined in histological sections. The gill arch and pharyngeal cavity became prominent (5 DAH), the digestive tract differentiated into anterior and posterior intestines, and the hepato-pancreas became evident (7 DAH). The fact that this period is short ensures the formation of a functional stomach in a short time. The transition of rosy barb larvae from live food to artificial powder food takes place in a shorter time than many other ornamental fish larvae, and thus, the feeding time with live food appears to be shorter. This is so crucial in terms of low live food costs and larval losses in the culture of the species.

Allometric Growth Measurements

Allometric growth models are presented in this study, which reveal the allometric growth parameters in the larval stage of the rosy barb. This method is widely used to analyze the growth relationships in the early larval stages of fishes (Peña and Dumas 2009; Çelik et al. 2011). Allometric growth of fish groups in their larval stages was studied based on family and species (Osse and Van den Boogaart 2004). There is no scientific literature on the growth of many ornamental fish of commercial importance. It is thought that the data on the larval development of the rosy barb, which is a commercially essential and popular aquarium fish, will contribute to the knowledge.

Ontogeny and Feeding Relationship

Histological methods have been used for monitoring the development of tissues and systems in the early larval stages of fishes, significantly to determine the structural metamorphosis of the stomach and entire digestive tract and turn into a functional stomach (Onal et al. 2008; Ramezani Fard et al. 2011). Limited enzyme activity and an undeveloped digestive system in the early life stage of fish prevent powder feed consumption (Onal et al. 2008). This situation necessitates using various zooplankton species (*Artemia* sp., *Brachionus plicatilis* and infusoria) in this stage. The mouth gap size of the larvae determines which of these live foods will be used in the first feeding. Especially live foods are the highest cost in larval feeding in commercial fish culture (Onal 2006; Sepil and Şen 2024). Therefore, determining how long zooplankton feeding will be followed by powder artificial feeds depending on the mouth gap size and digestive system. So, these physiological and morphometric developments are closely related to the effective use of microscopic and histological methods in larvae ontogeny studies.

Generally, rosy barbs are thought to be among the species with high commercial returns that hobbyists easily prefer in mixed-species aquariums, especially due to their veil-tails, attractive colors, and ease of maintenance. In this respect, it is very important to know the larval development stages of this species and other ornamental fish species well for the aquarium fishing industry.

FUNDING

This study was conducted using university facilities without any institutional financial support.

ETHICAL CONSIDERATIONS

The study was carried out following experimental animal ethical rules, grand number 2021/11-10.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interests to any authors.

ACKNOWLEDGMENTS

This study was approved by Van Yuzuncu Yıl University, Animal Researches Local Ethic Committee in the season held on 25/11/2021 (Decision no. 2021/11-10). We also thank the editorial team and referees for their valuable help and suggestions in editing this article.

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ROLE OF AUTHORS: *AS – carried out the experimental design of the aquariums used in the trial, the feeding of the fish and the egg collection, conducted water quality monitoring throughout the trial, and performed daily measurements and allometric calculations of the larvae. ZAÇ and BEA – performed the histological processing and evaluated the sections. ZAÇ, BEA and ARO – performed immunohistochemical stainings and cell counts. AS and ARO – wrote and revised the manuscript. The final version of the text was read and approved by all authors.*

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Social capital, structural capital, human capital, spiritual capital, and cooperative performance: A path model

JR A. Mantog[1,*] and Annie L. Deriada[2]

[1]*Faculty, Davao Oriental State University – Cateel Extension Campus*
[2]*Faculty, Central Mindanao University*

[*]*Corresponding Author: jrmantog04@gmail.com*

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ABSTRACT

Cooperatives were created to foster productivity, food security, and safety. However, the literature revealed a significant number of non-operating cooperatives. It can also be noted that cooperatives have weaknesses in terms of sustainability and are unable to account for the role of intangible resources in the organization. Thus, this study investigated the effect of social capital, human capital, structural capital, and spiritual capital on cooperative performance as the basis for the strategic priorities of the cooperatives. Survey questionnaires were distributed among 201 randomly selected officers and members of six farmers' and producers' cooperatives in District I, Davao Oriental. Multiple regression analysis was utilized to determine the most influencing factors that affect performance, while path analysis was employed to generate the best fit model. Based on the findings, this study proposed a best fit model for cooperative performance which is based on intangible assets. In the best fit model, structural capital (0.37), social capital (0.29), and spiritual capital (0.11) showed a direct effect on cooperative performance. The model also showed that structural capital and social capital have an indirect effect on cooperative performance through spiritual capital. The findings of this paper would guide the cooperative sector to improve its performance by taking into account the role of intangible assets like structural capital, social capital, human capital, and spiritual capital, which are found to have direct and indirect effects in improving cooperative performance, thus helping the government in realizing its national economic goals.

Keywords: cooperative performance, human capital, social capital, spiritual capital, structural capital

INTRODUCTION

The cooperative sector has been found to play a significant role in the economic development of society through the creation and provision of employment opportunities, thus reducing the rate of poverty in the country (Hammad Ahmad Khan et al. 2016). The establishment of cooperatives will bring benefits and improve the standard of living in society, especially among low- and middle-income households.

Additionally, government servants, who are the majority members of the cooperative, can benefit by borrowing money from the cooperative in times of need (Hammad Ahmad Khan et al. 2016). A cooperative is an independent and properly recorded group of individuals with a common bond of interest. They voluntarily join together to realize their private, economic, and cultural needs and expectations by making equal contributions to the capital required and patronizing their products and services. Members embrace a good share of the risks and benefits of the undertaking following universally accepted cooperative principles (Republic Act 9520 2009).

In 2013, it was reported that there were more than 10,000 cooperative organizations in Malaysia, but their contribution to economic growth was relatively low and failed to achieve the goals set in the National Cooperative Policy. The weak performance of the cooperative sector has raised concerns about the factors affecting the cooperative's ability to overcome its shortcomings through effective policy implementation. In the master list of registered cooperatives of the Cooperative Development Authority (CDA) in the Philippines, as of 31 December 2016, approximately 38% of registered cooperatives in District 1 Davao Oriental had been dissolved. The reason for dissolution has not been studied. In the Davao Region, the number of cooperatives facing bankruptcy has soared. From 2012 to 2016, over 500 out of 4,000 cooperatives declared bankruptcy. This has raised the highest concern for financial institutions, which now prioritize reducing the risk of loan defaults (Cruz and Sabado 2022). The study by Masuku et al. (2016) concluded that the cooperatives were not performing well financially, as they were making losses. On the other hand, many cooperatives are performing well in terms of their financial performance. They are managed effectively and efficiently. The study by Castillo (2003) revealed that profitability, liquidity, and solvent operations were evident in the top four cooperatives in Region IX, which had been in operation for 25 years or longer, making them mature and solid. Assets and net surplus were in the millions of peso range, and the company's product lines had grown from feed milling to service provision, such as extension services, veterinary care, marketing, banking, production credit, meat processing, among other things. Despite the potential growth of cooperatives, the study by Deriada (2005) found that cooperative weaknesses in the identified important core organizational capacity indicators were savings mobilization, sufficient budget, innovativeness and skill development in entrepreneurship, participation of members, and continuous training and education. There has been limited study on cooperatives in Davao Oriental, specifically on the factors affecting the dissolution and failure of cooperative operations.

In the context of cooperatives, social capital, human capital, and structural capital are considered important resources that can affect their performance. Social capital refers to the network of relationships among individuals, organizations, and institutions, which can create norms of reciprocity and trust that enable collective action (Adler and Kwon 2002). Human capital refers to the knowledge, skills, and abilities of individuals that contribute to their productivity and creativity (Khalique et al. 2013). Structural capital refers to the organizational and technological infrastructure that supports knowledge sharing and innovation (Khalique et al. 2013). Nakhata (2018) defines spiritual capital as the spiritual strength (power) that controls and encourages people to act in any situation.

Several studies have explored the relationship between these forms of capital and cooperative performance. For example, some studies have found that social capital can facilitate information sharing and coordination among cooperative members, which can lead to better performance (Liang et al. 2018). Other studies have found that human capital, such as education and training, can improve the skills and productivity of cooperative members, which can also enhance performance (Hammad Ahmad Khan et al. 2016). Finally, some studies have examined the role of structural capital, such as technology and communication systems, in enabling cooperative members to share knowledge and collaborate effectively (Lv and Han 2015, Li et al. 2019).

The study by Hammad Ahmad Khan et al. (2016) also revealed that structural capital has a significant relationship with cooperative performance while human capital has been found to have a negative relationship. The study conducted by Ariyanto and Chalil (2017) revealed that human capital, structural capital, relational capital, and spiritual capital have a positive and significant impact on organizational performance. In addition, Hashim et al. (2015) found that intellectual capital, which consists of human capital, customer capital, structural capital, social capital, technological capital, and spiritual capital, has a significant influence on organizational performance.

The researcher has not come across published studies on path analysis regarding the effect of intangible resources on cooperative performance conducted in Davao Oriental, looking into the variables that contribute to cooperative performance and that will become the basis for strategic improvement for planners in maintaining the

economic growth and stability of the cooperative. Thus, this study would fill this gap.

This study investigates the relationship between social capital, human capital, structural capital, and spiritual capital on cooperative performance and aims to identify what are the most influencing factor of cooperative performance. Additionally, it seeks to determine the best fit model that significantly influences cooperative performance.

METHODS

Research Design

The study utilized a descriptive–correlational and causal-comparative research design which aimed to examine the causal relationships between a dependent variable, which is cooperative performance and four independent variables: social capital, human capital, structural capital, and spiritual capital (Maheshwari 2018). The survey approach was applied in this study. According to Ardales (2008), the survey is used when a researcher wants to collect data from a large population particularly those that cannot be directly observed. Its focus encompasses any measurement procedure wherein the researcher selects a sample of respondents from a population and administers a questionnaire to them.

The theoretical framework of this study is anchored in the resource-based view (RBV) theory. The RBV analyzes and interprets internal resources of organizations, emphasizing the importance of resources and capabilities in formulating strategies to achieve sustainable competitive advantages. According to RBV, an organization that possesses strategic resources has the potential to develop a competitive edge over its rivals. In this study, the internal resources of cooperatives – namely, social capital, human capital, structural capital, and spiritual capital – are examined under the assumption that they directly influence cooperative performance. The RBV asserts that not all resources of a firm contribute to competitive advantage, only those that are heterogeneous and immobile can be strategic (Utami and Alamanos 2022). Thus, by leveraging these unique resources, cooperatives may enhance their performance and sustain their competitive advantages.

Respondents of the Study

The respondents were the board of directors, committee members, and regular members of registered cooperatives in the first district of Davao Oriental. Farmers' cooperatives and other types of cooperatives have existed in the province for a long time. They contribute significantly to creating jobs, generating income, and providing essential services and products. From agriculture and fishing to trade and services, cooperatives are key drivers of economic activity, stimulating local development and offering livelihood opportunities. After Typhoon Pablo hit Davao Oriental, specifically in the eastern part, additional cooperatives were created and served as beneficiaries of the financial grants and assistance from the government and non-government organizations. Community linkages were created to foster productivity, food security, and safety; however, it can be noted that there is a problem in the cooperatives as literature revealed a significant number of non-operating cooperatives in the province. It can be noted that cooperatives have weaknesses in terms of sustainability and are unable to account for the role of intangible resources in the organization. Thus, there is a necessity to conduct research on the role of social capital, human capital, structural capital, spiritual capital, and cooperative performance of the cooperatives in District 1 of Davao Oriental.

The selection of Davao Oriental for the research study is justified because of its notable cooperative history, diverse cooperative sector, unique socioeconomic conditions, and the potential it holds to provide insights for policy and practical applications. By studying cooperatives in this particular region, researchers aim to develop a comprehensive understanding of cooperative operations and their impact on local development and governance mechanisms. The cooperatives were chosen as respondents of the study based on the following qualifications: a) must be farmers'/fishers' cooperatives, producer's cooperatives; b) must have been operating for at least five years and beyond; c) must have at least 15 regular members and above; and d) must be compliant with registration with the Cooperative Development Authority (CDA). Based on the criteria, there were six qualified cooperatives with a total of 419 members. The study applied stratified random sampling in selecting the respondents. The population was stratified based on the types of cooperatives (farmers' and producers' cooperatives), while proportionate stratified random sampling was employed to determine the number of respondents selected from each stratum. Random numbers were used to select respondents based on the sampling frame, which is the list of cooperative members. A Sample Size Calculator Online (Creative Research Systems 2020) was used to estimate the 201-sample size for a population of 419. The total population of 419 was entered into the system, along with the corresponding confidence level (95%) and a confidence interval of 5 or 5% margin of error; the system calculated and generated the 201-sample size.

Research Instrument

The survey questionnaire utilized in this study was adapted from different authors whose research works are in line with the variables being studied. The survey questionnaire consisted of structured and open-ended questions. The questions for social capital were adapted from the World Bank Social Capital Integrated Questionnaire (Grootaert et al. 2004). The questions for human capital and structural capital were modified questions from the survey questionnaire of Sharabati et al. (2010) and Hammad Ahmad Khan et al. (2016). The questions about spiritual capital came from the study of Harry Hui Ng Mok Lau et al. (2011). Furthermore, the questions for cooperative performance were derived from Mellor's study (2009). All variables were measured using a 5-point Likert scale, as shown in Tables 1 and 2. The questions were contextualized based on the cooperative setting in the province. The questionnaire was prepared in English and translated into the Cebuano dialect to be easily understood by the respondents. To test the reliability of the questionnaire, pilot testing was conducted to determine whether the questions were reliable and could be easily understood by the respondents. The initial analysis of the pre-tested questionnaire has produced a Cronbach Alpha value of 0.825. Consequently, eight items were deleted from the original 139 items to obtain the maximum reliability result of a 0.937 Cronbach Alpha value. This indicates that the pre-tested questionnaire was highly reliable. Further, the questionnaire was also subjected to a validity test with the help of an expert who reviewed the different questions and ensured their validity. The questionnaire was reviewed by an expert in the Cebuano language who provided the exact translation of the questions.

Table 1. Likert scale used in measuring social capital, structural capital, human capital and spiritual capital.

Scale	Qualitative Description	Range of Means	Qualitative Interpretation
1	Strongly Disagree	1.00 – 1.80	Very Weak
2	Disagree	1.81 – 2.60	Weak
3	Neither Agree nor Disagree	2.61 – 3.40	Moderate
4	Agree	3.41 – 4.20	Strong
5	Strongly Agree	4.21 – 5.00	Very strong

Table 2. Likert scale used in measuring cooperative performance.

Scale	Qualitative Description	Range of Means	Qualitative Interpretation
1	Strongly Disagree	1.00 – 1.80	Very Low
2	Disagree	1.81 – 2.60	Low
3	Neither Agree nor Disagree	2.61 – 3.40	Moderate
4	Agree	3.41 – 4.20	High
5	Strongly Agree	4.21 – 5.00	Very High

Data Gathering Procedure

Before conducting the study, the researcher sought permission from the Barangay Captain and Board of Directors (BOD) Chair of the cooperatives by sending a letter requesting to conduct the study in their barangay. In addition, an ethical clearance was secured from the university ethics board. After receiving approval for the letter and securing ethical clearance, the researcher located the participants, and data gathering commenced thereafter. There were two ways to administer the survey questionnaire to the respondents. The first method involved individual visits. The target respondents were visited individually at their homes. In consideration of the availability of the respondents (farmers and processors), the survey was conducted on Saturdays and Sundays, as these were their resting days. Follow-up visits were conducted for those who were unavailable during the first visit. If still unavailable during the follow-up visit, a replacement was selected from the shuffled names in the bowl. The other method involved group administration, where the survey questionnaires were administered in groups. The group also administered questionnaires after the end of the meeting or processing period, duly coordinated with the Chairman of the respective cooperatives. Enumerators from the municipality where the cooperative is located facilitated the administration of the questionnaire and ensured that respondents understood the instructions clearly. After the interview, the filled-out

questionnaires were retrieved, collected, tallied, tabulated, analyzed, and interpreted accordingly with the aid of a statistician (Abulela and Harwell 2019).

Data Analysis

In this study, descriptive and inferential statistics were employed. Data were processed by assigning code numbers to categorical variables for ease of statistical analysis.

The Pearson Product-Moment Correlation was utilized to determine the relationship among social capital, human capital, structural capital, spiritual capital, and cooperative performance, while multiple regression was utilized to determine the influencing factors among independent variables (social capital, human capital, structural capital, and spiritual capital) that most influence cooperative performance.

Path analysis is a multiple regression statistical analysis method used to assess causal models by investigating the relationships between a dependent variable and two or more independent variables. Using this method, one can estimate the magnitude and significance of causal connections between variables (Crossman 2019). This method was used to assess the best fit model for cooperative performance. For the causal model to be considered fit for cooperative performance, the following criteria were considered: the Comparative Fit Index (CFI) is equal to the discrepancy function adjusted for sample size. The CFI ranges from 0 to 1, with a larger value indicating better model fit. Acceptable model fit is indicated by a CFI value of 0.90 or greater (Hu and Bentler 1999). The Root Mean Square Error of Approximation (RMSEA) should be less than 0.05, which is related to the residual in the model. RMSEA values range from 0 to 1, with a smaller RMSEA value indicating better model fit. Acceptable model fit is indicated by an RMSEA value of 0.05 or less (Hu and Bentler 1999). The results of the analysis are presented using tables and graphs.

RESULTS

Relationship Between Social Capital, Human Capital, Structural Capital, Spiritual Capital, and Cooperative Performance

The statistical results of the Pearson Product Moment Correlation analysis are shown in Table 3. The correlation analysis implied that all the independent variables are highly associated with the performance of cooperatives ($P < 0.05$). The study found a strong, positive, and significant relationship between the three independent variables, namely social capital, human capital, and structural capital with cooperative performance, with r-values of 0.71 ($P < 0.05$), 0.75 ($P < 0.05$), and 0.80 ($P < 0.05$), respectively. On the other hand, spiritual capital shows a moderate, positive, and significant relationship with an r-value of 0.69 ($P < 0.05$).

Factors Influencing Cooperative Performance

Based on the result of the multiple regression analysis, the findings reveal that structural capital, social capital, and spiritual capital have a positive effect on cooperative performance, while human capital does not show a direct influence on cooperative performance (Table 4).

The results of this study showed that 73.10% of the variations in cooperative performance can be explained by the variation in social capital, human capital, structural capital, and spiritual capital, while the remaining 26.90% of the variations can be explained by other variables not incorporated in the study, such asthe role of tangible resources of the cooperative. The regression model of the study is shown as follows:

$$y = 0.936+0.370x\_1+0.288x\_2+0.109x\_3$$

y = cooperative performance
x_1 = structural capital
x_2 = social capital
x_3 = spiritual capital

This model represents that for each unit of structural capital (x_1), cooperative performance increases by 0.370 units, assuming other factors remain the same. Similarly, for each unit of social capital (x_2), cooperative performance increases by 0.288 units, while all other factors are kept constant. For every unit of spiritual capital (x_1), cooperative performance increases by 0.109 units, with all other factors remaining unchanged. The constant 0.936 is the intercept, which represents the cooperative performance value when all independent variables (structural, social, and spiritual capital) are zero.

Statistical Model for Cooperative Performance

Hypothesized model 1. Figure 1 depicts the hypothetical model of the study. It was used as a template in future iterations in finding the best fit path model of cooperative performance in terms of social, human, structural, and spiritual capitals. As shown in the figure, all explanatory variables have a direct effect on cooperative performance. Among all explanatory variables, structural capital has the highest effect with a path coefficient of 0.35, which confirms the result of the regression analysis. On the other hand, human capital has the lowest effect with a path coefficient of 0.07. This model can explain 93% of the data.

Table 5 shows the characterization of Figure 1 in terms of different parameters. It shows that all of the parameters exhibited by Figure 1 do not fit which implies that even if it can explain 93% of the data, it is not the best fit model of cooperative performance. For instance, the minimum discrepancy over degrees of freedom (CMIN/DF) is 98.073, which is far beyond the highest threshold of 2.00. Additionally, the Tucker-Lewis Index (TLI) has a negative value but requires being greater than 0.95.

Hypothesized model 2. Figure 2 shows the hypothetical model 2 of the study. The model is being improved by showing spirituality as having an indirect effect on cooperative performance. The one-headed arrow from spiritual to structural shows an indirect effect on cooperative performance through structural capital. As shown in the figure, structural capital still has the highest impact (0.37) on cooperative performance compared with the previous model, followed by social capital and spiritual capital. This result also confirms the result of the regression analysis that human capital does not directly influence cooperative performance. This model can explain 94% of the data

Table 3. Degree of the relationship between cooperative performance and social, human, structural, and spiritual capitals.

Types of Capital	Pearson r	Degree of relationship	p-value	Statistical Inference
Social Capital	0.71	Strong positive relationship	0.000	Significant
Human Capital	0.75	Strong positive relationship	0.000	Significant
Structural Capital	0.80	Strong positive relationship	0.000	Significant
Spiritual Capital	0.69	Moderate positive relationship	0.000	Significant

Table 4. Summary of stepwise multiple regression analysis.

Predictor	Coefficient	R square
Structural Capital	.370	0.731
Social Capital	.288	
Spiritual Capital	.109	
Constant	.936	

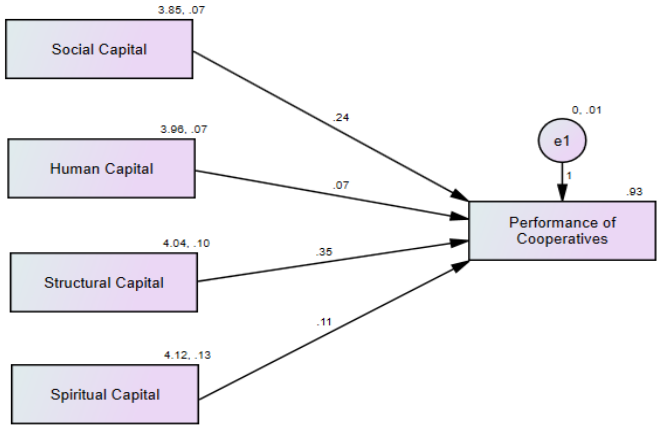

Figure 1. Hypothesized model 1 (CMIN/DF = 98.07, p-value = 0.000, GFI = 0.697, CFI = 0.308, TLI = -0.153, NFI = 0.309, RMSEA = 0.697).

Table 5. Characterization of hypothesized model 1.

Types of Model Fit	Parameters	Results	Critical Values	Remarks
Parsimonious Model Fit	CMIN/DF	98.073	< 2.00	Not Fit
Absolute Model Fit	p-value	0.000	> 0.05	Not Fit
	RMSEA	0.697	< 0.05	Not Fit
	GFI	0.410	> 0.95	Not Fit
Incremental Model Fit	CFI	0.308	> 0.95	Not Fit
	TLI	– 0.153	> 0.95	Not Fit
	NFI	0.309	> 0.95	Not Fit

Table 6 shows the characterization of Figure 2 in terms of different parameters. It shows that five (5) parameters which are p-value, Goodness of Fit Index (GFI), Comparative Fit Index (CFI), TLI, and Normed Fit Index (NFI) satisfy the required critical values. The remaining two (2) parameters do not satisfy. This implies that while Figure 2 can explain 94% of the data, as with the previous model, and showed a majority of the parameters, it is still not the best fit model of cooperative performance.

Hypothesized model 3. Figure 3 shows the hypothetical model 3 of the study. In this model, social capital indirectly affects cooperative performance through spiritual capital. As shown in the figure, structural capital still has the highest impact (0.37) on cooperative performance compared with the previous model followed by social and spiritual capital. This result also confirms the result of the regression analysis that human capital does not directly influence cooperative performance. This model can explain 94% of the data.

Table 7 shows the characterization of Figure 3 in terms of the different parameters. It shows that only the GFI and CFI satisfy the required critical values compared with model 2 which satisfies 5 parameters. This implies that it can explain 94% of the data similar to the previous model, and although it showed two (2) parameters that fit the model, it is still not the best fit model of cooperative performance.

Hypothesized model 4. Figure 4 shows the hypothetical model 4 of the study. In this model, structural capital (0.37), social capital (0.29), and spiritual capital (0.11) showed a direct effect on cooperative performance. The figure also depicts that structural capital and social capital indirectly affect cooperative performance through spiritual capital. Though human capital shows no influence on cooperative performance based on the results of the multiple regression analysis, this should not be discredited as part of the intangible resources of the firm that contribute to cooperative performance. Based on the model generated, human capital directly affects structural, social, and spiritual capitals and indirectly affects cooperative performance through these three (3) capitals. This model can explain 94% of the data. Table 8 shows the characterization of Figure 4 in terms of different parameters. It shows that all parameters of parsimonious, absolute, and incremental model fits, which are Degrees of Freedom (DF), p-value, Root Mean Square Approximation (RMSEA), GFI, CFI, TLI, and NFI fit the model. Thus, this model is considered the best fit model of cooperative performance.

DISCUSSION

Relationship between Social Capital, Human Capital, Structural Capital, Spiritual Capital, and Cooperative Performance

The study found that four variables have a significant positive relationship with the performance of cooperatives in Davao Oriental. The result of correlation analysis is consistent with the study of Ettehadi and Seyyedi (2016) that there is a strong positive relationship between social capital, human capital, structural capital, and spiritual capital with organizational performance. The study of Hashim et al. (2015) found that intellectual capital which consists of human capital, customer capital, structural capital, social capital, technological capital, and spiritual capital has a significant influence on organizational performance.

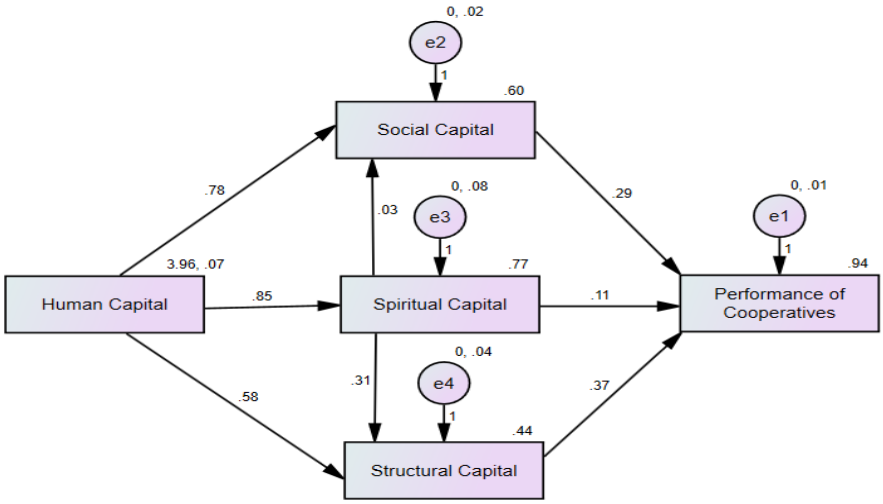

Figure 2. Hypothesized model 2 (CMIN/DF = 2.517, p-value = 0.081, GFI = 0.998, CFI = 0.996, TLI = -0.982, NFI = 0.994, RMSEA = 0.259).

Table 6. Characterization of hypothesized model 2.

Types of Model Fit	Parameters	Results	Critical Values	Remarks
Parsimonious Model Fit	CMIN/DF	2.517	< 2.00	Not Fit
Absolute Model Fit	p-value	0.081	> 0.05	Fit
	RMSEA	0.259	< 0.05	Not Fit
	GFI	0.998	> 0.95	Fit
Incremental Model Fit	CFI	0.996	> 0.95	Fit
	TLI	0.982	> 0.95	Fit
	NFI	0.994	> 0.95	Fit

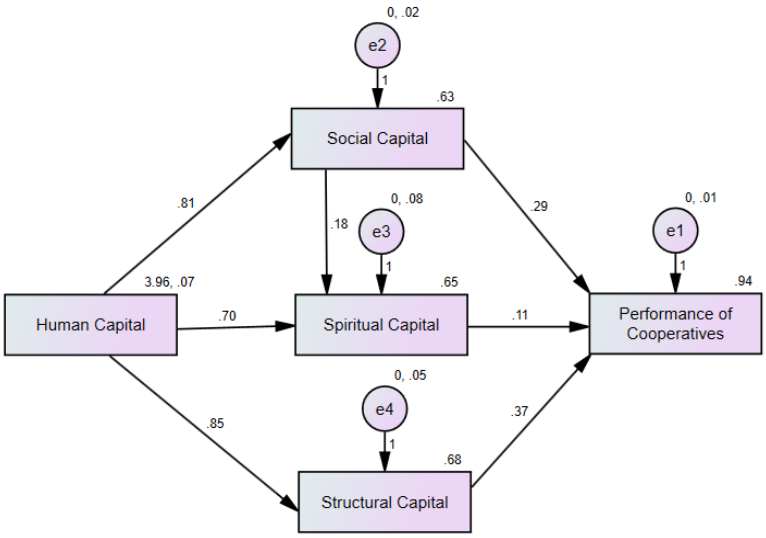

Figure 3. Hypothesized model 3 (CMIN/DF = 14.460, p-value = 0.000, GFI = 0.953, CFI = 0.952, TLI = -0.840, NFI = 0.949, RMSEA = 0.259).

Table 7. Characterization of hypothesized model 3.

Types of Model Fit	Parameters	Results	Critical Values	Remarks
Parsimonious Model Fit	CMIN/DF	14.460	< 2.00	Not Fit
Absolute Model Fit	p-value	0.000	> 0.05	Not Fit
	RMSEA	0.259	< 0.05	Not Fit
	GFI	0.953	> 0.95	Fit
Incremental Model Fit	CFI	0.952	> 0.95	Fit
	TLI	0.840	> 0.95	Not Fit
	NFI	0.949	> 0.95	Not Fit

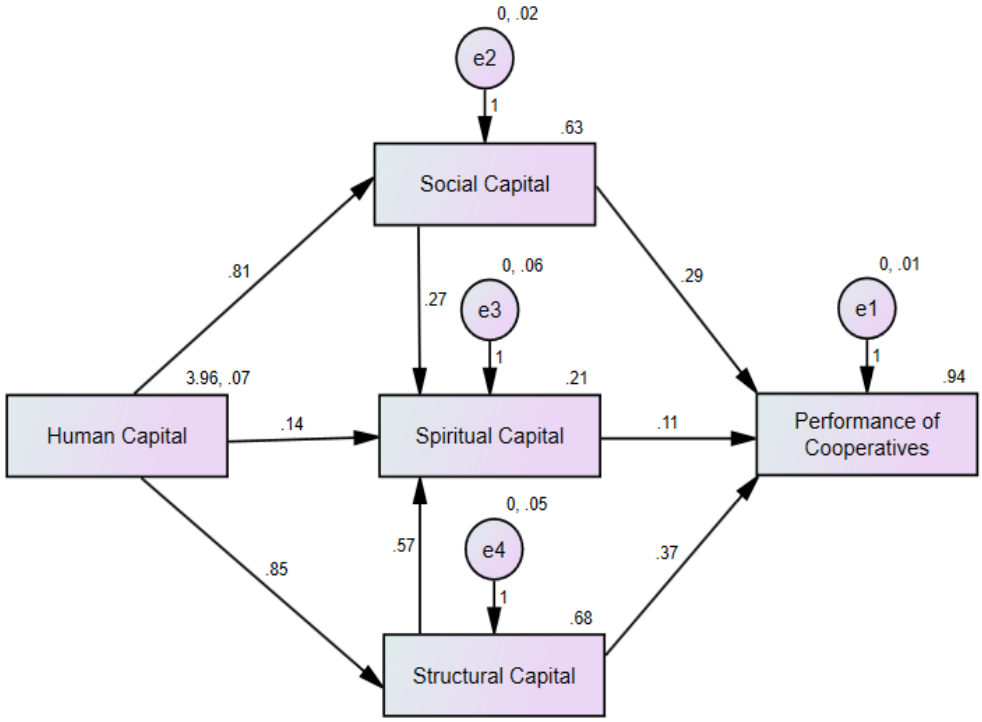

Figure 4. Hypothesized model 4 (CMIN/DF = 1.360, p-value = 0.257, GFI = 0.998, CFI = 0.999, TLI = -0.996, NFI = 0.997, RMSEA = 0.042).

Table 8. Characterization of hypothesized model 4.

Types of Model Fit	Parameters	Results	Critical Values	Remarks
Parsimonious Model Fit	CMIN/DF	1.360	< 2.00	Fit
Absolute Model Fit	p-value	0.257	> 0.05	Fit
	RMSEA	0.042	< 0.05	Fit
	GFI	0.998	> 0.95	Fit
Incremental Model Fit	CFI	0.999	> 0.95	Fit
	TLI	0.996	> 0.95	Fit
	NFI	0.997	> 0.95	Fit

Social Capital to Cooperative Performance. The result of the analysis reveals that cooperatives with strong social connections and networks tend to perform better (r = 0.71, $P < 0.05$). According to Liang et al. (2018), social capital has a significant and positive effect on the economic performance of cooperatives. Further, results also showed that there is a positive relationship between certain dimensions of social capital and members' participation in cooperative meetings and training. In the study of Sis et al. (2013), domains of social capital like social integration, social awareness, and infrastructure domains had the highest effect on the performance of cooperatives.

Structural Capital to Cooperative Performance. Based on the results of the correlation, cooperatives in Davao Oriental with well-established systems and processes perform better (r = 0.80, $P < 0.05$). Hammad Ahmad Khan et al. (2016) also revealed that structural capital has been found to have a significant relationship with cooperative performance, while human capital has been found to have a negative relationship, which is inconsistent with the results of this study.

Human Capital to Cooperative Performance. This study found that cooperatives with skilled and educated members tend to have higher performance (r = 0.75, $P < 0.05$). Moreover, the study conducted by Ariyanto and Chalil (2017) revealed that human capital, structural capital, relational capital, and spiritual capital are found to have a positive and significant impact on the organization's performance.

Khalique and Mansor's (2016) findings demonstrate that structural and social capital positively impact the hotel industry's performance in Malaysia. On the other hand, human capital, technological capital, and spiritual capital show insignificant contributions to enhancing the performance of the hotel industry. Spiritual capital has been found to have an insignificant contribution to performance, which is in contrast to the results of this study, in which this type of capital shows a significant contribution to organizational performance.

Spiritual Capital to Cooperative Performance. According to the results of the study by Moghadam and Makvandi (2019), a direct and strong correlation exists between spiritual capital and employee job performance. This is consistent with the results of this study that cooperatives that emphasize shared values and beliefs tend to perform better (r = 0.69, $P < 0.05$), but this relationship is slightly weaker than the others. This finding is in line with the findings of other researchers (Badakhshani 2017; Moghadam and Makvandi 2019) who confirmed the relationship between spiritual capital and organizational performance. Accordingly, it is highly suggested by different authors that promoting spiritual capital can improve the performance of organizations. Therefore, it is recommended that organizational managers promote factors such as spiritual valuation, speaking with God, spirituality, and spiritual influencing in the organization.

Factors Influencing Cooperative Performance

Among the explanatory variables, structural capital was the most influential factor contributing significantly to cooperative performance. Structural capital highlights the intangible resources held by the company in terms of processes and procedures, databases, work culture, and others that facilitate the workers' ability to create wealth for the organization and stakeholders, which would enhance the performance of cooperatives. The efficiency of this process is very significant because it includes internal procedures that allow for knowledge integration and sharing of capabilities, resulting in wealth creation for the organization (Azzahra 2018). It also remains an asset for the firm even when there are employees who will leave the firm (Muhammad and Ismail 2009; Khalique et al. 2013). According to Liang et al. (2019), social capital shows a positive effect on the economic performance of cooperatives, which supports the results of this study. Moreover, the study of Neubert et al. (2017) found that spiritual capital impacts business success even after controlling for other forms of capital. Therefore, it is suggested that cooperatives should always consider the proper implementation of the systems and programs of the cooperative. Besides, officers and members should be informed of the policies, procedures, and processes and their respective functions, duties, and responsibilities.

Human capital was found to have no significant influence on cooperative performance. This result is consistent with the study by Leal et al. (2014), which showed that human capital had no significant influence on organizational performance and satisfaction, unlike structural and relational capital. Additionally, the findings of Buang et al. (2023) indicated that the relationship between human capital and cooperative performance is not significant. In this study, human capital does not directly influence cooperative performance; however, it mediates the effect of other independent variables on cooperative performance. Similarly, the study by Birhane et al. (2023) reported that human capital plays a partial mediating role in the association between capital structure and organizational performance.

Statistical Model for Cooperative Performance

According to Liang et al. (2019), social capital shows a positive effect on the economic performance of cooperatives, which supports the results of this study. In their study, they concluded that social capital through the internal structure relationship enhanced performance and innovativeness. Social integration, as part of the social capital, is a vital part of the performance of cooperatives. In business, social capital contributes to an organization's success and is attributed to personal relationships and networks, both within the organization and outside of it. The term social capital promotes personal relationships within a company that help build trust and respect among employees, leading to enhanced company performance (Kenton 2019). The sense of cooperation, life satisfaction, security, empathy, respect of members for their beliefs, and capacity for differences are increased among members through educational production functions and market regulation (Sis et al. 2013). Furthermore, the social relationships in the organization are considered an intangible resource that can contribute to organizational success (Bhandari and Yasunobu 2009).

According to Obeidat et al. (2017), structural capital is concerned with the organization's structures and mechanisms, which eventually impact firm innovation, thus making it a vital organizational resource. Structural capital in this study shows a direct effect on cooperative performance. It implies that this intangible resource, which consists of assets that are intangibly included in the company's infrastructure technologies and organizational structure, enables the movement of knowledge to advance the operational effectiveness and efficiency of the firm (Ariawan et al. 2016). Hence, supportive infrastructure within a cooperative would improve its performance since this capital remains even if the employees leave the cooperative. Therefore, the cooperatives sector should focus on building up its infrastructure such as information systems, databases, and processes to ensure its success, especially in the long term.

The study of Neubert et al. (2017) found that spiritual capital impacts business success even after controlling for other forms of capital. Spiritual capital is an important asset for both individuals and organizations. It supplies a framework on how to govern business processes without conflicting with standards, ethics, and laws. Accordingly, it results in improved organizational performance (Abdullah and Sofian 2012). This capital also directly affects cooperative performance, implying that qualities rooted in an individual's perspective encourage their behavior to act accordingly; knowledge, confidence, and spiritual practices of an individual or an organization are vital to organizational success (Ariawan et al. 2016). Previous studies have found that spiritual capital serves as a guide on how to utilize human capital, structural capital, and relational capital in entrepreneurial activities (Sullivan 2000). This literature supports the result of the model that spiritual capital shows a direct effect on structural capital. Accordingly, spiritual capital promotes sustainable development and brings wealth and happiness to all the stakeholders of business firms (Florin et al. 2003). Spiritual capital is also important for both individuals and organizations. According to the results of the study by Moghadam and Makvandi (2019), a direct and strong correlation exists between spiritual capital and job performance in employees. This finding is in line with the findings of other researchers (Badakhshani 2017; Moghadam and Makvandi 2019) who confirmed the relationship between spiritual capital and organizational performance.

Despite no direct effect of human capital on cooperative performance, it indirectly affects social, spiritual, and structural capital. It cannot be discredited in modeling because there are many kinds of literature that state human capital contributes to cooperative performance but do not specifically clarify whether it is a direct or indirect effect. A recent study on Malaysian ministerial officers found that human capital and organizational performance are highly correlated (Tastan and Davoudi 2015). Researchers have argued that human capital and service delivery significantly contribute to organizational performance (Beh-Pajooh 2010; Saifuddin et al. 2014; Ferreira and Franco 2017; Neubert et al. 2017). Most researchers believe that firms that invest in human capital will have a competitive advantage over others (Beh-Pajooh 2010; Saifuddin et al. 2014). Furthermore, Cisneros and Perlines (2018) stated that human capital represents competence and an individual's potential, such as people-embodied knowledge, experiential knowledge, skills, and capabilities. It is also considered an attitude regarding motivation, behavior, and ethical conduct. Additionally, human capital can also encompass intellectual agility, includingadaptation, innovation, and imitation. Human capital comprises all other elements, includingcreativity, education/training, changeability, employee demographics, employee loyalty, entrepreneurial spirit, emotional intelligence, formal relationships, flexibility, influencing behavior identity of individuals, vocational qualifications, informal relationships, workforce training, and other work-related aspects (Cisneros and Perlines 2018).

Human capital directly affects social capital in the organization. In the process of economic growth, the investment in social fabric and human knowledge

creates economic progress. Economic growth is determined by human capital within social relationships (Soumyananda 2016). Despite the economic progress brought by human capital through social relationships in the organization, sentiments of not being needed anymore are evident in the social organization of displaced agricultural farmers due to mechanization (Banudan et al. 2023).

Human capital also has a direct effect on structural capital with path coefficients of 0.85. In the study by Namvar et al. (2011) exploring the role of human capital in a firm's structural capital in the Iranian e-business industry, it was found that human capital significantly influences innovation capital, relational capital, and process capital, which are the dimensions of structural capital. Additionally, structural capital stems from human capital. The mixture of knowledge and intangible resources derived from the procedures inside the business includes procedural innovativeness, access to information for codification into knowledge, and elements of efficiency (Edvinsson and Malone 1997). Model 4 also shows that human capital directly affects spiritual capital. Spiritual capital is possessed by individuals; thus, it is fundamentally noted in an individual's perspective. The qualities of humanspiritual capital boost behavior that aligns with the knowledge, confidence, and spiritual practices of individuals or organizations (Ariawan et al. 2016). This best fit model for cooperative performance is an effective framework for cooperatives to consider when structuring their strategic goals and priorities.

This study has proven that the resource-based theory is significant to organizations, where the internal resources have the opportunity to influence cooperative performance and sustained competitiveness against rivals. The cooperatives' internal resources, namely social capital, human capital, structural capital, and spiritual capital, have influenced the performance of cooperatives. This means that these capitals, directly and indirectly, affect the productivity and profitability of the cooperative. Therefore, community planners, policymakers, extension workers, and government agencies must consider these capitals as vital for cooperative success. Since this study found that structural capital is the most influential factor in cooperative performance, government agencies such as the Cooperative Development Authority can provide training and programs to strengthen the structural capital of cooperatives. These may include capacity-building initiatives for officers and members, the development of systems to enhance cooperative operations, and strict monitoring and evaluation of the implementation of these systems and processes. It is also recommended that cooperatives undergo ISO Certification, which would help strengthen their processes and procedures. This certification will strengthen the processes and procedures of the cooperatives. This study only focused on a successful cooperative; therefore, the researcher recommends further research on the effects of social capital, human capital, structural capital and spiritual capital on the performance of cooperatives from the perspective of passive or failed cooperatives. Additionally, investigating the roles of both tangible and intangible assets in different types of cooperatives, such as agricultural cooperatives, credit cooperatives, multi-purpose cooperatives, and others, would be interesting.

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ETHICAL CONSIDERATIONS

As part of the ethical considerations in conducting the study, the researcher secured prior informed consent before the participants answered the survey questionnaire. This showed proof of their voluntary participation in the study, being informed of the purpose of the study, the risks, and the benefits of participating in the survey. Moreover, the confidentiality of data was given utmost consideration. The risks in conducting the study were identified and proposed ways to minimize them. The respondents were located in other municipalities far from the researcher's location. A personal vehicle was used during data collection for ease and safety. Wearing personal protective gear was also practiced to protect against possible vehicle accidents resulting in injury.

The peace and order situation of the area was also considered. To minimize the risks of possible violence, the researcher closely coordinated with the Punong Barangay to ensure peace and order in the area during data collection. Most of the respondents did not provide accurate information due to confidentiality matters. The anonymity of the respondents was respected to uphold privacy and preserve dignity during data collection and analysis.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests for any authors.

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Representasyon ng piling editoryal karton sa panahon ng pandemya: Isang eco-critical discourse analysis

Arnel T. Noval

College of Arts and Sciences, Departamento ng Filipino at Ibang mga Wika, Cebu Technological University - Main Campus, Cebu City, Philippines

Corresponding Author: arnelnoval663@gmail.com

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ABSTRAK

Nilayon ng pag-aaral na mahimay ang diskurso ng piling editoryal karton sa panahon ng pandemya bilang kabahagi ng diskursong ekolohikal. Tiniyak din nitong masuri ang mga tampok na semyotika (*semiotic features*) ng piling editoryal karton na mabisang komponent sa pagpapaabot ng diskursong may kinalaman sa kapaligiran, mga isyung pangkalikasan at panlipunang dulot ng *COVID-19* pandemya. Gumamit ang pag-aaral ng *eco-critical discourse analysis* sa pagsusuri sa semyotika ng limang piling editoryal karton ayon sa sitwasyon, wika at *semiotic strategies*, target, lohikal na mekanismo, at *script opposition* at binigyang pagpapakahulugan ang mga lantad at di-lantad na mensaheng ipinapahiwatig nito. Natuklasan sa pag-aaral na ang nangungunang diskursong ekolohikal na nasipat sa piling editoryal karton ay may kaugnayan sa paglaganap na dulot ng *COVID-19* pandemic at nang walang habas na pagputol ng mga puno na naging sanhi ng pagbaha sa bansa. Ang pandemyang ito ay nagsiwalat sa iba pang mga isyung panlipunang kinahaharap ng bansa gaya ng mababang pasahod sa mga nars, walang kasiguraduhang pagpapatupad sa mga naipangakong proyekto/programa ng gobyerno, at ang mataas na bayarin sa kuryente na ipinalutang sa mga lantad na mensaheng ipinahiwatig ng mga nakasulat na teksto at mga di-lantad na mensaheng ipinahihiwatig ng mga guhit at ekspresyon sa mukha ng mga karakter sa karton. Bilang kongklusyon, ang semyotika kabilang na ang *linguistic*, *semiotic*, at *visual tools*, at iba pang aspektong berbal at di-berbal ay nakatutulong sa paghahatid sa mensaheng nais ipabatid ng *cartoonist.* Ang paggamit ng pang-uuyam at nakatatawang ekspresyon sa mukha ng mga karakter sa editoryal karton ay isang mabisang kasangkapan sa pagpapalutang ng diskursong pangkapaligiran at isyung pangkalikasang kinakaharap ng lipunan sa panahon ng pandemya. Inirerekomenda ng pag-aaral na magsagawa ng mas malawakang pag-aaral sa diskurso ng editoryal karton sa bawat rehiyon ng bansa gamit ang *eco-critical discourse analysis*, *ecolinguistics*, *green studies*, at iba pang lapit sa pagsusulong sa pangangalaga at preserbasyon ng kalikasan.

Mga Susing Salita: editoryal, ekolohikal na diskurso, karikatura, pagpapakahulugan, semiotic features, visual metaphor

Representation of selected editorial cartoons during the pandemic: An eco-critical discourse analysis

ABSTRACT
(English)

This study aims to examine the discourse of the selected editorial cartoons during the pandemic as part of the ecological discourse. It also ensures to analyze the semiotic features of the editorial cartoons, which are an effective component in conveying the discourse related to environmental and societal issues caused by the COVID-19 pandemic. The study utilized eco-critical discourse analysis to analyze the semiotic features of five selected editorial cartoons according to the situation, language, semiotic strategies, target, logical mechanism, and script opposition, which were used to interpret the intentional and unintentional messages they convey. The study revealed that the leading ecological discourse found in the selected editorial cartoons was related to the outbreak caused by the COVID-19 pandemic and the reckless cutting of trees, which resulted in flooding in the country. The pandemic has revealed other social issues that the country is facing, such as low wages for nurses, uncertain implementation of government-sponsored and high electricity bills, conveyed in the intentional messages in the written texts and the unintentional messages in the drawings and characters' facial expressions in the cartoons. In conclusion, the semiotic features, including linguistic, semiotic, and visual tools, along with other verbal and non-verbal aspects, help impart the message that the cartoonist wants to convey. This only proves that the use of sarcasm and humorous facial expressions of the characters in the editorial cartoons are effective tools in unveiling the environmental discourse and issues faced by society during the pandemic. The study recommends conducting more comprehensive research on the discourse of editorial cartoons in each region of the country using eco-critical discourse analysis, ecolinguistics, green studies, and other approaches to promote the conservation and preservation of nature.

Keywords: caricature, editorial, ecological discourse, meaning, semiotic features, visual metaphor

INTRODUKSYON

Ang *COVID-19 o coronavirus disease 2019* ay nakaapekto sa lahat ng aspekto ng mga gawain ng tao sa buong mundo mula sa edukasyon, pananaliksik, isports at iba pang larangan. Kabilang din ang transportasyon, pagsamba, sosyal na pagtitipon/interaksyon, ekonomiya, pagnenegosyo, at politika. Sa katunayan, ang buong mundo ay nasa pagkabalisa dahil sa banta ng pandemya, ang reyalidad ng sitwasyon ay mapanghamon at ang sektor ng edukasyon ay nananatiling isa sa pinakanaapektuhan ng paglaganap ng *COVID-19* (Onyema et al. 2020). Sa Pilipinas, ang pandemya ay nagdulot ng maraming problema sa bansa na nakaapekto hindi lamang sa sektor ng kalusugan maging sa lahat ng mga aspekto ng pambansang buhay. Naapektuhan nito ang pamamalakad sa gobyerno, pagpapatakbo ng negosyo sa industriya, at buhay ng mga ordinaryong tao sa bansa (Manila Bulletin 2020).

Walang bansa o lahi sa mundo ang sanay mula sa pandemyang dulot ng *COVID-19.* Ang mundo ay tila nabalisa sa mabilis na pagkalat ng epekto nito. Sa loob lamang ng ilang buwan ng paglaganap, binago nito ang pamumuhay ng pangkalahatan, bilyon-bilyong katao ang napilitang manatili sa kanilang mga tahanan, mag-obserba ng *self-isolation*, magtrabaho at matuto sa kani-kanilang mga tahanan (Onyema et al. 2020). Ayon sa World Health Organization (2020), ang bilang ng impeksyon at namamatay ay patuloy na tumataas sa buong mundo. Upang maiwasan ang impeksyon, inirekomenda ng *WHO* ang pagpigil, mitigasyon, *contact tracing*, *self-isolation*, *social distancing*, pagsusuot ng *face mask*, pagpapahusay ng sistemang pangkalusugan, paghuhugas ng kamay, at paglilinis o pag-*disinfect* ng mga nahahawakan.

Nagdulot ang paglaganap ng *COVID-19* nang biglaang pagtigil sa karaniwang kalakaran at pamumuhay ng mga tao sa mundo. Ang mga *cartoonist* sa buong mundo ay kumilos sa pamamagitan ng pagpapaabot sa mga tao at pagpapalawak ng kamalayan tungkol sa *virus*, habang pinagsusumikapan namang maging magaan at masaya ang sitwasyon. Lumikha sila ng mga *COVID-19* karton at komiks na isang mahusay na paraan upang maagapan ang *stress* na naidulot ng *virus.* Bagaman ang mga karton at komiks istrip ay madalas na inaanalisa kaugnay sa konsepto ng kapangyarihan at pagtutol, may mga pag-aaral sa mga karton at komiks istrip sa panahon ng pandemya ang nagpapakita na nakatutulong ito upang mawala sa isipan ng mga tao ang tensyonadong kalagayan at nakatutulong din ito sa paglikha ng kamalayan sa realidad ng pandemya sa paraang magaan sa kalooban (Alabi 2020).

Ang pandemyang naganap sa Pilipinas ang nagtulak sa mananaliksik upang isagawa ang pag-aaral na nakatuon sa pagsusuri sa mga editoryal karton na nailathala sa panahong may banta ng kalabang hindi nakikita – ang *COVID-19*. Naniniwala ang mananaliksik na mahalaga at napapanahon ang pag-aaral sa diskursong pangkapaligiran at mga usaping pangkalikasan sa pamamagitan ng pagsusuri sa piling editoryal karton sapagkat mabisa itong kasangkapan sa paglalarawan ng totoong kalagayan ng lipunan partikular sa panahon ng pandemya sa Pilipinas. Ang editoryal karton ay kasasalaminan ng iba't ibang sosyo-politikal na sitwasyon, mga usaping pangkapaligiran, indibidwal, at mahahalagang pangyayari sa lipunan na maaaring maging tulay sa katotohanan at imahinasyon ng *cartoonist* na ipinapahayag sa pamamagitan ng katatawanan o satirikong pamamaraan. Ang katatawanan o satirikong pamamaraang ito ay maaaring maglatag ng mga diskursong pangkapaligiran at usaping pangkalikasan sa panahon ng pandemya gaya ng pagkalat ng nakamamatay na sakit o *virus*, pang-aabuso sa kalikasan at iba pang usaping dulot nito tulad ng pagtaas ng mga bilihin, gasolina, kuryente, mababang sahod ng mga nars, kakulangan sa ayuda, at iba pang usaping panlipunan. Ayon kay Bantugan (2020), ang mga editoryal karton ay sumisibol mula sa mga kalunus-lunos na katotohanan na humihimok sa maugong na panawagan ng pagkilos alinsunod sa panlipunang katarungan at ang pagiging patas. Binibigyang-diin nito ang mga partikular na konteksto na kailangang ayusin ng mga nasa kapangyarihan ngunit patuloy na isinasawalang-bahala bagaman nakasalalay rito ang buhay o kamatayan.

Sa pamamagitan ng pagsusuri sa eko-kritikal (*eco-critical*) na diskurso ng lipunan, mas mapapalawak pa ang kasanayan ng mga guro sa pagsusuri sa mga diskursong pangkapaligiran, usaping pangkalikasan, at mga sanga-sangang isyung panlipunan sa panahon ng pandemya. Dahil dito, mabibigyan ang mga guro ng pagkakataong makabuo ng mga gawaing pampagkatutong kontekstuwalisado at mailapat ang mga inobatibong estratehiya sa pagtuturo na direktang sasangkot sa mga mag-aaral sa mga usaping ito. Kaugnay nito, nagiging mulat ang mga mag-aaral at ang komunidad sa mga napapanahong usapin sa lipunan. Sa ganitong paraan, mahihimok sila na maging aktibo sa pakikilahok upang malutasan o maagapan ang mga usaping ito. Ang pag-aaral na ito ay magiging dagdag sa lawak ng kaalaman hinggil sa pagsasalimbayan ng berbal at di-berbal na pagpapahiwatig ng karikatura kabilang na ang semyotika at mga pagpapakahulugan nito batay sa eko-kritikal na diskurso ng lipunan. Ayon kay Chandler (2011), mahalaga ang semyotika sapagkat itinuturo nito na ang realidad ay isang sistema ng mga simbolo. Ang pag-aaral ng semyotika ay makatutulong din upang maging mulat tayo sa realidad bilang isang konstruksiyon at sa mga tungkuling ginagampanan ng ating sarili at ng iba sa pagbuo nito. Nilalayon ng pag-aaral na masuri ang kalagayan ng bansa sa panahon ng pandemya sa pamamagitan ng eko-kritikal na pagsusuri sa diskurso sa piling editoryal karton upang makapagsagawa ng malalimang pag-aanalisa sa mga usaping ipinalulutang nito at mabigyan ng naaangkop na pagpapakahulugan ang semyotika na mamamalas sa mga karikatura. Sisipatin nito ang pagsasalimbayan ng berbal at di-berbal na pahiwatig gaya ng mga nakasulat na teksto, guhit, simbolo, at mga ekspresyon sa piling karikatura.

Nakatuon ang pag-aaral sa pagsusuri sa representayon ng piling editoryal karton sa panahon ng pandemya sa pamamagitan ng *eco-critical discourse analysis.* Ang pagsibol ng *eco-critical discourse analysis* ay bunga ng patuloy na pag-aangkop at pag-unlad ng panlipunang kilusan sa *critical discourse analysis* (CDA). Ang *CDA* ay isang *practically-oriented* na anyo ng *discourse analysis* na naglalayong matugunan ang mga suliraning panlipunan (Haig 2001). Tinatangka ng *CDA* na tukuyin ang kahulugan ng talakayan at kung paano nakabubuo ang diskurso ng ganitong uri ng kahulugan. Nilalayon nitong ihayag ang epekto ng ideolohiya sa talakayan sa pamamagitan ng pang-ibabaw na antas (*surface level*) ng estruktura ng wika, para sa kaayusan sa lipunan at mga relasyon sa kapangyarihan, ang kontra-aktibong impluwensya ng diyalogo at pilosopiya at kung paano umusbong at gumagana ang mga komponent nito. Tinatangka nitong ilantad ang ugnayan sa pagitan ng wika, ideolohiya, at kapangyarihan (Ahmed at Ali 2022).

Pinangangatwiranan naman nina Freeden et al. (2013) na ang *CDA* ay hindi lamang isang paraan ng pagsusuri kundi isang kilusang panlipunan. Maaaring kabilang dito ang pagsusuri ng leksikon, sintaks, lokal at pandaigdigang kahulugan (semantiks), *speech act*, at iba pang kontekstuwal na relasyon (pragmatiks), argumentasyon, retorika, estilo, *narrative framework*, o anumang iba pang tradisyunal na oraganisasyon ng diskurso. Sinuportahan naman ito ni Haig (2001), binanggit niya na ang *CDA* ay isang anyo ng *discourse analysis* na gumagamit ng *sytematic-functional linguistic* upang pag-aralan kung paanong ang pormal na lingguwistika ay nagtatampok ng mga teksto tulad ng bokabularyo at gramatika na nauugnay sa sosyal na kapangyarihan.

Idinagdag naman ni Haig (2001) na maraming isyung pangkapaligiran ang nagsasangkot sa tunggalian ng kapangyarihan sa pagitan ng magkasalungat na mga grupo at ang mga tunggaliang ito ay madalas na nagaganap sa wika. Samakatuwid, makatutulong ang *CDA* na maging mas sistematiko at kritikal na kamalayan sa wika kung saan tinatalakay ang mga bagay sa kapaligiran. Ang ganitong kamalayan ay makatutulong na maunawaan ang

ideolohikal na pagpapalagay ng mga tekstong pangkalikasan. Kamakailan, ang pinsala sa ekolohiya at polusyon sa kapaligiran ay lubhang nakababahala. Kung kaya, sinimulan ng mga eksperto sa iba't ibang larangan ang pag-aaral kaugnay sa mga isyung pangkapaligiran batay sa iba-ibang pananaw. Upang maiwasan ang mabilis na pagkasira ng ekolohikal na kapaligiran, sumibol ang ekolinggwistika bilang larangang nakatuon sa mga usaping pangkapaligiran (Zhang et al. 2023). Ang ekolingguwistika (*ecolinguistics*) ang nagbigay ng puwang sa pagsibol ng *eco-critical discourse analysis*. Ipinakilala ni Stibbe (2015b) ang isa sa mga pangunahing pokus ng ekolingguwistika na pag-aaral, na tinatawag na *ecolinguitics discourse analysis* o *eco-critical discourse analysis*. Samakatuwid, ang paggamit ng mga terminong ito ay maaaring magkapalitan. Binanggit nina Zhang et al. (2023) na ang pagsusuri sa eko-kritikal na diskurso ay isa sa mahahalagang bahagi ng pananaliksik sa ekolinggwistika, ibig sabihin, ito ay isang kritikal na pagsusuri sa diskurso ng paggamit ng wika mula sa pananaw ng kapaligirang ekolohikal ng wika.

Ayon kay El-Hameed (2016), si Halliday ang kinokonsiderang nanguna sa larangan ng *eco-critical discourse analysis* matapos ang kanyang maimpluwensyang panayam na pinamagatang "*New Ways of Meaning*" at nailathala noong 2001. Ang pangunahing halimbawang ibinigay niya sa kanyang panayam ay ang paglaganap ng metapora ng "*economic growth*." Inilarawan niya ang mga salita sa wikang Ingles bilang "*large, yellow and tall*", sinuri bilang positibo at mabuti ngunit ang mga ito ay nakasasama sa ekolohiya. Ang mga pamaraan ng *ecocriticism* sa *discourse analysis* ay magkakaiba, na nakatuon sa kung paano hinuhubog ng wika at komunikasyon ang pag-unawa ng tao sa mga isyu sa kapaligiran. Ang mga pamamaraang ito ay mula sa tekstuwal na analisis na sumusuri sa mga nakasulat na teksto, hanggang sa analisis sa kombersasyon na gumagalugad sa sinasalitang wika sa mga talakayang nauugnay sa kapaligiran, at iba pang pamaraan (Discourse Analyzer 2024).

Winika ni Dash (2019) na ang ekolingguwistika ay nagbibigay ng mga pangangailangan sa lingguwistika sa pangangalaga ng ekolohiya sa pamamagitan ng wika upang mapanatili ang *ecological sustainability* sa pamamagitan ng paggamit, pagbuo, at pagsulong ng wika nang magkasabay. Ang ekolingguwistika ay ang pag-aaral na nakatuon sa kritikal na pagsusuri ng wika ng potensyal na pagtaas ng pagkasira ng ekolohikal na aspeto ng buhay at naglalayong makahanap ng isang paraan upang magbigay ng inspirasyon sa sangkatauhang protektahan ang kalikasan (Mansyur et al. 2021). Ipinaliwanag ni Stibbe (2015a) na sa ekolingguwistika at *critical discourse analysis*, nilalayon nitong maipakita ang representasyon ng mga isyung ekolohikal sa diskurso at mapalawak ang kritikal na kamalayan sa wika. Sa kasalukuyan, marami ng mga kasangkapang pangwika ang ginagamit ng mga tao upang maipaabot, maisulong, at mapalawak ang mga usaping pangkalikasan at iba pang suliraning panlipunan tulad ng editoryal karton.

Malaki ang naitutulong ng paggamit ng editoryal karton sa paglalantad ng mga usaping pangkapaligiran at napapanahong isyung panlipunang danas ng mga tao lalo na sa panahon ng pandemya na nagdulot ng malaking pinsala at pangamba sa pamumuhay, kalusugan, at ari-arian. Binanggit nga nina Xue at Xu (2021) na ang pandemyang *COVID-19* na isang pampublikong emerhensiya sa kalusugan ay nagdulot ng malawakang pagpapahirap sa sangkatauhan sa siglong ito at naging dahilan nang matinding banta sa buhay at kalusugan ng tao.

Ang editoryal karton ay mabisang paraan ng pagtalakay sa mga isyung panlipunan at isinasalamin nito ang pananaw ng nakararaming mga miyembro ng pangkalahatang publiko sa isang partikular na kilusan (Pinto at Mustaffa 2023). Isinaad nina Freeden et al. (2013) na ang karikatura ay ang sining ng pagbaluktot, paghila, pag-unat at pagsisiyasat sa pagkakahawig upang mahanap ang mga mahahalagang punto. Ayon naman kay El-Hameed (2016), ang *cartoon* o karton ay mga guhit na idenisinyo upang maipaabot ang isang mensahe. Gumagamit ang *cartoonist* ng mga karikatura at simpleng biswal na imahe upang ipakita ang mga ideya at maunawaan ng mga mambabasa, lalo na kapag gumagamit ng mga simbolong biswal para sa mga komplikadong konseptong pampolitika.

Nakatutulong sa mga mambabasa ang mga metapora sa karton sa paglikha ng isang positibo o negatibong opinyon at paghuhusga kung kaya maaari itong isaalang-alang bilang isang mapanghimok at ideolohikal na kasangkapan ng *cartoonist* na may kamalayan sa paggamit (Gil 2018). Sa pag-aaral ni Oluremi (2019), ang *cartoon* at *humor* ay mga reserba ng midya upang mapanatili ang hindi maikakailang presensya at impluwensya sa komunikasyon ng matagal ng institusyong panlipunan na kilala bilang politika. Batay sa komunikatibong gampaning ginagampanan ng *cartoon* at *humor*, kapwa pinangangalagaan ng dalawa ang simbolikong ugnayan nito sa politika. Isinasaad na ang mga politikal karton ay makapangyarihang komunikatibong sandatang ginagamit upang patamaan o pasaringan ang gobyerno nang hindi gumagamit ng dahas o nagdudulot ng pinsala sa apektadong tao at/o institusyon.

Sa pag-aaral ni Bantugan (2020), patuloy na pumupuna ang mga editoryal karton sa malawak na agwat ng kapangyarihan, kahit na sa panahon ng *COVID-19* kung kailan ang mga dati nang walang kapangyarihan ay nagiging mas walang kapangyarihan dahil sa kakulangan ng *mobility*, mga mapagkukunan, mga pagpipilian, at suporta mula sa

gobyerno. Ipinalulutang ng editoryal karton ang kalunos-lunos na realidad na humihimok ng panawagan sa panlipunang hustisya at katarungan, at binibigyang-diin ang mga partikular na konteksto na kailangang ayusin ng mga nasa kapangyarihan ngunit patuloy na napababayaan gayong nakasalalay sa sitwasyong ito ang buhay o kamatayan.

Ang mga karton ay isang koleksyon ng mga elemento na binibigyan ng iba't ibang pagpapakahulugan ng bawat tao. Nangangahulugan ito na ang anumang bagay ay maaaring gamitin upang mailarawan at manudyo sa mga katiwalian sa lipunan, gayundin ang pagbibigay ng kabatiran sa mga *social pattern* at pagsasaalang-alang sa kung paanong ang mga pangyayari ay nakalilikha ng magkatulad na pagpapakahulugan para sa tiyak na pangkat ng lipunan (Pinto at Mustaffa 2023). Sa panahon ng pandemya, ang mga editoryal karton ay naghatid ng mga impormasyon sa pamamagitan ng natatangi nitong paraan at sa paggamit ng mga simbolo, figura o guhit, nakasulat na teksto, at mga ekspresyon ng mga karakter. Ito ang nagbibigay-diin sa kahalagahan ng semiotika ng mga editoryal karton sa pagpapalawak ng kabatiran ng mga mambabasa kaugnay sa mga napapanahong usapin ng lipunan partikular na sa mga usaping dulot ng pandemya.

Ang semyotika ay isang pagsisiyasat sa kung paano nalilikha ang kahulugan at kung paano ito ipinapahayag. Nag-uugat ito sa akademikong pag-aaral sa kung paano nabubuo ang kahulugan ng mga palatandaan at simbolo (*visual* at *linguistic*). Pangunahing kasangkapan ito upang matiyak na ang sinadyang pagpapakahulugan ay malinaw na nauunawaan ng tatanggap nito. Madalas may magagandang dahilan kung bakit hindi nauunawaan ng isang tao ang tunay na intensyon ng isang mensahe. Sa ganitong pagkakataon, makatutulong ang semyotika na malutas ang pagkalito at matiyak ang kalinawan ng kahulugan (Sign Salad nd). Ang palatandaan (*semiotic*) ay maaaring tumutukoy sa isang nosyon o bagay na gumaganap sa gampanin ng isang palatandaan. Sa semiotika, ang multisiplidad sa pagitan ng *signifier* at *signified* ang lumikha ng konsepto ng kahulugan. Ang *signifier* ay maaaring isang larawan o isang grapiko na kumakatawan sa isang bagay tulad ng mga salita o titik. Samantala, ang *signified* ay ang magkakaugnay na katotohanan ng isang ideya (hinalaw sa Freeden et al. 2013).

Ayon kay Chandler (2011), ang palatandaan (*sign*) ay isang natatanging kumbinasyon ng *signifier* na may partikular na *signified*. Ang *signifier* ay ang larawan o titik na bumubuo ng isang salitang may materyal na elementong nagsisilbing representasyon ng pandama ng isang tao. Sa kabilang banda, ang *signified* naman ay ang konseptong nasa isip. Tumutukoy ito sa nosyon ng isang bagay at hindi ang mismong bagay. Nakikilala ang mga *signifier* sa editoryal karton na kadalasang nasa malaking titik at naka-*bold*. Ilan sa *signifiers* ay may mga *signified* o kahulugang katulad ng kanilang porma (*signifier*) batay sa kung paano ito ginagamit sa mga editoryal karton. Gayundin, ang pantukoy na "ang" ay kadalasang ginagamit sa mga *signified* (kahulugan) ng palatandaan sa kadahilanang ito ay nagbibigay ng distinksyon (Mendoza 2016).

Kinokonsidera ng *semiotic analysis* na pag-aaral ang mga karton/karikatura bilang imahe o larawan na naghahatid ng kahulugan at nagsisilbing tahimik na paraan ng komunikasyon (hinalaw sa Pinto at Mustaffa 2023). May mga pag-aaral na nailathala na sumuri sa diskurso ng mga editoryal karton gaya ng pag-aaral ni Gil (2018), Adedun et al. (2017), at Oluremi (2019). Sa pag-aaral ni Gil (2018), sinuri niya ang paggamit ng mga metaporang multimodal sa mga karton ng Espanya na inilathala sa iba't ibang pahayagan na naglalarawan sa panloob na krisis pampolitika ng partidong sosyalista noong 2016. Tinangka naman sa pag-aaral nina Adedun et al. (2017) ang ekolingguwistikong pagsusuri mula sa eko-kritikal na perspektiba ng mga napiling editoryal ng mga pahayagan sa Nigeria. Tinatangka rin sa pag-aaral ni Oluremi (2019) ang pragmeme ng katatawanan sa piling *political events* sa Nigeria sa taong 2016 na isiniwalat sa mga kartong pampolitika. Ang mga pag-aaral ay kapwa nakatuon sa ugnayang pampolitika at isyung pangkalikasang isinasalamin sa mga kartong pampolitika ng isang bansa.

Bilang paglalahat, pinagtitibay lamang ng mga kaugnay na literatura at pag-aaral na ang kasalukuyang pag-aaral ay mahalaga, napapanahon, at naaangkop upang maihatid ang mga diskursong sumasangkot sa kapaligiran at isyung pangkalikasan sa panahong nakararanas ang bansa ng mga banta ng *COVID-19*. Sinipat sa pag-aaral ang diskurso ng piling editoryal karton sa panahon ng pandemya bilang kabahagi ng diskursong ekolohikal kabilang na ang semyotika nito na mabisang paraan sa pagpapaabot ng diskursong nauugnay sa kapaligiran at sa mga isyung pangkalikasan batay sa sitwasyon ng karton, wika at *semiotic strategies*, target, lohikal na mekanismo at *script opposition*. Natatangi ang kasalukuyang pag-aaral sapagkat nakatuon ito sa mga sanga-sangang isyung naidudulot ng *COVID-19* at ng mga suliraning panlipunang naganap sa panahon ng pandemya bilang kabahagi ng ekokritikal na diskurso sa Pilipinas.

PAMAMARAAN

Disenyo ng Pag-aaral

Ginamit sa pag-aaral ang kwalitatibong metodo ng pananaliksik sa paraang *eco-critical discourse analysis* sa pagsusuri sa piling editoryal karton sa panahon ng pandemya. Ginagamit ang berbal at di-berbal na komunikasyon upang maihatid ang mga ideya at katotohanan. Ang mga elemento ng pagiging

dominante at hindi pagkapantay-pantay ay ang pangunahing mapagkukunan ng *CDA* upang mapanghawakan ang lahat ng *pattern* na ginamit sa komunikasyon sa pagkuha ng malalim na pag-unawa (Van Dijk 2001).

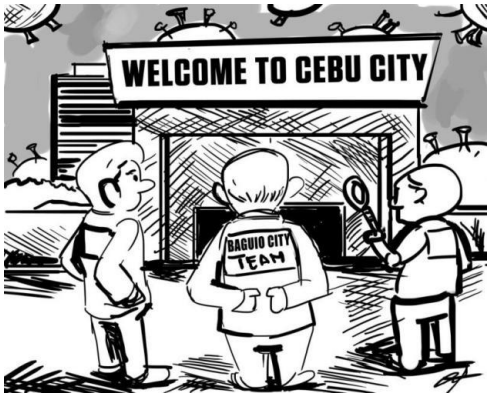
Iniangkla ang pag-aaral sa *Semantic Script Theory of Humor* at *Visual Metaphor*. Ayon kay Mazid (2008), ang *Semantic Script Theory of Humor* ay nakabatay sa argumento ng *script/schema opposition* sa nakatatawang diskurso. Sinuportahan ito ni Negro (2017) sa teoryang *Visual Metaphor* na nagsaad na ang "*metaphor in political cartooning exemplifies a critical perspective on a political issue or a recent event*."

Sa pag-aanalisa ng mga datos, ginamit ang modelong *bottom up* sa pagsusuri na nakabatay sa modelo nina Attardo at Raskin (sa El-Hameed 2016). Kinasasangkutan ang modelo ng pagsusuri sa sitwasyon ng karton, wika at drowing, *semiotic strategies*, target, lohikal na mekanismo at *script opposition.* Sinimulan ang pag-aaral sa pamamagitan ng paglikom ng mananaliksik ng mga editoryal karton na nailathala sa panahon ng pandemya. Limang editoryal karton ang napili ng mananaliksik sa mga pahayagang ma-a*access online* gaya ng Sunstar at Manila Bulletin na inilathala noong Hulyo hanggang Nobyembre 2020. Pagkatapos ng paglikom ng mga larawan, sinuri ng mananaliksik ang ekolohikal na diskurso sa piling editoryal karton sa panahon ng pandemya at hinimay ang semyotika nito na may kaugnayan sa kapaligiran at sa mga isyung pangkalikasang nakatuon sa pagsusuri sa sitwasyon ng karton, wika at drowing, *semiotic strategies*, target, lohikal na mekanismo at *script opposition.*

Sinundan ito ng paglalahad at pagpapakahulugan ng mga natuklasan ng isinagawang pagsusuri. Ang mga nalikom na editoryal karton ay sinuring mabuti bilang ambag sa ekokritikal na lapit sa paghihimay sa diskursong saklaw ng kapaligiran at mga isyung pangkalikasan at ang mga sanga-sangang suliraning panlipunang nararanasan ng lipunan.

Pinanggalingan ng mga Datos

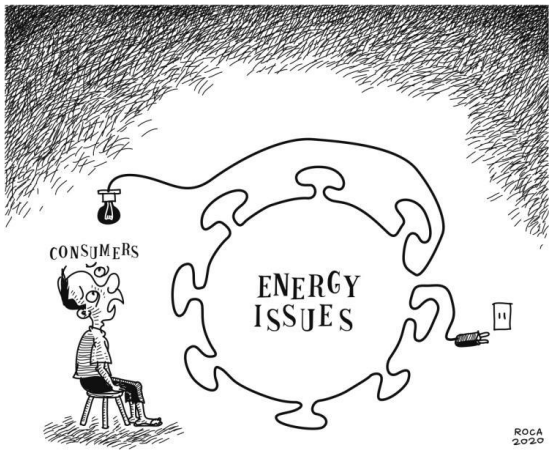
Limang editoryal karton ang napili ng mananaliksik sa mga onlayn na pahayagang Sunstar at Manila Bulletin na inilathala noong Hulyo hanggang Nobyembre 2020 kabilang nito ang 1. *Welcome, Baguio City* (Figyur 1; Santisas 2020b), 2. *Gov't nurse finally get nurses long-delayed pay increases* (Figyur 2; Santiago 2020b), 3. *Telling vs. showing* (Figyur 3; Santisas 2020a), 4. *Environment, energy issues linked to pandemic* (Figyur 4; Santiago 2020a), at 5. R*eforestation – long-range answer to massive flooding* (Figyur 5; Santiago 2020c). Sinuri ng mananaliksik ang ekolohikal na diskurso sa piling editoryal karton sa panahon ng pandemya (Talahanayan 1) at hinimay ang semyotika na nauugnay sa kapaligiran at sa mga isyung pangkalikasan na nakatuon sa pagsusuri sa sitwasyon ng karton, wika at *semiotic strategies*, target, lohikal na mekanismo at *script opposition*. Bilang pagsasaalang-alang sa etikal na konsiderasyon, sumulat ang mananaliksik sa Manila Bulletin at Sunstar upang humingi ng pahintulot na masuri ang piling editoryal karton. Tiniyak din ng mananaliksik na mabigyan ng karampatang pagkilala ang lahat ng mga sangguniang sinangguni ng kasalukuyang pag-aaral.



Figyur 1. *Welcome, Baguio City* (*posted July 2020*) *on Sunstar's website* (*Copyright 2018 Sunstar Publishing Inc. All rights reserved worldwide*).

Figyur 2. *Government nurse finally get nurses long-delayed pay increase* (*posted July 2020*) *on Manila Bulletin's website (Copyright 2024 Manila Bulletin the Nation's Leading Newspaper. All rights reserved).*

Figyur 3. *Showing vs. telling* (*posted July 2020*) *on Sunstar's website* (*Copyright 2018 Sunstar Publishing Inc. All rights reserved worldwide*).



Figyur 4. *Environment, energy issues linked to the pandemic (posted August 2020) on Manila Bulletin's website (Copyright 2024 Manila Bulletin the Nation's Leading Newspaper. All rights reserved).*

Figyur 5. *Long-range answer to massive flooding* (*posted November 2020) on Manila Bulletin's website (Copyright 2024 Manila Bulletin the Nation's Leading Newspaper. All rights reserved).*

Talahanayan 1. Mga editoryal karton na sinuri sa pag-aaral (*Editorial cartoons analyzed in the study*).

Pamagat ng Editoryal Karton	Petsa ng Publikasyon	Websayt
Welcome, Baguio City	09 July 2020	Sunstar https://www.sunstar.com.ph/article/1862968/Cebu/Opinion/Editorial-Welcome-Baguio-City
Government nurses finally get nurses long delayed pay increase	21 July 2020	Manila Bulletin https://mb.com.ph/2020/07/21/govt-nurses-finally-get-nurses-long-delayed-pay-increases/
Telling vs. Showing	23 July 2020	Sunstar https://www.sunstar.com.ph/article/1864593/Cebu/Opinion/Editorial-Telling-vs-showing
Environment, energy issues linked to pandemic	09 August 2020	Manila Bulletin https://mb.com.ph/2020/08/09/environment-energy-issues-linked-to-pandemic/
Reforestation – long-range answer to massive flooding	19 November 2020	Manila Bulletin https://mb.com.ph/2020/11/19/reforestation-long-range-answer-to-massive-flooding/

Limitasyon ng Pag-aaral

Nakatuon ang pag-aaral sa pagsusuri sa *semiotic features* ng limang editoryal karton batay sa sitwasyon ng karton, wika at *semiotic strategies*, target, lohikal na mekanismo at *script oppositions* na nailathala sa Sunstar at Manila Bulletin noong Hulyo hanggang Nobyembre 2020. Limitado lamang ang bilang ng editoryal karton ang sinuri sa pag-aaral at hindi sumasaklaw sa pangkalahatang diskursong pangkapaligiran at mga isyung pangkalikasan sa bansa. Kaugnay nito, ang interpretasyon ng mananaliksik sa mga editoryal karton ay ilan lamang sa maaaring interpretasyong mahihinuha sa mga ito. Napili ang mga editoryal karton na ito dahil nailathala ito sa panahon ng kasagsagan ng paglaganap ng pandemya. Dagdag pa rito, naging batayan din ng mananaliksik ang pagpili ng mga ito ang *accessibility* nito sa *internet* at ang mga inklusyong at ekslusyong kriterya sa inilahad na Talahanayan 2.

Talahanayan 2. Inklusyon at eksklusyong kriterya sa pagpili ng editoryal karton *(Inclusion and exclusion criteria for selecting editorial cartoons).*

Kriterya	Inklusyon	Eksklusyon
Petsa	Mga editoryal karton na nailathala noong Hulyo-Nobyembre 2020	Mga editoryal karton na nailathala bago at pagkatapos ng Hulyo-Nobyembre 2020
Paksa	Mga editoryal karton na nakatuon sa diskursong pangkapaligiran, usaping pangkalikasan at ang mga sanga-sangang usaping panlipunang dulot ng pandemya	Mga editoryal karton na walang kaugnayan sa paksa
Lokasyon	Lokal o nasyonal na setting	Internasyonal na setting
Konteksto	Diskursong pangkapaligiran, usaping pangkalikasan at ang mga sanga-sangang usaping panlipunang dulot ng pandemya	Mga usaping walang kinalaman o kaugnayan sa pandemya
Publikasyon	Lokal at nasyonal na pahayagan	Internasyonal na pahayagan

RESULTA

Inilalahad sa bahaging ito ang prosesong isinagawa sa paghihimay-himay sa piling editoryal karton na nalikom. Ginawang sandigan ng pagsusuri ang mga layuning inilahad sa papel. Matutunghayan din sa bahaging ito ang pagtatalakay sa resulta at interpretasyong nabuo batay sa pagsusuring isinagawa.

Pagsusuri sa Editoryal Karton 1: *Welcome, Baguio City*

Nakabase ang sitwasyon ng editoryal karton sa mataas na kaso ng impeksyong dulot ng *COVID-19* sa Cebu bilang isa sa mga pangunahing lungsod na naapektuhan ng pandemya (Talahanayan 3). Tatlong tao ang nag-uusap ang makikita sa sitwasyong nasa karton (Figyur 1). Makikita sa editoryal karton na ang *team* mula sa Baguio City batay sa pagkaguguhit ay nagtataglay ng kaalamang sa pagsugpo sa pandemya batay sa mga di-berbal na pagpapahiwatig gaya ng *magnifying glass* na sumisimbolo sa kaalaman at ang postura at kasuotan ng tatlong karakter na nagpapahiwatig ng pagiging kalmado at pagkakaroon ng malaking posisyon sa lipunan. Sa kabuoan, makikita sa editoryal karton na inimbita ang *team* Baguio City upang magbahagi ng kanilang karanasan at mga pamamaraan sa pagsugpo sa pandemya nararanasan sa Cebu City.

Talahanayan 3. Pagsusuri sa editoryal kartong "*Welcome, Baguio City*" (*Analysis of the editorial cartoon "Welcome, Baguio City*).

Semiotic Features	Pagsusuri sa Editorya Karton
Sitwasyon	Pagtaas ng impeksyong dulot ng COVID-19 sa Cebu
Wika at *Semiotic Strategy*	Mainit na pagtanggap ng mga taga-Cebu sa mga bisitang mula sa Baguio na mga ekspertong tutulong sa pagsugpo ng pandemya COVID-19
Target	Mga ekspertong bisita mula sa Baguio
Lohikal na Mekanismo	Pagkokontrast sa pagitan ng tatlong eksperto mula sa *Team Baguio* at ang mga guhit ng bayrus
Script Opposition	Pagtanggap ng mga taga-Cebu na makikita sa *banner* na "*Welcome to Cebu City*" at panganib ng pandemya

May kaugnayan naman ang wika at ang *semiotic strategy* sa pagpapahayag ng mensahe sa pagtanggap sa mga bisitang nagmumula sa Baguio at sa paglaganap ng *COVID-19* na nararanasan sa Cebu. Ang kapsyon na "*Welcome to Cebu City*" ay nagpapahayag ng pagtanggap ng mga taga-Cebu sa *team* ng Baguio City. Makikita rin ang nagkalat na guhit ng *virus* malapit sa bukana ng Lungsod ng Cebu, ipinapahayag lamang nito na ang Cebu ay may mataas na kaso ng impeksyong dulot ng *COVID-19*. Mapapansin din ang guhit ng taong nakapamulsa, ang isa naman ay nasa likod ang kamay at taong may hawak na *magnifying glass*. Isinisimbolo nito na ang mga taong ito ay mga eksperto o may sapat na kaalaman at ang kanilang pagbisita ay maaaring may kinalaman sa pagsugpo sa pandemya.

Ang target ng editoryal karton ay ang pagbisita ng *team* Baguio City sa Cebu City upang magbahagi ng kanilang karanasan sa pagsugpo sa *COVID-19.* Sa kabilang banda, mapapansin sa karton na ang *team* mula sa Baguio City ay hindi nakasuot ng *personal protective equipment* (PPE) gaya ng *face mask, face shield*, at iba pa na maaaring magdulot sa kanila ng panganib mula sa impeksyong dulot ng *COVID-19* pandemya.

Sa *logical mechanism* naman, mapapansing may pagkokontrast sa pagitan ng tatlong taong papasok sa Lungsod ng Cebu at sa mga nagkalat na guhit ng mga *virus* upang maibunyag ang mensaheng

nais maihatid ng editoryal karton. Nagsasalungatan ang ideya ng pagtanggap at ang panganib na maaaring maidulot ng *virus* sa sinumang bibisita sa lungsod. Nagsasalungatan din ang konsepto ng pagiging eksperto ng mga bisita mula sa Baguio City ngunit hindi man lang sila nakasuot ng PPE batay sa guhit na nasa editoryal karton.

Ang *script opposition* sa editoryal karton ay makikita sa *banner* na nakasabit sa may bukana ng lungsod ng Cebu na "Welcome to Cebu City" at sa likod ng taong nasa gitna na *"Baguio City Team."* Nagsasalungatan ang mga script na ito na nagpapahayag ng pagtanggap at banta ng *virus* sa mga bibisita sa Cebu.

Pagsusuri sa Editoryal Karton 2: *Government nurses finally get nurses long delayed pay increase*

Ang sitwasyon ng editoryal karton ay nakabatay sa matagal nang ipinaglalaban ng mga nars sa Pilipinas na maitaas ang kanilang sahod (Talahanayan 4). Dalawang karakter ang makikita sa editoryal kartong ito (Figyur 2). Una, ang braso na nakabarong na may hawak ng hugis perang papel na may kapsyong "*pay increase"*. Pangalawa, ang nars na inilalarawan sa karton na may punit-punit na uniporme na patuloy na ginagampanan ang kanyang sinumpaang tungkulin sa kabila ng banta ng pandemya.

Ang wika at *semiotic strategy* ay nagkakaisa sa pagpapahayag sa mensahe ng editoryal kartong ito (Talahanayan 4). Mailalarawan sa ekspresyon ng nars ang mensahe ng karton. Nanlaki ang mga mata ng nars nang makita niya ang hugis perang papel na iniabot ng brasong nakabarong. Ang perang papel ay sumisimbolo sa matagal nang pakikipaglaban ng mga nars sa Pilipinas na magkaroon ng kompensasyong nararapat para sa serbisyong kanilang iniaambag sa sektor ng kalusugan. Samantala, mapapansin din ang nakabarong na braso na kumakatawan sa disenteng kalagayan ng mga lider ng bansa. Ang paghawak ng nakabarong na braso sa perang papel ay nagpapahayag sa kapangyarihan ng mga lider ng bansa na kontrolin ang pagtaas ng sahod ng mga nars. Isinisimbolo naman ng imahen ng nars ang reyalidad ng buhay ng mga nars sa bansa na hindi napapasahod nang tama sa kabila ng panganib na dulot ng *COVID-19*. Masasalamin ang nakatatawang mensahe ng editoryal karton sa naging reaksyon ng nars o paglaki ng mata matapos maibigay ang pagtaas ng suweldo na matagal na nilang isinusulong.

Bagamat inihayag sa larawan ang kaawa-awang sitwasyon ng mga nars sa bansa, ang totoong target ng editoryal karton ay ang mga lider ng bansa na nagbubulag-bulagan sa kanilang kalagayan. Isinisimbolo ng brasong nakabarong ang mga lider ng bansa na matagal nang nagbibingi-bingihan sa isinusulong na pagtaas ng suweldo para sa mga nars sa bansa.

Sa *logical mechanism*, nagsasalungatan ang kalagayan o postura ng brasong nakabarong at ang madungis na uniporme ng nars (Talahanayan 4). Naglalaban ang konsepto ng barong na sumisimbolo sa pagiging disente ng mga lider sa bansa at ang gutay-gutay na uniporme ng nars na sumisimbolo sa mahirap na kalagayan ng mga nars na nagsasakripisyo ng kanilang buhay sa panahon ng pandemya.

Ang *script opposition* sa editoryal karton na ay mamamalas sa paglaki ng mga mata ng nars matapos maiabot ang perang papel na may kapsyong "*pay increase*" (Talahanayan 4). Tila ipinapahayag sa ekspresyon ng mukha ng nars na hindi siya makapaniwala na matutupad na ang kanilang isinusulong.

Talahanayan 4. Pagsusuri sa editoryal kartong "*Government nurses finally get nurses long delayed pay increase*". (*Analysis of the editorial cartoon* "*Government nurses finally get nurses long delayed pay increase*").

Semiotic Features	Pagsusuri sa Editorya Karton
Sitwasyon	Matagal na panawagan ng mga nars para sa pagtaas ng sahod
Wika at *Semiotic Strategy*	Panlalaki ng mata ng nars, hugis perang papel at brasong nakasuot ng barong
Target	Lider ng bansa na nagbubulag-bulagan at nagbibingi-bingihan sa panawagang maitaas ang mga nars sa bansa
Lohikal na Mekanismo	Naglalaban ang konsepto ng brasong nakasuot ng barong at ang madungis na uniporme ng nars habang tinutupad ang kanyang tungkulin
Script Opposition	Paglaki ng mga mata ng nars matapos maiabot ng brasong nakabarong ang perang papel na may nakasulat na "*pay increase"*

Pagsusuri sa Editoryal Karton 3: *Telling vs. Showing*

Nakabatay ang sitwasyon ng editoryal karton sa magagandang pangako ng mga politiko noong eleksyon at sa pagtupad ng kanilang ipinangako sa termino ng kanilang panunungkulan (Talahanayan 5). Direktang tinukoy sa karton ang mayor ng Lungsod ng Cebu na nagtatalumpati at nangangakong pagtutuonan ng kanyang pamumuno ang paglikha ng mga trabaho, pagtataguyod ng negosyo, suporta sa edukasyon, pagpapabuti ng kalusugan ng mga residente at pag-upgrade ng sistema ng sanitasyon (Figyur 3).

Sa wika at *semiotic strategy*, may dalawang eksenang inilahad sa editoryal karton (Talahanayan 5).

Una, ang pagtatalumpati ng mayor ng Cebu kasabay ang pagbanggit ng mga pangakong makikita sa kapsyon ng larawan gaya na lamang ng *Carbon Market Modernization, traffic and garbage, education, recovery, COVID-19 response, CCMC*, at *flood control*. Sa kabilang banda, ang pangalawang eksena ay nagpapakita na ang lahat ng kanyang ipinangako ay nakalagay sa isang kartilya ayon sa kanyang pagmamando o pamamahala. Mahihiwatig sa dalawang eksena ang konsepto ng pangangako at pagtupad ng mga pangako. Ang konseptong ito ay umaalinsabay rin sa nauusong pahayag sa *social media* na "*expectations vs. reality*" at nawa'y lahat ng ipinangako ay hindi magagaya sa ibang proyektong napako.

Direktang tinatarget sa editoryal karton ang mayor ng Lungsod ng Cebu. Patunay nito ay ang mga biswal na representasyong tulad ng postura o suot na barong, suot na salamin, hugis ng mukha at ayos ng buhok na karaniwang mapapansin sa kanyang pagtatalumpati.

Inihayag naman sa lohikal na mekanismo ang mga pangako ng mayor ng lungsod ng Cebu sa kanyang mga residente kaugnay sa kanyang mga proyekto. Ganunpaman, maraming pangako ang napapako. Mapapansin sa editoryal karton ang dalawang pagkilos ng karakter – una, ang pagtatalumpati na sumisimbolo sa pagbanggit ng mga pangako sa panahon ng pangangampanya at ang pangalawa ay ang pagkarga ng mayor sa mga pangakong proyekto gamit ang kartilya na sumisimbolo sa pagpapatupad ng mga proyekto.

Nagsasalungatan ang *script opposition* sa dalawang eksenang inilalarawan sa editoryal karton – labanan ng konsepto ng pangako at pagpapatupad. Sa unang eksena ng karton, makikita ang pagtatalumpati ng mayor ng Lungsod ng Cebu na puno ng mga pangako. Ang pangalawang eksena naman ay nagpapakita na ikinakarga ng mayor sa kartilya ang kanyang mga ipinangako. Masasabing may tono ng pang-uyam sa mga eksenang ito sapagkat mahihinuha sa larawan kung paano ipatutupad ng mayor ang kanyang mga ipinangakong proyekto o matutulad lamang ito sa ibang pangako ng mga politiko na napako.

Talahanayan 5. Pagsusuri sa editoryal kartong "*Telling vs. Showing*" (*Analysis of the editorial cartoon* "*Telling vs. Showing*").

Semiotic Features	Pagsusuri sa Editorya Karton
Sitwasyon	Magandang pangako ng mga politiko sa panahon ng eleksyon at ang pagtupad nito sa panahon ng panunungkulan
Wika at *Semiotic Strategy*	Pagtatalumpati ng Mayor ng lungsod ng Cebu sabay pagbibitaw ng pangakong mga proyekto at kartilya na naglalaman ng mga pangako
Target	Mayor ng lungsod ng Cebu
Lohikal na Mekanismo	Expectation vs. Reality Pagbibitiw ng pangako at pagtupad ng pangako
Script Opposition	Nagsasalungatang konsepto ng pagbitiw ng pangako sa panahon ng pangangampanya at pagtupad ng pangako sa panahon ng panunungkulan

Pagsusuri sa Editoryal Karton 4: *Environment, energy issues linked to pandemic*

Nakabatay ang sitwasyong mamamalas sa editoryal karton sa mataas na bayarin ng kuryente sa unang mga buwan ng *Enhanced Community Quarantine* (ECQ) (Talahanayan 6). Makikita sa karton ang nakatingalang karakter sa nakapatay na ilaw at hugis virus na kable ng kuryente (Figyur 4). Mahihinuha rito na ang paglaganap ng *COVID-19* ay nakaapekto hindi lamang sa sektor ng kalusugan at edukasyon kundi maging sa mga usaping pang-enerhiya, isa na rito ang pagtaas ng singil sa kuryente.

Makikita naman sa wika at *semiotic strategy* ang ekspresyon ng mukha ng karakter ang katatawanang mensahe ng semyotika ng editoryal karton. Nanlaki ang mata ng karakter matapos maputulan ng kuryente sa panahong may *virus* na lumalaganap. Isinisimbolo ng pagtingala ng karakter sa ilaw ang hindi abot-kamay na bayarin na nakadaragdag sa paghihirap ng mga konsyumer. Dagdag pa nito, ang hugis *virus* na kable ng kuryente na may kapsyon sa gitna na "*Energy issues*" ay nagpapahiwatig na mas ginagawang komplikado ng *COVID-19* ang isyu sa enerhiya sa bansa dahil na rin sa ipinatupad na bagong patakaran sa pagbayad ang *fit-all rates* na bayarin sa kuryente na naging sanhi ng pagtaas ng bayarin sa kuryente. Ang nakatatawang ekspresyon ng mukha ng karakter ay pang-uuyam sa mga insensitibo at pabayang *electric distributors* sa bansa.

Ipinakita sa editoryal karton ang nakaaawang postura ng karakter at ang nakatatawang ekspresyon sa kanyang mukha ngunit ang totoong target nito ay ang *electric distributors* sa bansa sa kanilang insensitibong at pabayang pagpapatupad sa mga patakarang nagpahirap sa mga konsumedor sa panahong nakararanas ang bansa ng pandemya.

Ipinaaabot ng *cartoonist* ang lohikal na mekanismong kaugnay sa mataas na bayarin ng kuryente na hindi na abot-kamay ng mga karaniwang mamamayan sa bansa. Ang pagtingala ng karakter sa patay na ilaw ay sumisimbolo sa agwat ng

konsumedor at presyo ng bayarin sa kuryente. Kaugnay nito, ang kableng may hugis *COVID-19* ay mahihiwatigan na ang paglaganap nito ay nakaapekto sa paglala ng mga isyung pang-enerhiya sa bansa. Bilang pangkalahatan, mahihinuha sa editoryal karton na maraming konsyumer ang mahihirapan sa pagbabayad ng bayarin at ang iba ay tuluyan nang mapuputulan ng kuryente.

Namalas naman ang *script opposition* sa pagitan ng karaniwang konsumedor na inirerepresenta ng pigurang tao sa larawan at sa kanyang pagtingala sa nakapatay na ilaw na may kableng hugis *virus*. Ang kableng ito na iginuhit ayon sa hugis ng *virus* na *COVID-19* ay nagpapahiwatig na ang *virus* ay may malaking epekto sa bayarin ng kuryente. Kaya, mapapansin din ang karakter sa editoryal karton ay nakatingala sa ilaw dahil sinisimbolo nito ang agwat sa pagitan ng konsumedor at bayarin sa kuryente na siyang isyung pangkapaligirang masasalamin dito.

Talahanayan 6. Pagsusuri sa editoryal kartong "*Environment, energy issues linked to pandemic*" (*Analysis of the editorial cartoon* "*Environment, energy issues linked to pandemic*").

Semiotic Features	Pagsusuri sa Editorya Karton
Sitwasyon	Mataas na bayarin sa kuryente sa unang mga buwan ng *Enhanced Community Quarantine* (ECQ)
Wika at *Semiotic Strategy*	Panlalaki ng mga mata ng karakter matapos maputulan ng kuryente, pagtingala ng karakter na sumisimbolo sa mataas na bayarin, hugis bayrus na kable
Target	*Electric distributors* sa bansa
Lohikal na Mekanismo	Pagtingala ng karakter sa nakapatay na ilaw na sumisimbolo sa agwat ng konsumedor at mataas na bayarin sa kuryente. Dagdag pa ang hugis virus na kable na nagpalala sa usapin ng enerhiya sa bansa
Script Opposition	Ang karaniwang konsumedor na kumakatawan sa maraming Pilipinong nahihirapan sa mataas na bayarin na pinalala pa ng pandemya na nagdulot sa pagtaas ng presyo ng kuryente

Pagsusuri sa Editoryal Karton 5: *Reforestation - Long-Range Answer to Massive Flooding*

Nakabatay ang sitwasyon ng editoryal karton na walang habas na pamumutol ng punongkahoy ng mga malalaking kompaya at industriya at sa pinsalang naidudulot ng pagsalanta ng mga bagyo sa bansa (Talahanayan 7; Figyur 5). Isa na rito ay ang Bagyong Ulysses na nagpalubog sa Lungsod ng Marikina at iba pang karatig lugar gaya ng Cagayan at Isabela.

Ang wika at *semiotic strategy* ay kapwa nagpapalutang sa mapang-uyam at nakatatawang mensahe ng editoryal karton na mapapansin sa karakter na kumakatawan sa gobyerno na sumusuntok sa hangin at sa ekspresyon ng mukha ng *illegal logger*. Mapapansin sa larawan na naubos na sa kakaputol ng *illegal logger* ang mga puno sa bundok. Tinatakpan ng kamay nito ang mukha ng gobyerno na nakikipagsuntukan sa hangin. Lutang na lutang na tila natatakpan ang paningin ng gobyerno at waring nakikipaglaban o gumagawa ng aksyon kahit hindi tukoy ang puno't dulo ng malalang pagbaha sa bansa. Maaari ring ipinapakahulugan nito na marami sa gobyerno ang nasusuhulan ng malalaking kompanya at industriyang nagpuputol ng mga punongkahoy. Dahil dito, nagkukunwari ang iilang taong-gobyerno na hindi nakikita ang ugat ng problema kaya hindi nabibigyan ng pangmatagalang solusyon ang isyu sa baha gaya na lamang ng pagsuntok sa hangin.

Bagamat direktang inuyam sa editoryal karton ang gobyerno dahil sa pagsuntok nito sa hangin na waring nasusuhulan, ang totoong target sa kartong ito ay ang mga malalaking kompanya at industriya sa bansa sa walang habas at iligal na pagputol ng mga puno na naging sanhi ng matinding pagbaha sa bansa.

Sa lohikal na mekanismo, ang pagpigil o pagtakip sa mukha ng *illegal logger* sa gobyerno ay di-lohikal. Inaasahan na ang gobyerno ang siyang pumipigil sa mga tiwali ngunit mamamalas sa editoryal karton na tila ang *illegal logger* ang pumipigil sa gobyerno.

Kapansin-pansin ang *script opposition* sa pagitan ng gobyerno at *illegal logger* sa editoryal karton. Makikita na payat ang karakter na kumakatawan sa gobyerno at may matabang pangangatawan naman ang sa *illegal logger* na representasyon ng mga malalaking kompanya at industriya sa bansa na nagsisiputol ng puno bagay na nagpapahiwatig na mas makapangyarihan o kontrolado ng *illegal logger* ang tao na mamamalas sa editoryal karton.

Talahanayan 7. Pagsusuri sa editoryal kartong "*Reforestation - Long-range Answer to Massive Flooding*" (*Analysis of the editorial cartoon* "*Reforestation - Long-range Answer to Massive Flooding*").

Semiotic Features	Pagsusuri sa Editorya Karton
Sitwasyon	Walang habas na pagputol ng punongkahoy at ang pinsalang dulot ng Bagyong Ulysses na nagpalubog sa lungsod ng Marikina at mga karatig lugar gaya ng Cagayan at Isabela
Wika at *Semiotic Strategy*	Ipinalulutang ang mapang-uyam at nakatatawang mensahe sa karakter na sumusuntok sa hangin at sa ekspresyon sa mukha ng *illegal logger*
Target	Gobyernong nasusuhulan ng malalaking kompanya at industriyang walang habas sa pagputol ng mga puno
Lohikal na Mekanismo	Ang pagpigil o pagtakip ng *illegal logger* sa mukha ng karakter na kumakatawan sa gobeyrno
Script Opposition	Payat na pangangatawan ng karakter na kumakatawan sa gobyerno at matabang pangangatawan ng *illegal logger* na representasyon ng malalaking kompanya at industriya sa bansa na nagsiputol ng mga puno

PAGTATALAKAY

Ipinakikita sa editoryal kartong *Welcome, Baguio City* ang isyung pangkapaligiran kaugnay sa paglaganap ng *COVID-19* pandemya na masasalamin sa target sa pamamagitan ng semyotika at biswal na mga kagamitang makikita sa editoryal karton. Mapapansin dito ang guhit ng *virus* na nakapalibot sa *banner* na may kapsyong "*Welcome to Cebu City*." Ang banta ng *virus* ay higit na mapanganib sa mga bisitang papasok sa lungsod. Makikita rin ang tatlong tao na batay sa pagkaguguhit ng kanilang imahen at postura ay nagpapahiwatig na sila ay eksperto sa pasugpo ng virus na lumalaganap sa lungsod. Ang bagong tuklas na *β-coronavirus* ay pinangalanang *2019-novel coronavirus* na pinaniniwalaang unang kumalat sa Wuhan, China, noong Disyembre 2019. Noong Pebrero 2020, ang *2019-novel coronavirus* ay opisyal na pinangalanan ng World Health Organization (WHO) na kilala ring *COVID-19* (Guo et al. 2020). Binanggit sa artikulo ng ABS-CBN News na ang Lungsod ng Cebu ang may pinakamaraming kumpirmadong kaso ng *COVID-19* sa bansa (Ganibe 2020). Dagdag pa nito, anim na lugar sa bansa ang binabantayan ng Department of Health (DOH) dahil sa patuloy na pagtaas ng kaso ng *COVID-19* kabilang na ang Manila at Quezon City, Cebu Province, Cebu City, Mandaue at Lapu-Lapu City (de Guzman 2020). Ang mataas na kaso ng *COVID-19* sa Lungsod ng Cebu ang naghimok sa *Local Government Unit (LGU)* ng Baguio upang tumulong sa pamamagitan ng pagbabahagi ng kanilang kaalaman at kasanayan upang maiwasan ang paglaganap ng pandemya. Gayunpaman, mapapansin din sa editoryal karton na ang *team* mula sa Baguio City ay hindi nakasuot ng *personal protective equipment* (PPE) gaya ng *face mask, face shield,* at iba pa na maaaring magdulot sa kanila ng panganib at dumagdag pa sa lumulubong bilang ng mga taong may *COVID-19* sa bansa. Mahihinuha na hindi naging handa ang *team* Baguio City sa kanilang pagbisita sa Lungsod ng Cebu batay sa guhit na nasa editoryal karton. Mas magiging mainam kung ang pagbabahaginan ng impormasyon at kaalaman ay isinagawa sa pamamagitan ng *online meeting* gamit ang mga aplikasyong onlayn gaya ng *Zoom*, *Google Meet* at iba pa upang hindi mailagay sa panganib ang kalusugan ng *team* mula sa Baguio City.

Isinasalamin ng editoryal kartong *Government nurses finally get nurses long delayed pay increase* ang isyu ng mababang pasahod ng mga nars sa kabila ng hirap na kanilang dinaranas sa pagsugpo sa *COVID-19* na maituturing na kabahagi ng isyung panlipunan. Ang *semiotic devices* at biswal na metapora ay nakatulong sa pagpapaabot ng ideya ng *cartoonist* ukol sa isyu. Iginiit pa nga ng Filipino Nurses United (FNU) na matagal na panahon na silang nagbibigay ng serbisyo sa bayan kahit katumbas nito ay ang mababang sahod at 'di makataong kondisyon ng kanilang trabaho (Nishimori 2020). Sa kabila ng mahalagang papel at di-matatawarang kontribusyon ng mga nars sa larangan ng kalusugan, nananatili pa ring mababa ang suporta sa kanila. Ang tinatayang sahod ng isang rehistradong nars sa Pilipinas ay PHP 36,000 kada buwan sa taong 2024. Ang mga nagsisimulang nars ay may mas mababang sahod ngunit karaniwang nakatatanggap ng dagdag sahod alinsunod sa kanilang natatanggap na promosyon at karanasan (Senate Bill No. 2694 2024). Samantala, ang renumerasyon ng mga *staff nurse* sa Singapore ay iba-iba batay sa antas ng karanasan, espesyalisasyon, at ang kompleksidad ng tungkulin. Sa pangkalahatan, ang isang *staff nurse* ay sumasahod ng humigit-kumulang na SGD 7,100 kada buwan (aabot ng PHP 310,445 ayon sa Oanda Fx Data Services nd). Ngunit, maaari itong magsimula sa SGD 2,084 at umaabot hanggang SGD 6,900 kada buwan (Dynamic Health Staff nd). Sa madaling salita, ang sahod ng *staff nurse* sa Singapore ay aabot ng PHP 91,122.30 hanggang PHP 301,700.00 kada buwan (Oanda Fx Data Services nd). Sa Tsina naman, ang isang rehistradong nars ay karaniwang kumikita ng CNY 279,400 bawat taon o PHP 2,269,999 (ayon sa conversion rate ng Oanda Fx Data Services nd), maaaring umabot sa pinakamababa na average na sahod na CNY 137,400 hanggang sa

pinakamataas na average na CNY 437, 300 at may kasama rin na mga benepisyo tulad ng tahanan at transportasyon (World Salaries nd). Nangangahulugan ito na ang mga nars sa Tsina ay kumikita ng PHP 1,116,313 hanggang PHP 3,552,866 bawat taon (Oanda Fx Data Services nd). Isinisiwalat nito hindi lamang ang isyu ng paglaganap ng COVID-19 maging ang isyung panlipunang kinaharap ng mga nars at sektor ng kalusugan kaugnay sa dami at bigat ng trabaho at mababang kompensasyon. Maaari rin itong magdulot ng *braindrain* dulot ng mababababang sahod, kakulangan ng mga nars na mangangalaga sa maysakit, at *overworked* at *stressed* na mga nars na magdudulot pagbaba ng kalidad ng serbisyong pangkalusugan.

Malinaw namang ipinalutang sa editoryal karton na *Telling vs showing* ang isyu sa pagtugon ng Lungsod ng Cebu laban sa *COVID-19* at sa pagtupad ng ibang proyekto tulad ng *flood control, sanitation*, at iba pa. Mapapansing ang berbal at di-berbal na aspekto ng karton ay nagkakaisa sa pagpapahayag ng mensahe ng *cartoonist.* Nakikipag-interak ang semyotika sa *script* nito upang maihayag ang kabuoang mensahe. Ang suot na barong, postura, salamin, hugis ng mukha at ayos ng buhok ay direktang tumutukoy sa mayor ng Lungsod ng Cebu habang ang mga kapsyon na nasa itaas ng kanyang ulo ay representasyon ng mga pangakong proyekto. Kinapapalooban ito ng dalawang eksenang nagtutunggalian ang konsepto. Ang unang eksena, ang pagtatalumpati at pagbitiw ng mga salitang ipinangako sa kanyang pangangampanya habang ang ikalawang eksena naman ay ang pagkarga ng mayor sa mga naipangakong proyekto gamit ang kartilya na sumisimbolo sa pagpapatupad ng mga proyektong ito. Naglalaban ang konsepto ng pagbitiw at pagtupad sa mga pangako. Binanggit nga ni Alquitran (2018) na noon pa man ay uso na ang pangako ng mga politiko at kalimitan ay puro hangin lamang. Idinagdag pa niya na buwenas lamang kung may isang natupad sa sampung pangako ng mga politiko. Ayon kay Piquero (2024), ang naantala at hindi pa natatapos na mga proyekto ay naging pangunahing suliranin sa Lungsod ng Cebu katulad ng pagkaantala ng Cebu City Medical Center (CCMC). Ang CCMC na nasa yugto pa ng construction na umabot na ng siyam na taon at may kaukulang dalawang bilyong pisong pondo nagagasta ay hindi pa rin natatapos at tanging ang tatlong palapag pa lamang ang ganap na operasyonal. Mahihinuha sa editoryal karton ang pang-uuyam na kadalasang ipinapangako ng mga politiko sa panahon ng pangangampanya ay kadalasang napapako sakaling sila na ang ibinoto at nakaupo sa posisyong kanilang pinangarap.

Tinalakay sa editoryal kartong *Environment, energy issues linked to pandemic* ang isyu sa enerhiya sa bansa na mas lalong pinalala ng pandemya. Mapapansin na ang semyotika, berbal man o di-berbal ay tumutugma sa usaping umiiral sa karton. Dalawang pigura ang makikita rito, ang pigura ng lalaking nakaupo habang nakatingala sa nakapatay na ilaw at ang kableng hugis *virus* na hindi nakasaksak. Ang mga *semiotic features* na ito ay tumutugma at maiuugnay sa mensaheng nais maihatid ng *cartoonist* ukol sa isyu ng enerhiya sa bansa. Mahihinuha rin sa mga representasyong ito na may agwat ang tao sa presyo ng bayarin ng kuryente ayon sa mga biswal na representasyon. Ang agwat na ito ay bunga ng pagpapatupad ng *fit-all rates* na bayarin sa lahat ng mga konsyumer ng kuryente. Samantala, ang hugis *virus* na kable ng kuryente ay sumisimbolo sa epekto ng *COVID-19* sa pagtaas ng presyo ng kuryente. Ang nakatatawang paglaki ng mga mata bilang ekspresyon ng karakter sa editoryal karton ay paraan sa pang-uuyam sa mga *electric distributor* sa kanilang insensitibo at pabayang pagpapatupad ng polisiyang *fit-all rates* na bayarin sa kuryente. Binanggit sa Manila Bulletin (2020) na ang pandemya ay nagdulot ng maraming suliranin na nakaapekto hindi lamang sa sektor ng kalusugan kung ‘di maging sa iba pang aspekto ng nasyonal na buhay. Dagdag pa nito, nagkaroon ng problema sa mataas na bayarin sa kuryente sa unang mga buwan ng *Enhanced Community Quarantine* (ECQ). Sa pananaliksik ni Lagunsad (2020) binanggit niya na noong 16 Marso 2020 ang mga lider ng bansa ay nagdesisyon na isailalim sa *ECQ* ang buong Metro Manila bilang pagtugon sa banta ng *COVID-19.* Sa ilalim nito, ipinatupad ang mahigpit na polisiya at pagpapatupad ng mga panuntunan sa lahat ng *Local Government Unit* (LGU) kabilang na ang pagbabawal sa paglabas ng bahay maliban lamang kung may bibilhing pangangailangan gaya ng pagkain at gamot dala ang kanilang *quarantine pass*, suot ang *face mask*, at pag-obserba sa *social distancing*. Ipinagbabawal din ang *movement o* paggalaw anuman ang edad o kalagayang pangkalusugan, minimal lang ang aktibidad sa ekonomiya maliban sa *utility services* (pagkain, kuryente, tubig, at iba pa) at kritikal na sektor ng ekonomiya, walang pisikal na klase, ang pag-eehersisyo sa labas ng bahay, mga pagtitipon, pamamasada at pagsakay sa pampublikong transportasyon, *domestic flights* at limitadong *international flights* (PhilStar 2020). Inihahayag lamang nito na tila may ugnayan ang pandemya sa sanga-sangang kinakaharap na suliranin ng bansa na mas lalong nagpapalala nito. Sa isang advisory, ipinaalam ng Manila Electric Company (MERALCO) ang pagtaas ng electricity rate ng PHP 0.1543 per kilowatt-hour (kWh) na dumagdag sa kabuoang rate ng isang household sa PHP 11.7882 per kWh sa Setyembre 2024 mula sa PHP 11.6339 per kWh sa Agosto 2024 (Cordero 2024). Sa Tsina, sa nakalipas na ilang taon, bahagyang tumaas ang average industrial power rate na umabot sa humigit-kumulang USD 0.088 per kWh (o may katumbas na PHP

5.17917 batay sa Oanda Fx Data Services nd) sa 2024 mula sa USD 0.084 per kWh (o PHP 4.94375 batay sa Oanda Fx Data Services nd) noong 2019 (Xiao at Zhou 2024). Sa Thailand naman, ang presyo ng kuryente para sa residential ay nagkakahalaga ng THB 4.293 per kWh o USD 0.127 (o PHP 7.47448 ayon sa Oanda Fx Data Services nd). Umaabot naman sa THB 4.300 o USD 0.128 (o PHP 7.53334 ayon sa Oanda Fx Data Services nd) ang presyo ng kuryente para sa mga negosyo. Ipinatupad ang presyong ito noong March 2024 kasama na rito ang cost of power, distribusyon, transmisyon, buwis at iba pang mga bayarin (GlobalPetrolPrices.com nd). Ang pagkakaroon ng mataas na bayarin sa kuryente ay maaaring magkaroon ng epekto sa mahihirap na pamilya gaya ng limitadong access sa kuryente, pagiging hadlang sa kalidad na edukasyon at mga ekonomikong oportunidad, pagtaas ng panganib sa sunog, pagtaas ng gastusin sa enerhiya na nagiging hadlang sa pag-unlad ng maliliit na negosyo. Nakaaapekto rin ito sa pisikal at mental na kalusugan ng mga tao na nagpapababa sa kalidad ng buhay at nagpapalawak sa siklo ng kahirapang nararanasan.

Tinalakay sa editoryal kartong *Reforestation - long-range answer to massive flooding* ang isyung pangkalikasan ukol sa walang habas na pagputol ng mga punong kahoy na naging sanhi sa matinding pagbaha sa bansa. Malinaw ang nakatatawang mensaheng makikita sa ekspresyon sa mukha ng matabang *illegal logger* na representasyon ng mga kompanya at industriyang nagpuputol ng mga kahoy habang pinipigilan ang payat na lalaking kumakatawan sa gobyerno na sumusuntok sa hangin. Ipinapahiwatig nito na tila nagbubulag-bulagan ang gobyerno o nasusuhulan kaya tila hindi nila nakikita ang tunay na dahilan ng matinding pagbaha sa bansa. Sa ulat ng ABS-CBN News, inamin ng Gobernador ng Cagayan na ang dahilan ng matinding pagbaha sa kanilang lugar ay dulot ng pagkakalbo ng kagubatan at *illegal mining* sa kanilang lugar (Ochona 2020). Kaugnay nito, isinisisi ng mga *peasant group* ang pagbaha sa Cagayan at Isabela matapos ang paghagupit ng Bagyong Ulysses sa talamak na pagmimina ng *black sand* at iligal na pagputol ng mga puno (Journal Online 2020). Inihahayag lamang nito na bagamat mayroong pandemya ang bansa, patuloy pa rin ang iligal na mga gawain na nakapipinsala sa kalikasan na nagiging mitya ng pagbaha at iba pang pinsala dulot ng natural na kalamidad kung kaya nararapat itong pagtuonan ng pansin ng kinauukulan. Ayon sa Global Forest Watch (nd), mula sa taong 2002 hanggang 2023, nabawasan ang Pilipinas ng 190,000 ha ng pangunahing kagubatan *(humid primary forest)* sumasakop ito sa kabuoang 13% ng mga nawalang puno sa kaparehong panahon. Bumaba ng 4.1% ang kabuoang erya ng pangunahing kagubatan sa Pilipinas sa mga panahong ito. Ang *deforestation* sa Pilipinas ay nagdudulot ng iba't ibang usaping pangkapaligiran at panlipunan. Ang pagkasira ng kagubatan ay maaaring maging sanhi ng *soil erosion*, *landslide*, at pagkaubos ng mapagkukuhanan ng tubig. Maaari ring magkaroon ng sosyal impak ang *deforestation* sapagkat madalas itong humahantong sa *displacement* ng mga lokal na komunidad at paglaho ng mga tradisyonal na kabuhayan (Salas 2023).

Sa isinagawang pagsusuri sa piling editoryal karton mahihinuha sa semyotika nito ang mga lantad na pagpapahiwatig sa diskursong pangkalikasan at ang mga usaping pangkalikasan at panlipunang umiiral gaya ng pagtaas ng impkesyong dulot ng *COVID-19* pandemya, pagtaas ng sahod ng mga nars sa Pilipinas, maayos na pagpapatupad ng mga proyekto ng gobyerno, mataas na bayarin ng kuryente sa panahon ng pandemya, at ang walang habas na pagputol ng mga puno ng *illegal loggers.* Nakatulong naman ang mga guhit, karakter, at mga ekspresyon ng mukha sa pagbibigay-diin sa mga di-lantad na pagpapahiwatig sa pagpapaabot ng mensahe ng piling editoryal karton. Mahihinuha sa editoryal karton na ang *team* mula sa Baguio City bagaman kakikitaan ang mga karakter ng pagiging eksperto at may kaalaman sa pagsugpo sa *COVID-19* pandemya, ang hindi nila pagsusuot ng *personal protective equipment (PPE)* ay indikasyon ng kanilang hindi pagiging handa sa pagbisita sa Lungsod ng Cebu na maaaring magdulot sa kanila ng panganib na mahawaan ng *virus.* Mas magiging ligtas at praktikal ang pagsasagawa ng *online meeting* sa pagbabahaginan ng impormasyon hinggil sa pagsugpo sa *COVID-19* gamit ang mga aplikasyong onlayn gaya ng *Zoom*, *Google Meet*, at iba pa. Makikita naman ang guhit na karakter ng isang nars na may punit-punit na uniporme at ang brasong nakasuot na barong na may hawak na pera na nagpapahiwatig sa masaklap na kalagayan ng mga nars sa bansa sa panahon ng pandemya at ang panawagan sa pagtataas ng pasahod ng mga nars. Makikita naman sa isa pang editoryal karton ang dalawang larawan - pagbitiw ng pangako at ang pagpapatupad ng pangako. Mahihinuha rito ang sitwasyon na sa panahon ng eleksyon ay napakaraming magagandang proyekto ipinapangako ngunit kung nasa puwesto na ay tila nakalilimutan na ang kanilang naipangako sa taumbayan. Samantala, ang guhit na kuryenteng hugis virus at taong nakatingala sa ilaw ay nagpapahiwatig sa mataas na bayarin ng kuryente sa panahon ng pandemya at tila hindi na maabot ng mga konsumedor ang bayarin. Panghuli, malinaw ang nakatatawang mensaheng makikita sa ekspresyon sa mukha ng matabang *illegal logger* na representasyon ng mga kompanya at industriyang nagpuputol ng mga kahoy habang pinipigilan ang payat na lalaking kumakatawan sa gobyerno na sumusuntok sa hangin. Ipinapahiwatig nito na tila nagbubulag-bulagan ang gobyerno o nasusuhulan kaya tila hindi nila nakikita ang tunay na dahilan ng matinding pagbaha sa bansa. Ang mga lantad at di-lantad na pagpapahiwatig na ito ay kapwa

nakatulong sa pagbibigay-diin sa mensaheng ipinapaabot ng mga editoryal karton. Sa pamamagitan ng mga nakatatawang ekspresyon, panunudyo, at pang-uuyam, naiengganyo ang mga mambabasa na makilahok sa mga usaping isinisiwalat ng mga editoryal karton at nagmumulat sa kanilang kamalayan hinggil sa mga usaping umiiral lalo na sa mga usaping pangkalikasan at panlipunan sa panahon ng paglaganap ng *COVID-19* pandemya kabilang na rin ang usapin sa mataas na bayarin sa kuryente, pagpapatupad ng mga naipangakong proyekto, *salary increase* ng mga nars, at ang iligal na pagputol ng mga punongkahoy.

Sa pangkalahatan, natuklasan sa pag-aaral na ang mga nangungunang diskursong ekolohikal na nasipat sa piling editoryal karton ay may kaugnayan sa *COVID-19* pandemya at ng iligal na pagputol ng mga puno na naging sanhi ng pagbaha sa bansa. Ang pandemya ay nagsiwalat sa iba pang mga isyung panlipunang kinakaharap ng bansa gaya ng mataas na bayarin sa kuryente. Natuklasan din na ang *linguistic*, *semiotic* at *visual tools*, at iba pang aspektong berbal at di-berbal ay nakatutulong sa paghahatid sa mensaheng nais ipabatid ng *cartoonist*. Ang nakatatawang mensahe ng editoryal karton ay naipahihiwatig sa pamamagitan ng pang-uuyam sa gobyerno, sa mga kompanya at industriyang namumutol ng kahoy sa pamamagitan ng ekspresyon ng mukha ng mga karakter. Maiuugnay ito sa *Semantic Script theory* na ibinabatay ang argumento ng editoryal karton sa nakatatawang diskurso kaugnay sa usaping tinatalakay nito. Bilang karagdagan, isinasaad din ng teoryang *Visual Metaphor* na isinisiwalat ng mga karton ang kritikal na perspektiba hinggil sa usaping danas ng lipunan. Pinatutunayan nito na ang paggamit ng pang-uuyam at nakatatawang ekspresyon sa mukha ay isang mabisang kasangkapan sa pagpapalutang ng diskursong pangkapaligiran at isyung pangkalikasang nararanasan ng bansa sa panahon ng *COVID-19*. Mula sa mga natuklasan, naisakongklusyon na ang *semiotic features* sa piling editoryal karton gaya ng mga nakasulat na teksto, guhit, ekspresyon ng mukha at iba pa ay isang mabisang kasangkapan sa pagkikipag-ugnayan sa mga napapanahong isyung pangkalikasan at mga usaping panlipunang nauugnay sa pandemya. Ang mapang-uyam at nakatatawang estilo ng pagkabuo ng mga editoryal karton ay nakatulong sa pagbibigay-diin sa napapanahong isyu at nakakukuha ng atensyon upang maging mulat at malay ang mga mamamayan sa usaping pangkalikasan at sa sanga-sangang usaping panlipunang naidulot ng *COVID-19* pandemya.

Iminungkahi ng mananaliksik ang mas malawakang pag-aaral sa mga editoryal karton kabilang na ang iba pang mga rehiyonal na pahayagan upang magkaroon ng representasyon ang bawat rehiyon sa bansa. Ang pagpapalawak sa pag-aaral ay makatutulong upang mailahok ang pangkalahatang diskursong pangkapaligiran at isyung pangkalikasan sa pagsipat sa aktuwal na kalagayan ng kapaligiran sa bawat panig ng bansa. Bigyang-diin ang pagtuturo sa pagpapahalaga sa kalikasan sa lahat ng antas ng pag-aaral upang makalinang ng mga mag-aaral na hindi lamang handa para sa karera ng buhay kung hindi bilang mga tagapangalaga ng kalikasan at may pagkiling sa preserbasyon at pagpapanatili nito. Iminumungkahi rin sa mga mananaliksik sa hinaharap na magsagawa ng mga pag-aaral gamit ang *eco-critical discourse analysis*, *ecolinguistics*, *green studies* at iba pang lapit na nagbibigay-diin sa kahalagahan ng pagsipat sa kalikasan at iba pang usaping magpapatibay sa pagsusulong sa pangangalaga at preserbasyon ng kalikasan.

PINANGGALINGAN NG PONDO

Hindi nakatanggap ng pondo ang pag-aaral sa anumang mga ahensiya o institusyon.

ETIKAL NA KONSIDERASYON

Sumulat ang mananaliksik kay G. Roni Santiago, *Editor of the Comics Department* ng Manila Bulletin at kay Dr. Nestor B. Ramirez, *Executive Publisher* ng Sunstar upang humingi ng pahintulot na magamit ang mga editoryal kartong kanilang inilathala at maisama sa paglalathala sa kasalukuyang pag-aaral. Pinaunlakan at nagbigay ng permiso sina G. Roni Santiago at Dr. Nestor B. Ramirez na muling mailathala ang mga editoryal karton sa pamamagitan ng paglagda sa isinulat na liham pahintulot ng mananaliksik. Tiniyak din ng mananaliksik na mabigyan ng karampatang pagkilala ang lahat ng mga sangguniang sinangguni ng kasalukuyang pag-aaral.

PAGKILALA

Pinasasalamatan ng mananaliksik ang Manila Bulletin, Sunstar, G. Roni Santiago, at Dr. Nestor B. Ramirez sa pagbibigay ng permiso na maisama sa pag-aaral ang nailathala nilang editoryal karton at muli itong mailathala sa kasalukuyang pag-aaral. Lubos din ang pasasalamat ng mananaliksik sa mga mahuhusay na *cartoonists* na sina Roni Santiago at Enrico Santisas, sa paglathala ng kanilang mga editoryal karton online mula Hulyo hanggang Nobyembre 2020. Ang limang napiling editoryal karton na naging batayan ng pagsusuri ay ang mga sumusunod: *Welcome, Baguio City*, *Government nurse finally get nurses long-delayed pay increases*, *Telling vs. showing*, *Environment*, *energy issues linked to pandemic*, at *Reforestation – long-range answer to massive flooding*. Taos-pusong pasasalamat

din ang ipinapaabot ng mananaliksik sa mga tagasuri at rebyuwer na nagbahagi ng kanilang husay at dunong upang mapabuti pa lalo ang pag-aaral na ito.

CONFLICT OF INTEREST

Ang may-akda ng pag-aaral na ito ay nagdeklara nang walang anumang *conflict of interest* sa ibang may-akda.

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Dynamic interplay of plants, microorganisms, and arthropods: Exploring ecosystem

Mehmet Ramazan Rişvanlı* **and Remzi Atlıhan**

Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University, Van, Turkey

**Corresponding Author: risvanli@yyu.edu.tr*

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ABSTRACT

Plants, microorganisms, and arthropods continuously interact within the intricate system of the environment. These interactions can often lead to significant crop damage due to diseases and pests; however, there are also circumstances where microorganisms serve as necessary symbiotic plant partners. A range of beneficial microbes in the soil support plant development and health through direct and indirect mechanisms. These beneficial microorganisms, also known as "little helpers" are vital due to their ability to colonize various niches and their ubiquitous presence. Increasingly, such microorganisms are used as biological control agents and microbial fertilizers. They are specific to pests and diseases, with a minimal negative impact on humans and the environment. Plants face numerous environmental challenges and must respond appropriately to survive. Recent studies suggest that beneficial microbial biota in the soil can affect herbivores, highlighting the importance of these biological agents. Specifically, they can reduce the harmful effects of herbivorous insect pests, which damage plants are a major factor in global yield losses. Therefore, they are expected to be essential candidates to replace chemical insecticides in the near future. This review includes recent findings on many aspects of below-ground and above-ground plant-mediated interactions.

Keywords: plant-mediated interactions, pest management, symbiosis, three-way interactions

INTRODUCTION

The world population is estimated to increase by approximately 83 million annually and is expected to reach 9.7 billion by 2050 (UN 2022). The increasing population brings along significant problems. One of the biggest challenges in this regard is how to meet the growing demand for food by the world's expanding population. Currently, one out of every nine people in the world is struggling with hunger, and Food and Agriculture Organization (FAO) estimates, food demand will increase by over 200% by 2050 (FAO 2018, 2021). On the other hand, crop losses to an average of up to 50% annually worldwide due to plant disease and pests (Fried et al. 2017; Grabka et al. 2022).

This figure is roughly equivalent to the food that could feed one billion people annually.

Intensive agriculture practices reliant on chemical inputs were heavily emphasized in the past century to feed the rapidly growing human population. This farming approach has caused significant damage to natural resources over the past fifty years. Irresponsible practices are rapidly depleting and polluting clean water, soil quality, energy, and biodiversity (IFPRI 2016; FAO 2021). This situation accelerates the disruption of the natural balance that restricts the spread of diseases, pests, and weeds, leading to even more damage (Bramble 1989; Arora and Dhaliwal 1996; Dhaliwal et al. 2010). The multifaceted problems caused by using chemical pesticides to control pests, which in turn cause economic damage, so the is a need to develop alternative methods. Thus, changes in European Union legislation (European Parliament Directive 2009/128/EC) restrict chemical pesticides and fertilizers in agricultural production processes, highlighting the apparent need to develop alternative methods to control pests while maintaining crop yields.

Plants, microorganisms, and arthropods constantly interact with each other in the ecosystems they inhabit. These interactions have a significant impact on plant growth and productivity. Harmful or pathogenic microorganisms can reduce yield, while productivity can increase through mutualistic relationships established with beneficial microorganisms (Ballhorn et al. 2017; Wilkinson et al. 2019). Unfortunately, the effects of arthropods and microorganisms on plants have been studied independently by entomologists and phytopathologists. While phytopathologists concentrate on the study of plant diseases brought by microorganisms like fungi, bacteria, and viruses, entomologists study insects and other arthropods. Researchers can better understand the elements that affect plant health and productivity by adopting a more interdisciplinary approach and considering the interactions between plants, arthropods, and microorganisms. Through the research conducted, a more apparent appreciation has been obtained regarding the significance of this matter.

Surprisingly, research on the impacts of interactions between plants, microorganisms, and arthropods (PMA) on plant production is limited. Nevertheless, all these factors coexist in the same ecosystem, interact in various ways, and can cause more benefit or harm to the plant than expected. For example, the success of the control of the invasive species "prickly pear cacti" (*Opuntia* spp.) in Australia is attributed to the South American cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera, Pyralidae), which allows pathogens to enter the plant through the wounds it creates (Courtney and Forsberg 1988; Varone et al. 2014). This example illustrates that neither the host nor the microorganism alone can control this invasive species. Instead, control of the plant is achieved through mutual interaction. A meta-analysis encompassing data from 132 records across 35 studies published between 1969 and 2011 supports this example revealing that the combined effects of herbivorous arthropods and plant diseases have a far greater impact on plant performance than the sum of their individual effects. In the meta-analysis, it was shown that arthropod herbivores and phytopathogens typically had synergistic effects on plant performance, which means that when they coexisted, the effects of each stressor were increased. Overall, these findings contribute to this understanding on how multiple stressors can interact to affect plant performance and highlight the importance of considering different types of plant traits when studying these interactions (Hauser et al. 2013). The examples presented highlight the interdependence of PMA interactions because they include complex and dynamic feedback mechanisms that significantly impact each participant's fitness and survival. These deep interdependencies highlight the tripartite interactions' integrated character, wherein the behaviors of plants, microorganisms, and insects closely influence the ecological consequences and evolutionary trajectories of one another. If interactions among PMA, such as those in the example, are fully understood and effectively utilized in agricultural production systems, this could provide a significant advantage in pest management. Furthermore, the potential application of PMA interactions in sustainable crop production has been recommended to achieve crop protection targets under the United Nations Sustainable Development Goals for 2030. This study examined the potential benefits of PMA interactions in plant production and pest management.

CURRENT PERSPECTIVES ON THE INTERACTIONS AMONG PLANTS, ARTHROPODS, AND MICROORGANISMS

Recent advancements have increased the quality of products and the environment by using appropriate plant-microorganism combinations (Aneja et al. 2016; Bakker et al. 2018; Fernandez-Conradi et al. 2018; Woo and Pepe 2018; Coppola et al. 2019a; Coppola et al. 2019b; Contreras-Cornejo et al. 2020; Agbessenou et al. 2022). Indeed many countries, studies were conducted to obtain microbial fertilizer formulations containing soil-borne beneficial microorganisms. Microbial fertilizers control soil-borne diseases, decompose organic waste, improve soil structure and plant nutrition, reduce the need for chemical fertilizers as well as soil and water pollution (Bakker et al. 2018; Coppola et al. 2019a).

Historically, the effects of arthropods and microorganisms on plant health and yield have been

studied independently by entomologists and phytopathologists. Beneficial microorganisms have long been used in various disease-control measures, and successful results have been reported in numerous studies. For example, *Azotobacter* spp., *Bacillus* spp., *Pseudomonas* spp., and *Trichoderma* spp. are recognized biocontrol agents that effectively control many plant diseases, including leaf spot, gray mold, soft rot, stem rot, wilt, blight, and mildew (Figueiredo et al. 2010; Kim et al. 2011; Lazebnik et al. 2014; Pieterse et al. 2014; Vinale et al. 2014; Liu et al. 2018; Bakker et al. 2018; Vaello et al. 2018; Verma et al. 2019; Metwally 2020; Oljira et al. 2020; Adeleke and Babalola 2021; El-Maraghy et al. 2021). However, information on the effects of interactions between arthropods and microorganisms on plant health and yield is limited. Furthermore, studies on the effects of these interactions on plant growth and herbivore populations have only gained momentum in recent years, and continue to be revealed. For example, Pineda et al. (2010) showed how rhizobacteria prime plants improved defense against herbivorous insects, whereas Biere and Bennett (2013) addressed the function of endophytic fungi in affecting multitrophic interactions. In the same way, Macías-Rodríguez et al. (2020) demonstrated how arbuscular mycorrhizal fungi can change the population dynamics and diets of herbivores. To provide insights into sustainable pest management strategies, Zytynska (2021) underlined the significance of comprehending plant-microbe-arthropod interactions under changing climatic conditions. While Alınç et al. (2024) reported that root inoculation with beneficial soil bacteria improved plant defenses against herbivore feeding and egg deposition, Van Dijk (2021) offered a more comprehensive view of how these interactions impact ecosystem dynamics. Mathematical models have been used to simulate the results of plant-microbe-pest interactions in an attempt to support and extend these biological findings. For instance, the positive effects of mycorrhizal fungi on the health of the potato plant have been demonstrated in the mathematic models. A model simulating the interaction between the potato plant, mycorrhizal fungi, and the Colorado potato beetle (*Leptinotarsa decemlineata* Say), an important potato pest. The Colorado potato beetle showed that although mycorrhizal fungi can improve plant productivity, reliance on these fungi may result in changes in pest populations, highlighting the requirement for balanced management strategies. By simulating these interactions with mathematical models, researchers can gain valuable insights for using these interactions in plant protection and production (Atlihan et al. 2021; Seminara et al. 2021). Some significant studies on interactions between plants, microbes, and arthropods are provided in the table below.

Table 1. Three-way interactions between plants, arthropods, and microbes reported by different researchers. An overview at the various consequences. PGPF: Plant growth-promoting fungi, PGPR: Plant growth-promoting rhizobacteria.

Pest	Microorganism	Plant	Effect	Literature
Diabrotica undecimpunctata howardi Barber and *Acalymma vittatum* Fabricius	*Pseudomonas putida* Trevisan, *Serratia marcescens* Bizio, *Pseudomonas oryzihabitans* Kodama (formerly Flavimonas) and *Bacillus pumilus* Meyer & Gottheil (PGPR)	Cucumber	Reduced pest population and enhanced plant yield	(Zehnder et al. 1997)
Plutella xylostella L.	*Acremonium alternatum* Link (PGPF)	Courgette	Negative effect on the pest feeding and development	(Raps and Vidal 1998)
Cnaphalocrocis medinalis Guenée	*Pseudomonas fluorescens* (PGPR)	Rice	51.9% reduction in the pest population and a 16.5% increase in plant yield	(Commare et al. 2002)
Amrasca biguttulla biguttulla Ishida and *Aphis gossypii* Glover	*Pseudomonas fluorescens* (PGPR)	Okra	Reduced the pest population, increased plant yield	(Gandhi et al. 2006)
Pieris rapae L. and *Spodoptera exigua* Hübner	*P. fluorescens* and *Pseudomonas syringae* van Hall (PGPR)	Arabidopsis	Negative effect on *S. exigua* development, no effect on *P. rapae*	(Van Oosten et al. 2008)
Myzus persicae Sulzer	*Bacillus subtilis* Ehrenberg and *Bacillus amyloliquefaciens* Fukumoto (PGPR)	Pepper	No effect on the pest population, positive effect on the pepper germination and development	(Herman et al. 2008)
Spodoptera littoralis Boisduval	*Rhizobium leguminosarum* Frank (PGPR)	White clover	Increased *S. littoralis* performance	(Kempel et al. 2009)

Pest	Microorganism	Plant	Effect	Literature
			(enhanced feeding or development)	
Helicoverpa armigera Hübner	*Acremonium strictum* W. Gams (PGPF)	Broad bean	Reduced larval performance and fitness of the pest and effects carried over to the second generation	(Jaber and Vidal 2010)
Bemisia tabaci Gennadius	*B. subtilis* (PGPR)	Tomatoes	Decreased pupal development in the pest	(Valenzuela-Soto et al. 2010)
Macrosiphum euphorbiae Thomas	*Trichoderma longibrachiatum* Rifai (PGPF)	Tomatoes	Increased attraction of natural enemies to the plant	(Battaglia et al. 2013)
H. armigera	*Funneliformis mosseae* (Formerly *Glomus mosseae*) Nicolson & Gerd (PGPF)	Tomatoes	Negative effect on pest larval performance	(Song et al. 2013)
Thrips tabaci Lindeman	*Clonostachys rosea, Trichoderma* spp., *Hypocrea lixii* Patouillard, and *Fusarium* sp. (PGPF)	Onion	Reduced pest population, lower feeding punctures, and fewer eggs laid on inoculated plants.	(Muvea et al. 2014)
Leucinodes orbonalis Guenée	*T. longibrachiatum* (PGPF)	Aubergine	50% decrease in the pest population, 56% increase in plant yield	(Ghosh and Pal 2016)
M. persicae	*Bacillus velezensis* (PGPR)	Arabidopsis	Reduced pest settling, feeding, and reproduction.	(Rashid et al. 2017)
Spodoptera frugiperda Smith	*Trichoderma atroviride* Karsten (PGPF)	Maize	Decreased pest population and performance, the increased attraction of natural enemies to the inoculated plant	(Contreras-Cornejo et al. 2018)
B. tabaci	*Trichoderma harzianum* Rifai (PGPF)	Tomatoes	Approximately 35% mortality in the pest population	(Jafarbeigi et al. 2020)
S. littoralis and *Ma. euphorbiae*	*T. atroviride* and *T. harzianum* (PGPF)	Tomatoes	100% death rate on *S. littoralis* in 25 days and increased natural enemy attraction to the inoculated plant	(Coppola et al. 2019a; Coppola et al. 2019b)
Auchenorrhyncha Dumeril and *Coccoidea* spp.	*Trichoderma* spp (PGPF)	Grapevine	Increased attraction of natural enemies to the inoculated plant	(Parrilli et al. 2019)
Unaspis mabilis Lit & Barbecho	*Trichoderma* spp. (PGPF)	*Lansium domesticum* Corrêa	Reduced pest population and performance, including feeding and reproduction	(Silva et al. 2019)
Tuta absoluta Meyrick	*Trichoderma asperellum* Samuels, *Beauveria bassiana* Balsamo-Crivelli and *H. lixii* (PGPF)	Tomatoes, Nightshade	Decreased number of eggs, vitality, pupa formation, and adults of the pest	(Agbessenou et al. 2020)
S. littoralis and Ma. euphorbiae	*T. afroharzianum* Błaszczyk, *T. atroviride* (PGPF)	Tomatoes	Enhanced pest resistance at specific temperatures and induced differential defense gene expression in plants	(Di Lelio et al. 2021)
Phyllophaga vetula Horn and *Aphididae* spp.	*T. harzianum* (PGPF)	Maize	Positive effect on plant tolerance and defense responses to pests	(Contreras-Cornejo et al. 2021b)
Manduca sexta L.	*T. harzianum* and *Rhizoglomus irregulare* (formerly *Rhizophagus*	Tomatoes	Negative effect on the development of the pest	(Papantoniou et al. 2021)

Pest	Microorganism	Plant	Effect	Literature
	irregularis) Blaszk., Wubet, Renker & Buscot (PGPF)			
M. persicae and *Tetranychus urticae* Koch	*B. amyloliquefaciens*, *Pseudomonas* spp., *Trichoderma* spp. and *Cordyceps fumosorosea* (*formerly Isaria fumosorosea*) Wize (PGPR, PGPF)	Pepper	Decreased in the number of eggs laid of pest	(Pappas et al. 2021)
T. absoluta	*T. asperellum* (PGPF)	Tomatoes	Decreased larval feeding performance of the pest	(Agbessenou et al. 2022)
S. exigua	*T. harzianum* (PGPF)	Cotton, potato	Negative effect on the pest development, reproduction, survival rate, population parameters, and leaf consumption	(Risvanli 2022)
Nezara viridula L.	*T. harzianum* (PGPF)	Tomatoes	Reduction in pest growth rate	(Alınç et al. 2021)
T. urticae	*Bacillus* spp., *F. mossae*, *Pseudomonas* spp., *R. irregulare*, *Trichoderma* spp. and *C. fumosorosea* (PGPR, PGPF)	Tomatoes	Negative effects on the survival, egg production and feeding of the pest	(Samaras et al. 2023)
Diabrotica virgifera virgifera LeConte	*Trichoderma virens* Miller, Giddens & Foster and *Pseudomonas chlororaphis* Smith & Chester (PGPR, PGPF)	Maize	Suppressed pest larvae survival and development	(Huang et al. 2024)
N. viridula	*T. harzianum* (PGPF)	Tomatoes	Enhanced indirect plant defenses including natural enemy attraction to the inoculated plant	(Alınç et al. 2024)

Although it is known that synergistic interactions between plants, arthropods, and microorganisms are robust, the researchers' knowledge about these interactions and associated biological diversity is quite limited. In addition, there is limited data on interactions involving non-pathogenic microorganisms, and limited to commonly occurring species like *Trichoderma* spp., *Glomus* spp, *Bacillus* spp. (Verma et al. 2019; Rişvanlı and Fidan 2024). A more thorough and nuanced understanding of these intricate systems might be obtained by investigating this topic from many different perspectives, such as concentrating on less commonly recognized microbial taxa or their function in multitrophic interactions. This restricts the use of PMA interactions in pest control. However, current research has shown how many different beneficial microbes can be used to create novel, eco-friendly pest control methods. A study by Kızılkan et al. (2025) demonstrate that multiple soil-borne microorganisms could cause physiological alterations to the host plant, such as increased chlorophyll content and changed protein-to-carbohydrate ratios, which could negatively impact the population growth parameters of *Spodoptera exigua* Hübner, a major cotton pest. Utilizing such microbial variety not only fortifies plant defenses but also highlights the potential of using beneficial microorganisms to develop long-term pest management strategies.

PROMOTING PLANT GROWTH AND RESISTANCE THROUGH BENEFICIAL MICROORGANISMS: MECHANISMS AND IMPLICATIONS

Various groups of soil-borne microbes can positively affect plant development and defense activities, both directly and through plant-mediated mechanisms. These groups include mycorrhizal fungi, endophytic root fungi, rhizobium bacteria, plant growth-promoting fungi (PGPF) and plant growth-promoting rhizobacteria (PGPR) (Bezemer and van Dam 2005; Gehring and Bennett 2009). It is well established that beneficial microbes promote nutrient uptake and utilization, enhance resilience to abiotic stress, and contribute to the growth of shoots and roots. As an illustration, mycorrhizal fungi and plant roots develop symbiotic relationships that greatly increase the efficiency of water and nutrient intake, especially

for nitrogen and phosphorus. Additionally, they have the ability to alter the amount of plant hormones like auxins and cytokinins, which have a direct impact on plant growth (Harman 2006; Verma et al. 2019; Macías-Rodríguez et al. 2020; Noman et al. 2020; Adeleke and Babalola 2021). In addition to these advantages, microorganisms can enhance photosynthesis by reducing oxidative stress under abiotic stress conditions, enhancing stomatal conductance, and increasing chlorophyll content (Macías-Rodríguez et al. 2020; Noman et al. 2020). Generally, the beneficial microorganisms with known positive effects are selected from *Bacillus* spp., *Azotobacter* spp., *Trichoderma* spp., *Rhizobium* spp., *Azospirillum* spp., and *Saccharomyces* spp. (Bezemer and van Dam 2005; Gehring and Bennett 2009; Lugtenberg and Kamilova 2009; Pineda et al. 2015; Woo and Pepe 2018). These microorganisms alter plant physiology through two fundamental mechanisms, which are defined as "promoting/stimulating plant growth" and "induced systemic resistance (ISR)" (Harman et al. 2004; Jafarbeigi et al. 2020; Noman et al. 2020; El-Maraghy et al. 2021; Contreras-Cornejo et al. 2021a; van Dijk 2021). The ISR has some unique features compared to other types of induced resistance because it is also induced by non-pathogenic microorganisms that colonize the plant roots. When a beneficial microorganism stimulates the plant, the plant switches to a primed state, called priming, after being attacked by a pathogen or insect. Priming refers to creating a faster and more effective defense response against possible attacks the plant may face after exposure to biotic or abiotic stress (Aranega-Bou et al. 2014). The defense generated by priming is more energy-efficient than structural defenses (Steppuhn and Baldwin 2008). In addition, the plant will specialize its response to the specific trace of the herbivore after recognizing it, making this mechanism more advantageous in inducible defense (Maffei et al. 2012; Zebelo and Maffei 2015). Therefore, resistance breeding for inducible defense features may become a significant alternative to biological control as a pest management strategy. Studies have shown that beneficial soil microorganisms can enhance plant defenses and have a negative impact on pests such as spider mites and aphids in crops such as pepper (Pappas et al. 2021), suggesting that breeding programs should take these traits into account.

Numerous beneficial microorganisms are widely used in studies to control plant pathogens because of their ability to promote plant growth and induce systemic resistance (Contreras-Cornejo et al. 2020; Noman et al. 2020). The effectiveness of ISR protects most plant species from bacteria, fungi, viruses, nematodes, and even pests (Pineda et al. 2010; Saharan and Nehra 2011; Pineda et al. 2015; Agbessenou et al. 2022).

In general, the induction of ISR in plants occurs through activating the Jasmonic Acid (JA) and Ethylene (ET) signaling pathways. The JA and Salicylic Acid (SA) are the main components that coordinate the complex signaling pathway in the plant that provides resistance against pests and diseases. Plants can defend themselves against disease and pests by using pathways for SA and JA. When herbivores or necrotrophic pathogens damage the plant, the JA pathway is activated, causing genes to produce defense molecules. The SA pathway, on the other hand, is primarily activated in response to biotrophic pathogens and activates defense genes known as PR genes. Although these two pathways can sometimes work together, they can also act antagonistically (Pineda et al. 2013; Pieterse et al. 2014). Other hormones, such as abscisic acid, cytokinin, gibberellic acid, and auxin, play a role as the backbone of the signaling system. Upon activation of these signaling pathways, the plant tries to defend itself directly (by producing proteins that prevent feeding, such as protease inhibitors, polyphenol oxidase, and chitinase, and by producing toxins and other secondary metabolites) or indirectly (by activating defense mechanisms such as the production of volatile organic compounds to attract natural enemies). For example, SA activation leads to an effective defense against biotrophic and phloem-feeding-sucking herbivores by strengthening cell walls and producing pathogenesis-related (PR) proteins, which prevents pathogen colonization and herbivore feeding. In contrast, JA and ET activations are effective against necrotrophic pathogens and chewing insects through promoting the production of proteinase inhibitors that reduce herbivore performance and secondary metabolites like terpenoids and alkaloids (Figure 1) (Pineda et al. 2013; Pieterse et al. 2014). However, the success of induced resistance is determined by the characteristics of the pests (diet) and the interaction between the hormones involved in resistance (Pineda et al. 2013; Agbessenou et al. 2022). While promoting plant growth through beneficial microorganisms has long been known, the importance of induced defense has recently been realized. For example, rhizobacteria-induced systemic resistance, which is usually mediated through the JA and ET pathways, prepares plants for stronger and quicker reactions to subsequent herbivore or pathogen attacks (Pieterse et al. 2014; Erb and Reymond 2019). However, there is still much to be learned about the role of ISR in PMA interactions (Silva et al. 2019; Contreras-Cornejo et al. 2021a; El-Maraghy et al. 2021; Agbessenou et al. 2022)

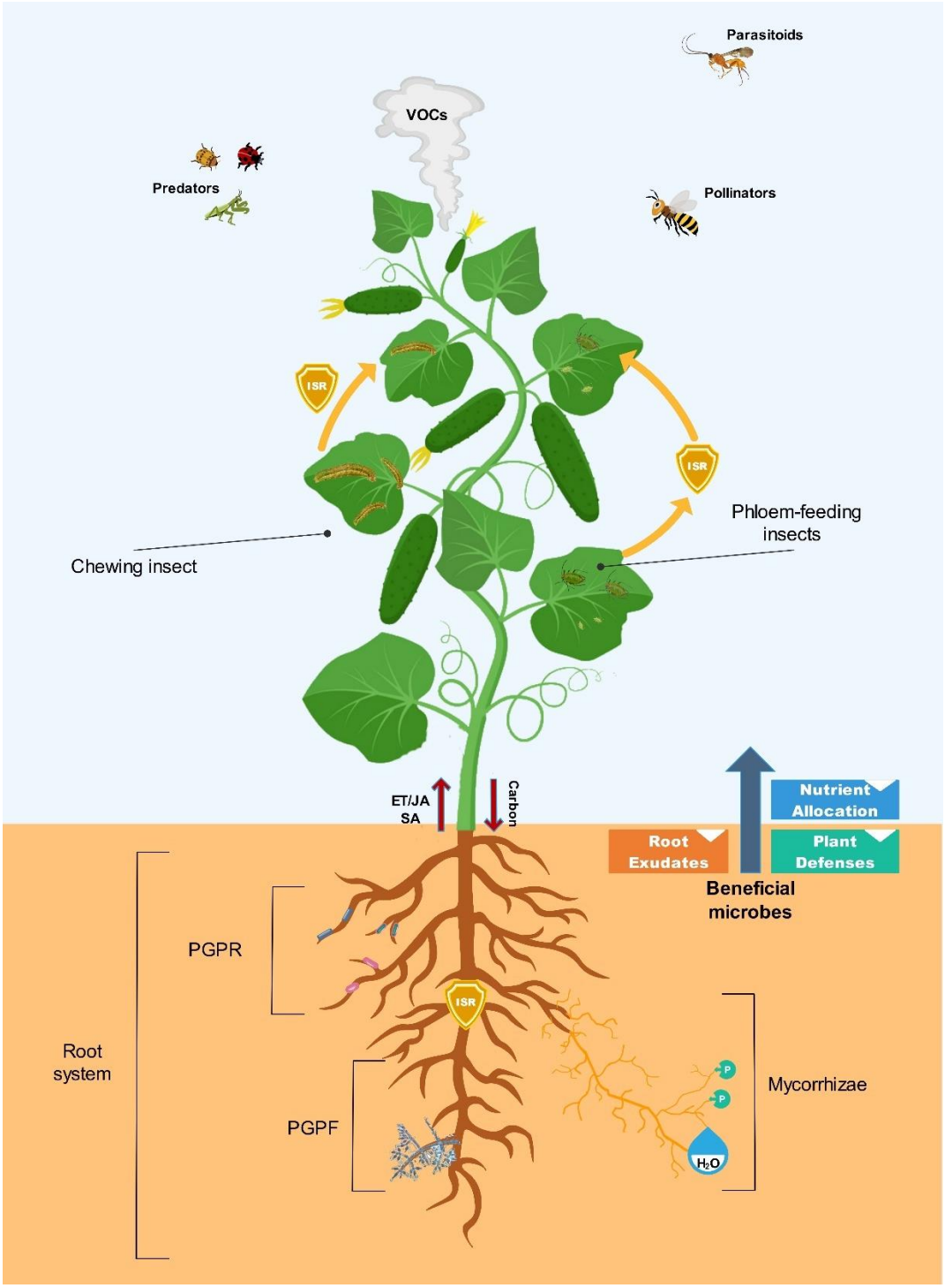

Figure 1. Below-ground and above-ground interactions in plants, microorganisms, and arthropods. Beneficial microorganisms interact with the plant's above-ground and below-ground parts, activating various defense mechanisms. ISR is triggered through these interactions, increasing resistance against diseases and pests. Changes in plant nutrition and defense result in a decrease in the population of harmful herbivores. While the population of piercing-sucking insects may appear to increase in the short term, the density of populations decreases due to the increased attraction of natural enemies to the plant. Increased pollinator visitation and richness of nutrients also lead to an increase in yield. (*VOCs: Volatile organic compounds, PGPF: Plant growth-promoting fungi, PGPR: Plant growth-promoting rhizobacteria).

THE EFFECT OF SOIL-BORNE MICROBES ON ABOVE-GROUND HERBIVORES

Certain microorganisms that form a symbiotic relationship with plants can influence the performance, population dynamics, and community structure of herbivorous insects that feed on them by altering the phenology, morphology, physiology, and biochemistry of plants. For example, positive effects of *Trichoderma* spp., arbuscular mycorrhizal fungi, and other endophytic fungi in the biological control of herbivorous insects have been reported (see Table 1). Furthermore, it is known that these beneficial microorganisms trigger plant defense against herbivores.

Beneficial soil-borne microorganisms can enhance plant resistance against above-ground herbivorous insects. However, this can also make the plant more nutritious and attractive to some herbivores, and the overall impact on insect performance is determined by the beneficial effects of enhanced plant growth and the detrimental effects of plant resistance combine. For instance, it was shown that applying plant growth-promoting rhizobacteria (PGPR) to tomato plants (*Solanum lycopersicum* L.) increased their biomass and nutrient content, which in turn made them more attractive to whiteflies (*Bemisia tabaci* Gennadius). Similarly, arbuscular mycorrhizal fungi (AMF) improved nitrogen acquisition in plants such as *Medicago truncatula* Gaertn., increasing the plants' nutritional value for aphids that feed on phloem (Wilkinson et al. 2019; Noman et al. 2020). These plant-mediated effects can be modulated by a variety of biotic and abiotic factors. For example, a specific type of microorganisms that promotes plant growth (e.g. mycorrhizal fungi or rhizobacteria), plant species and the feeding type of the herbivorous pest (e.g. phloem feeders or chewing insects) are significant biological factors that affect the result. Among abiotic factors, temperature, humidity, and the availability of nutrients in the soil are important determinants of these interactions (Silva et al. 2019; Noman et al. 2020). Therefore, the impact on above-ground herbivorous insects can be positive, negative, or neutral. But even with advancements in this field, there is still much to be explored, such as how microbial diversity affects defense responses and how multiple stressors interact with each other.

Although it is known that microorganisms can respond differently to herbivores under certain conditions, this situation also depends on the interactions between the microorganisms and the surrounding community of organisms, including other microorganisms, plants, and herbivores. For example, three different combinations of the mycorrhizal fungus species have shown different effects on host selection by leaf-miner fly (*Chromatomyia syngenesiae* Hardy) and seed-feeding insects (*Tephritis neesii* Meigen and *Ozirhincus leucanthemi* Vallot) (Gange et al. 2005). In addition, different combinations of PGPR strains in rice (*Oryza sativa* L.) also have a strong negative effect on leaf-folder caterpillars (*Cnaphalocrocis medinalis* Guenée) (Saravanakumar et al. 2007). Furthermore, enzymes involved in plant defense, such as chitinase, trypsin inhibitors, polyphenol oxidase, and lipoxygenase, are also present in similar species (Commare et al. 2002; Gange et al. 2003; Saravanakumar et al. 2007; Saravanakumar et al. 2008). There is a need for more realistic studies of these relationships, as different species and combinations of beneficial microorganisms may have different effects on herbivores and even higher trophic levels.

THE EFFECT OF ABOVE-GROUND HERBIVORES ON SOIL-BORNE MICROBES

There are studies on the effects of beneficial microorganisms on herbivorous insects; however, studies on their effects on beneficial microorganisms are limited. Recently, with the beginning of understanding these interactions, interest in this subject has increased. Herbivorous insects can cause a decrease, increase, or no effect on soil-borne microorganisms. For example, Gehring and Bennett (2009) reported that herbivory by *Neoclytus acuminatus* Fabricius reduced the number of AMF in *Pinus edulis* Engelm. Conversely, Friman et al. (2021) found that herbivory by *Helicoverpa armigera* Hübner larvae increased the activity of beneficial soil bacteria, possibly through root exudate-mediated signaling. Although the underlying mechanisms of these interactions have not been revealed much, it is assumed that the primary factor here is related to the amount of carbon compounds, such as sugars, amino acids, and organic acids shared by plants' roots (Gehring and Bennett 2009). Insect larvae's root herbivory can affect soil carbon and nutrient changes, including alterations in soil pH, moisture, and microbial biomass (Grayston et al. 2001). For example, Grayston et al. (2001) noticed that by increasing the amount of root exudates, which act as a carbon source for soil microbes, *Diabrotica virgifera* LeConte larvae's root herbivory decreased soil pH and changed nutrient availability. In the same way, Erb et al. (2009) showed that plants damaged by herbivores allocated more carbon and nitrogen resources to their roots, which affected the activity and composition of the soil microbial community. Plants increase the transfer of carbon compounds to their roots to tolerate damage from herbivores (Erb et al. 2009; Johnson et al. 2009). It is expected that this scenario will have an impact on the rhizosphere microbiomes. In addition to this factor, the microbiome can be altered by herbivore feeding

behavior because of altered plant root exudation. In the rhizosphere, root exudates are crucial to plant-microorganism interactions. Plant hormones impact root exudates, affecting the microbiome around the plant root (Eichmann et al. 2021). However, above-ground herbivores can alter the composition and quantity of root exudates, thereby affecting below-ground microorganisms (Kostenko and Bezemer 2020; Delory et al. 2021). Organic acids (e.g. including malic acid, citric acid, fumaric acid) and carbohydrates (e.g. glucose, fructose) are among the metabolites found in root exudates that trigger bacterial mobility and microorganism attraction to roots (Tahir et al. 2015; Eichmann et al. 2021; Chen and Liu 2024). For example, Arabidopsis contains high levels of malic acid in its root exudates after being infected with the bacterial leaf pathogen *Pseudomonas syringae* pv. tomato Okabe. This situation leads to the increased attraction of the ISR-stimulated microorganism *Bacillus subtilis* Ehrenberg to roots (Bais et al. 2006; Rudrappa et al. 2008). The underlying mechanisms become more complex with the plant defense mechanisms and synthesis of secondary metabolites created for herbivores (Soler et al. 2007; Erb et al. 2009). On the other hand, the primary plant defense mechanism stimulated by herbivore attacks can affect these microorganisms (van Dam and Heil 2011; Sánchez-Sánchez and Morquecho-Contreras 2017; Bernaola and Stout 2019).

These findings demonstrate how insects can impact beneficial microorganisms living in plant roots, and interactions between above-ground and below-ground environments should be considered in future research. Experimental evidence of these mechanisms is needed.

THE EFFECT OF SOIL-BORNE MICROBES ON NATURAL ENEMIES

Plants emit some volatile organic compounds (VOCs) when under herbivore attack to attract natural enemies of herbivores as part of a defense mechanism (Dicke et al. 2009). Emitting volatile organic compounds is a highly effective strategy for plants to survive. Low molecular weight terpenes such as methyl salicylate (MeSA), methyl jasmonate (MeJA), green leaf volatiles (GLVs), and monoterpenes (C_{10}) and sesquiterpenes (C_{15}) have been reported as solid chemical weapons of plants against pathogens or herbivores (Arimura et al. 2004; Clavijo Mccormick et al. 2014; Heil 2014). For instance, in response to herbivore damage, *Arabidopsis thaliana* L. and *Brassica oleracea* L. emit GLVs such as (Z)-3-hexenal and (E)-2-hexenal, which attract parasitic wasps like *Cotesia glomerata* L. (Dicke et al., 2009). Similarly, plants like *Cucumis sativus* L. emit sesquiterpenes like β-caryophyllene (C15) in response to herbivory by *Tetranychus urticae* Koch, which attract predatory mites like *Phytoseiulus persimilis* Athias-Henriot (Arimura et al. 2004). This is one of the main ways that plants defend themselves indirectly. The most important signaling pathway for the emission of these volatiles is the JA signaling pathway. Due to multitrophic effects, changes in the JA signaling pathway lead to changes in the volatile composition (Dicke et al. 2009; Snoeren et al. 2009; Soler et al. 2012). Therefore, it is also expected that the activation of the JA pathway by beneficial microorganisms, such as *Rhizobium* spp. or *Trichoderma* spp., affects the emission rate or composition of volatiles. A study investigating the changes in volatile emission due to microorganisms found that the sesquiterpenes emitted from *Glomus* spp. –inoculated mycorrhizal plants in response to herbivore attacks were more than those emitted from non-mycorrhizal plants (Fontana et al. 2009). Investigations have shown that soil-borne beneficial microorganisms, including *Pseudomonas* spp. and *Bacillus* spp., can mediate indirect defense against herbivores and alter their natural enemies' effectiveness. Research on how helpful bacteria affect indirect defense has revealed that variations in volatile organic compounds attract parasitoids like *Cotesia* spp. (Guerrieri et al. 2004; Hempel et al. 2009). Even when the number of plants colonized by beneficial microorganisms in an area is lower than that of non-colonized plants, they can increase the parasitoid attack rate, performance, and attraction like *Diaeretiella rapae* M'Intosh (Pineda et al. 2013; Coppola et al. 2017; Verma et al. 2019).

EFFECT OF SOIL-BORNE MICROBES ON POLLINATORS

Natural and agricultural ecosystems rely heavily on pollinators. It is known that plant-mediated interactions occur between soil microorganisms and pollinating insects; however, there is limited research on this topic. Current studies indicate that plants colonized by beneficial microorganisms, such as AMF, perform better in flower and seed production than non-colonized plants. This interaction increases the number of flowers, flowering amount, and nectar production. For instance, it has been noted that AMF colonization enhances flower production in a variety of vascular plants, including *A. thaliana, Chamerion angustifolium* L. (fireweed) and *Medicago sativa* L. (alfalfa) (Gange and Smith 2005; Cahill et al. 2008; Kessler and Halitschke 2009; Barber et al. 2012). As a result, it is expected to positively affect yield as plants with more flowers and nectar production when visited more by pollinators. Indeed, Chen et al. (2022) demonstrated that the application of AMF increased the number of flowers and fruits in Raspberry plants

(*Rubus idaeus* L.) by 33% and 35%, respectively. By increasing the number of flowers and fruit, pollinators were more likely to visit, indirectly increasing raspberry production. In addition, the synergistic effect of AMF and pollination led to a 43% increase in yield. There is ample evidence that plant-insect interactions are significantly influenced by the existing microbiomes of host plants, such as many flowering species that attract pollinators, including *R. idaeus* (Ushio et al. 2015; Shikano et al. 2017; Singh et al. 2020; Cusumano et al. 2022). For example, Barber et al. (2015) reported that *Acalymma vittatum* Fabricius, a harmful insect species in cucumber roots, caused a 34% reduction in leaf and fruit production and reduced pollinator visitation by 39%. Beneficial microorganisms are thought to impact this harmful species negatively and could, therefore, increase yield and pollination. Recent studies in pollination biology have focused on the role of the plant microbiome in plant-insect interactions (Good et al. 2014; Schaeffer et al. 2014; Mogren and Shikano 2021). Some microorganisms, such as bacteria, produce volatile compounds that act as semiochemicals, facilitating communication between plants and other organisms. These compounds function as pheromones, allomones, kairomones, attractants, or repellents, playing key roles in inter- and intra-species interactions. Typically, they are formed through the microbial transformation of fatty acids, amino acids, or carbohydrates. These volatiles convey critical information, such as the presence and quality of floral resources (nectar, pollen, oils), similar to plant volatiles (Nordlund and Lewis 1976; Leroy et al. 2011). For example, microbial volatiles can influence pollinator visitation rates and enhance yield by signaling the availability of high-quality nectar in the environment (Pineda et al. 2010; Knauer and Schiestl 2015; Pozo et al. 2015; Saini et al. 2019; Chen et al. 2022). While the direct effects of soil-borne microorganisms on pollinators are still unclear, yet it is clear that healthy soil and diverse microbial communities are important for supporting healthy plant communities and the ecosystem services they provide, including pollinator support.

In conclusion, soil-borne microorganisms can significantly affect plant-pollinator interactions positively and negatively and should be considered in developing sustainable agricultural practices. Studies highlight the importance of understanding the complex interactions between soil-borne microorganisms and pollinators to promote healthy pollinator populations and sustainable crop production.

RESULT AND FUTURE EXPECTATIONS

Interest in plants, microorganisms, and arthropods interactions has increased in recent years due to a growing recognition of the importance of these interactions for sustainable agriculture. These interactions can significantly impact plant growth and productivity and can be harnessed to improve crop yields and reduce the use of harmful chemical inputs.

An important reason for the increased interest in these interactions is the growing problem of pesticide resistance. Pests can evolve resistance to commonly used chemical pesticides, reducing their efficacy and increasing pest populations. Microorganism-based products such as biopesticides can provide an alternative mode of action to chemical pesticides, reducing the selection pressures for resistance to these chemicals. By using combinations of chemical and microorganism-based products with different modes of action, it may be possible to delay or prevent the development of pesticide resistance. In addition to their potential role in managing pesticide resistance, microorganism-based products can also provide other benefits. For example, they can have lower environmental impact than chemical pesticides and be part of an integrated pest management strategy that incorporates multiple tactics for pest control.

There has been limited use of microorganism-derived products, such as biopesticides and biofertilizers, because of concerns about their effectiveness, which is considered low and variable when used in field conditions. Although these products have shown to be effective against pests and can improve plant growth in laboratory studies, results obtained under field conditions can sometimes be inconsistent. Farmers and growers are often reluctant to use these products as they may not deliver the expected results. Overall, there is a growing recognition of the potential benefits of microbial-based products for sustainable agriculture, and efforts are underway to improve their efficacy and increase their adoption by farmers and growers. To encourage farmers to use biocontrol agents and biofertilizers, it is essential to highlight the advantages of beneficial microorganisms and the ecological problems caused by chemical pesticides and fertilizers. Microbial-based products have the potential to play an important role in achieving these goals. It is worth noting, however, that research in this area is ongoing, and there is hope that improvements can be made to the efficacy of microbial-based products. As such, these products may become more widely used in the future as their potential benefits become more widely recognized.

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ETHICAL CONSIDERATIONS

No human or animals were harmed in the conduct of this study.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interests to any authors.

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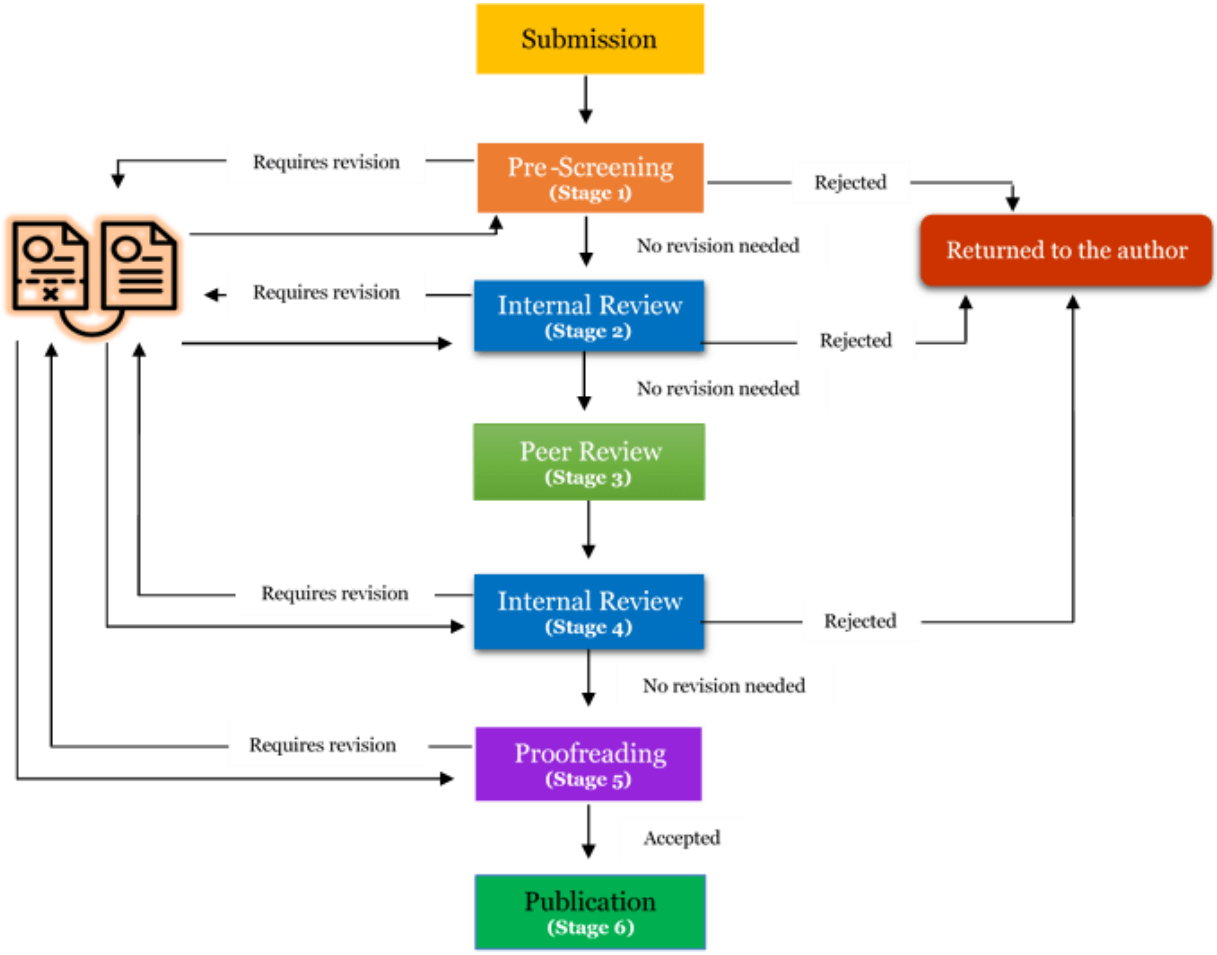

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