Year: 2024, Volume: 3, Issue: 1

SUSTAINABLE AQUATIC RESEARCH

www.saquares.com e-ISSN: 2822-4140

Editorial Team

EDITOR-IN-CHIEF
Erkan Can, Ph.D., MSc., BSc., Professor Department of Aquaculture, Faculty of Fisheries, University of Izmir Katip Çelebi, Cigli, Izmir, Türkiye

ASSOCIATE EDITORS
Sheila Mae S. Santander-de Leon, Ph.D., Associate Professor, Institute of Marine Fisheries and Oceanology, University of the Philippines Visayas, Iloilo, Philippines
Esin Özçiçek, Ph.D., Research Associate, Faculty of Fisheries, Munzur University, Tunceli, Türkiye
Keriman Yürüten Özdemir, Ph.D. Assistant Professor, Faculty of Engineering and Architecture, Department of Food Engineering, Kastamonu University, Kastamonu, Türkiye

HONORARY ADVISORY BOARD
Brian Austin, Ph.D., D.Sc. Professor Emeritus of Microbiology, Institute of Aquaculture, University of Stirling, Stirling, Scotland, U.K.
Murat Balaban, Ph.D. Professor of Chemical and Materials Engineering (retired), Chair of Food Process Engineering, University of Auckland, Auckland, New Zealand
Atilla Alpbaz, Ph.D. Professor of Aquaculture (retired), Faculty of Fisheries, Ege University, Izmir, Türkiye

LANGUAGE EDITOR
Sharon N. Nuñal, Ph.D., Associate Professor, Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Philippines

STATISTICAL EDITOR
Parviz Zare, Ph.D. Assistant Professor, Department of Aquatics Production and Exploitation, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

SECTION EDITORS

Aquatic Animal Nutrition
Christian E.W. Steinberg, Ph.D. Emeritus Professor, Freshwater Ecology, Humboldt Universität zu Berlin, Germany, Guest Professor, Kunming University of Science and Technology, China
Marine and Freshwater Biology
Veysel Aysel, Ph.D., Professor, Department of Biology, Faculty of Sciences, Dokuz Eylül University, Türkiye
Aquaculture
Volkan Kızak, Ph.D. Professor, Department of Aquaculture, Faculty of Fisheries, Munzur University, Türkiye
Fish Health and Welfare
Naim Sağlam, Ph.D. Professor, Department of Aquaculture, Fisheries Faculty, Firat University, Elazig, Türkiye
Fisheries

Hakkı Dereli, Ph.D., Professor, Department of Fisheries and Fish Processing, Faculty of Fisheries, University of Izmir Katip Çelebi, Izmir, Türkiye

Food Safety
Sharon N. Nuñal, Ph.D. Associate Professor, Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Philippines

Aquatic Living Resources

Tuğçe Şensurat Genç, Ph.D., Associate Professor, Department of Fisheries and Fish Processing, Faculty of Fisheries, University of Izmir Katip Çelebi, Izmir, Türkiye

Aquatic Biotechnology

Filiz Kutluyer Kocabaş, Ph.D., Associate Professor, Department of Aquaculture, Faculty of Fisheries, Munzur University, Tunceli, Türkiye

Biodiversity

Dilruba Seyhan Öztürk, Ph.D., Associate Professor, Department of Basic Sciences, Faculty of Fisheries, University of İzmir Katip Çelebi, Izmir, Türkiye

Water Quality and Pollution
Mohammad Gholizadeh, Ph.D., Associate Professor, Hydrobiology and Water Pollution, Department of Fisheries, Gonbad Kavous University, Iran

EDITORIAL BOARD

Aliakbar Hedayati, Ph.D. Professor, Agricultural Science and Natural Resources, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Science and Natural Resources
Gorgan, Iran

Davide Fezzardi, International Expert in Aquaculture, Rome, Italy

Güzel Yücel Gier, PhD, Professor, Dokuz Eylül University, Institute of Marine Sciences and Technology, Izmir, Türkiye

Ilker Zeki Kurtoğlu, Ph.D. Professor, Department of Aquaculture, Faculty of Fisheries, University of Recep Tayyip Erdoğan, Rize, Türkiye

Ivonne Lozano, MSc, PhD., Professor, Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile Santiago, Chile
Fabio Massa, International Expert in Aquaculture, Food and Agriculture Organization of the United Nations (FAO) (Retired), Italy

George Kehayias Ph.D. Professor, Department of Food Science & Technology, University of Patras, Agrinio, Greece

Margaret Crumlish, PhD, MSc., BSc., Professor, Institute of Aquaculture, University of Stirling, Scotland

Miquel Planas, Ph.D. Professor, Marine Ecology and Resources Department, Institute of Marine Research, Vigo, Spain

Murat Yiğit, Ph.D., Professor, Faculty of Marine Science and Technology, Canakkale Onsekiz Mart University, Çanakkale, Türkiye

Mustafa Can, Ph.D. Professor, Department of Engineering Sciences, University of İzmir Katip Çelebi, İzmir, Türkiye

Olena V. Honcharova, Ph.D., Professor, Faculty of Fisheries and Natural Use, Department of Bioresources and Aquaculture, Kherson State Agrarian-Economic University, Ukraine
Paran Gani, Ph.D. Professor, School of Applied Sciences, Faculty of Integrated Life Sciences, Quest, nternational University, Malaysia
Ramazan Serezli, Ph.D. Professor, Faculty of Fisheries, University of İzmir Katip Çelebi, İzmir, Türkiye
Sabrine Boucetta, Ph.D., Professor, Department of Natural and Life Sciences, University of 20 August 1955, Skikda, Algeria
Serge Utevsky, Ph.D. mProfessor, Department of Zoology and Animal Ecology, Karazin Kharkiv National University, Ukraine
Süleyman Akhan, Ph.D. Professor, Department of Aquaculture, Faculty of Fisheries, Akdeniz University, Türkiye
Zainal A. Muchlisin, Ph.D. Professor, Faculty of Marine and Fisheries, Syiah Kuala University, Indonesia
Adem Yavuz Sönmez Ph.D. AssociEate Professor, Department of Basic Sciences, Faculty of Fisheries, Kastamonu University, Kastamonu, Türkiye
Filiz Kutluyer Kocabaş, Ph.D., Associate Professor, Fisheries Faculty, Munzur University, Tunceli, Türkiye
Safak Seyhaneyildiz Can, Ph.D. Associate Professor, Dokuz Eylül University, Institute of Marine Sciences and Technology, Izmir, Türkiye
Saniye Türk Çulha, Ph.D. Associate Professor, Faculty of Fisheries, University of İzmir Katip Çelebi, Türkiye
Sheila Mae S. Santander-de Leon, Ph.D. Associate Professor, Institute of Marine Fisheries and Oceanology College of Fisheries and Ocean Sciences University of Visayas, Iloilo, Philippines
Victor Surugiu Ph.D. Associate Professor, Department of Biology, Universitatea Alexandru Ioan Cuza, Iaşi, Romania
Vijayaram Seerengaraj Ph.D. Department of Aquaculture, Fisheries College, Jimei University, Xiamen, China
Albaris B. Tahiluddin, Assistant Professor, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, Tawi-Tawi, Philippines
Övgü Gencer Ph.D. Research Associate, Department of Aquaculture, Ege University, İzmir. Türkiye

DESIGNERS

Safak Seyhaneyildiz Can, Ph.D., Associate Professor, Dokuz Eylül University, Institute of Marine Sciences and Technology, Izmir, Türkiye
Onur Karadal, Ph.D., Associate Professor, Department of Aquaculture, Faculty of Fisheries, Izmir Katip Celebi University, Izmir, Türkiye

PROOFREADER

Ms. Iqra Tariq, M.Phil Marine Biology, Institute of Aquaculture and Technology (SMC-Pvt.) Ltd. Centre of Excellence in Marine Biology, University of Karachi, Pakistan

TECHNICAL EDITOR

Deniz Çira Ph.D(c), Research Assistant, Department of Aquaculture and Aquatic Animal Diseases
Istanbul University-Cerrahpaşa Faculty of Veterinary Medicine, Istanbul, Türkiye

Aims & Scope

Aims

SUSTAINABLE AQUATIC RESEARCH (SAquaRes) aims to play an important role in advancing and understanding of aquatic sustainability. The most important aim of SAquares is "to put the research on aquatic sustainability at the focus of science. Sustainable life in the world will be realized with a sustainable aquatic ecosystem."

Scope

The scope of SAquaRes includes papers from non-traditional scientific areas such as sustainability science, social-ecological systems, ornamental, conservation, and restoration, and also the traditional priorities of its sections related to aquatic environments *(the list below is given in alphabetical order)*:

- Alternate Aquatic Energy Technologies
- Aquatic Sustainability
- Aquaculture and Fisheries
- Aquatic Environmental Interactions
- Aquatic biochemistry
- Aquaculture and environment
- Aquaculture and risk assessment
- Aquatic ecotoxicology
- Aquatic living resources
- Aquatic Biofuels
- Aquatic Biotechnology
- Climate Change and global warming
- Coastal Zone Management
- Ecofriendly aquaculture studies
- Environmental impacts of aquaculture
- Fish Health and Welfare
- Human and Environmental Risk Assessment
- Hydrology and Water Resources
- Impacts of global environmental changes
- Innovative livestock and farming systems
- Marine and Freshwater Biology
- Marine and Freshwater Pollution
- Seafood Quality and Safety
- Sustainable and Renewable Resources
- Sustainable Aquatic Ecosystem
- Sustainability assessment and design of aquacultural systems and decision support tools
- Water Quality and Pollution
- Wastewater Treatment
- And more research focused on sustainability

2024 •Volume 3 • Issue 1
www.saquares.com

How sustainable is sustainable living without sustainable aquatic research?

TABLE OF CONTENTS

Vol. 3 No. 1 (2024): SAquaRes
Published: 2024-04-30

Editorial

Original Articles

SAquaRes, e-ISSN: 2822-4140, is an anonymous peer-reviewed open-access journal that publishes articles in the English language with three volumes a year.

SUSTAINABLE AQUATIC RESEARCH
www.saquares.com

Sustainable Aquatic Research (2024) 3(1):1-4
DOI: 10.5281/zenodo.10890507
e-ISSN: 2822-4140

EDITORIAL NOTE

Where Have All the Good Editors Gone? - A Necessary Polemic

Christian E.W. Steinberg
Freshwater Ecology, Humboldt Universität zu Berlin, Germany
E-mail: *christian_ew_steinberg@web.de*, *ORCID:* *https://orcid.org/0000-0002-3132-8901*

Don't be afraid: America's greatest folksinger of all time, Pete Seeger ('Where have all the flowers gone?'), is not back as a zombie to critic aquaculture. It seems, however, that he was much more aware of the evolution of different human societies and cultures than many aquaculture editors are of the appearance and development of their journals.

In the new age, where a certain political elite has made alternative facts and fake news acceptable to the public, it is not surprising that strange papers appear even in highly ranked aquaculture journals: articles with fake bibliographies or missing or at least very poor identification of the organisms studied. They appear without any sanction or commentary and even pretend to be peer-reviewed. Who knows if results or even entire articles are homemade? Can we be sure that results or even entire articles are not just fabricated fairytales? Is it of any surprise that Open Access (OA) journals, including those from major publishers, do not fight these tendencies? Earning article processing charges appears to counts more than science! Where have all the good scientific editors gone?

In some subscription journals, and even more so in OA journals, you will miss the 'Letters to the Editor' section – usally a sign of the lively scientific life of a journal and the contest for the best interpretation of published results. Not so in many aquaculture journals. Which author wants to pay article processing fees in an OA journal for something intrinsic to science, namely discussing the results presented by colleagues? Discussion leads to paradigms being proposed and eventually recognized by a majority of scientists after academic pros and cons (Kuhn 2012). Do these journals believe that they are close to the absolute truth, like religions or communist and fascist ideologies, which not only don't need any discussion but actively suppress it? Where have all the good editors gone? And if they are still around, have they forgotten all about scientific education and good scientific practice?

One of the foundations of scientific work, which is clearly distinguished from esoteric homeopathic beliefs, is the traceability and reproducibility of studies from other laboratories. But how can this be guaranteed when dog (or cod) Latin terms are used for supplements of botanical preparations that are at best known regionally where the respective ethnopharmacy is applied, but are by no means in general use? Why do aquaculture journals not require the actual common scientific terms? This requirement is not a symptom of racism a la 'oppression of ethnic minorities' – it is the basis of scientific work and communication, which every scientific author should have learned in university. It contributes to the general understanding and acceptance of the more empirical science of aquaculture. Where have all the good editors gone?

Similar criticism applies when aquaculture and related journals, mainly OA journals, allow the use of product names instead of the usual, still rather superficial, characterization of the feed composition. This is simply advertisement and not science at all! Where have all the good editors gone?

Citation
Steinberg, C.E.W. (2024). Where Have All the Good Editors Gone? - A Necessary Polemic. Sustainable Aquatic Research, 3(1), 1-4.
Received: 16 March 2024, Accepted: 24 March 2024, Available online: 30 April 2024

If you believe the aquaculture journals, a Nile tilapia or any other farmed fish is the same all over the world. However, with breathtaking speed, epigenetic changes alter the phenotype during domestication, as recently summarized (Steinberg 2023). Studies of Nile tilapia provide evidence that such changes occur even within a single generation. Konstantinidis et al. (2020) showed that muscle DNA hydroxymethylation differs significantly between wild fish and their captive-bred offspring. Many differentially methylated sites are associated with genes involved in muscle growth, immunity, autophagy, and diet response. This shows that phenotypic traits often related to domestic animals (e.g., higher growth rate and different immune status) may be regulated epigenetically (Podgorniak et al. 2022). The tilapia example should suffice to illustrate theinexcusable gap that is created when aquatic species are treated worldwide as a 'monolith' and the study material is not further characterized. With this mindset, many studies are not comparable, as was already mentioned more than a decade ago (Hua and Bureau 2012). Where have all the good editors gone?

When supplementing aquafeeds or replacing marine proteins and lipids with plant materials from traditional medicine, the 'scientific' justification is always that this has a long tradition in ethnopharmacy. Are the authors serious? Do they really think that fishes and shrimps are little humans? Or that humans are fishes or shrimps that have come ashore? Against this background, it is not surprising that most authors deliberately forget to test the supplemented ethnopharmaceuticals for ecotoxicity. After all, they have already been tested on humans. Where have all the good editors gone? This nonsense must stop!

Algae or fungi? When large inedible phytoplankton species, often filamentous cyanobacteria, are infected by chytrids, nutrients within the host cells are transferred to the zooplankton via zoospores (Kagami et al. 2014). This loop may be important in shaping aquatic food chains and may be used technically in the aquaculture industry to improve low-value aquatic lipid sources. Recent classification agrees that these organisms, the chytrids, are parasitic unicellular fungi (Adl et al. 2005), rather than algae, and later confirmed (Galindo et al. 2021; Thome et al. 2023). However, this does not impress the many authors, most of them from the aquaculture business, who continue to consider the various chytrids in aquafeeds as microalgae. But if such inaccuracies occur in these small things, what happens to the really important things? Where have all the good editors gone?

Crassostrea gigas or *Magallana gigas*? This species, or rather the scientists who study it, has a long history of persistence. Although there is convincing biomolecular evidence that the Atlantic and Pacific species groups do not have the same origin, i.e. do not belong to a single genus (Salvi and Mariottini 2017; Salvi and Mariottini 2021; Willan 2021), the Pacific oyster is still referred to as *Crassostrea gigas* in most recent papers and the battle for the old name continues (Backeljau 2018). Can persistence perhaps be translated as convenience or laziness? A clarifying word from the good editors could certainly help here.

Litopenaeus vannamei or *Penaeus vannamei*? The correct nomenclature has been, and still is, the subject of heated and not always objective debate. In the meantime, molecular taxonomy seems to have won the day (Hurzaid et al. 2020; Balasubramanian et al. 2021; Katneni et al. 2021) and the previous morphological split of the genus *Penaeus* by Pérez Farfante and Ken (1997) seems obsolete. Thirty-one splinters are now reunited in the genus *Penaeus* (Vance and Rothlisberg 2020) and the most cultivated shrimp species is *Penaeus vannamei*, rather than *Litopenaeus vannamei* (Figueredo et al. 2023).

Taxonomic revisions, however, are often influenced by methodological progress and subsequent acceptance or rejection, which can be based on personal rather than scientific arguments. This can make the animal objects appear like a hunted game, rather than an object of scientific studies.

In all cases, workers suffering from 'revision shock' have used non-taxonomic courses of action to express their dissidence by attempting to suppress the taxonomy of others (i.e., by recommending avoidance, personal attacks, or omission, respectively) (Willan 2021). If these studies are already negligent in the selection and identification of their subjects, what can be expected in the actual experimental work to find something new? The same carelessness? Who knows!

Yet, the current nomenclature hullabaloo in aquaculture journals, exemplified by three examples, can be easily remedied if good editors would simply wake up or show up and specify which of the taxonomic databases authors must refer to if they want their paper processed or even accepted. This request does not impinge on scientific freedom; rather, it contributes significantly to scientific clarity, comprehensibility, and acceptance by the scientific community and the public, which is what we should all want. Where have all the good editors gone?

Now to conclude seriously, almost without any polemic. The concerns listed above are based on experiences with editors and journals while writing my *Aquatic Animal Nutrition* (Steinberg 2018, 2022) over the past 10 years, they are not homemade or fake, but are meant to stimulate discussion. Certainly, an editor cannot know all the issues of aquaculture or aquatic ecology. However, journals usually have several editors-in-chief, associate editors, or at least an editorial board of qualified scientists from different disciplines. These scientists can set scientific standards and requirements for manuscripts to be submitted. To overcome the nomenclature hullabaloo, taxonomic databases are available free of charge (my favorite taxonomic database is AlgaeBase, which uses beautiful phrasing: ‘This name is of an entity that is currently accepted taxonomically’ or ‘This name is currently regarded as a synonym of...’, indicating that taxonomy is a matter of opinion, not absolute truth). The editors only have to indicate which database for fishes, invertebrates, macrophytes or algae and microorganisms has to be consulted, as each journal mentions the style of references in detail. In addition, the animals, plants, and microorganisms to be studied must be identified at least to the species level, unless the genus is monospecific. Basic and applied ecology on the multi-species genus level is scientific nonsense, because different species within a genus can have contrasting ecological requirements – basic knowledge even for ecological freshmen. For probiotic microorganisms, even the strain used must be reported. The same is true for aquafeeds and supplements. Simply providing product names is not science, it is advertising.

To start or increase discussion in aquaculture journals, editors need only take a quick look at high-impact or multidisciplinary journals. Then all they have to do is add another category of article types, namely ‘Letters to the Editor’.

I am convinced that good editors are still out there!

References

Adl SM, Simpson AGB, Farmer MA, Andersen RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup Ø, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor MFJR (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J Eukaryot Microbiol 52 (5):399-451. https://doi.org/10.1111/j.1550-7408.2005.00053.x

Backeljau T (2018) *Crassostrea gigas* or *Magallana gigas*: A Community-based Scientific Response. National Shellfisheries Association Quarterly Newsletter 1:3. https://doi.org/https://shellfish.memberclicks.net/assets/docs/Current_Newsletters/qnl_2018_1.pdf

Balasubramanian CP, Katneni VK, Shekhar MS, Kumar JA, Vijayan KK (2021) Restoration of old genus name *Penaeus* based on molecular phylogenetic affiliations using complete mitochondrial genome. Curr Sci 121 (3):423-428. https://doi.org/10.18520/cs/v121/i3/423-428

Figueredo A, Lira C, Vera-Caripe J, De Donato M, Lodeiros C (2023) The Pacific white shrimp, the most cultivated shrimp species, is it *Litopenaeus* or *Penaeus vannamei*? Rev Aquacult 15 (1):7-13. https://doi.org/10.1111/raq.12725

Katneni VK, Shekhar MS, Jangam AK, Paran BC, Selvaraj A, Krishnan K, Kaikkolante N, Prabhudas SK, Gopalapillai G, Koyadan VK (2021) Phylogenetic relations and mitogenome-wide similarity metrics reveal monophyly of *Penaeus* sensu lato. Ecol Evol 11 (5):2040-2049. https://doi.org/10.1002/ece3.7148

Konstantinidis I, Sætrom P, Mjelle R, Nedoluzhko AV, Robledo D, Fernandes JMO (2020) Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after just one generation of domestication. Epigenetics 15 (10):1052-1067. https://doi.org/10.1080/15592294.2020.1748914

Kuhn TS (2012) The Structure of Scientific Revolutions. 50th anniversary. 4th edn. University of Chicago Press,

Pérez Farfante I, Ken B (1997) Penaeoid and sergestoid shrimps and prawns of the world. Keys and diagnoses for the families and genera. Mem Mus Natl Hist Nat 175:1-233

Podgorniak T, Dhanasiri A, Chen X, Ren X, Kuan P-F, Fernandes J (2022) Early fish domestication affects methylation of key genes involved in the rapid onset of the farmed phenotype. Epigenetics 17 (10):1281-1298. https://doi.org/10.1080/15592294.2021.2017554

Salvi D, Mariottini P (2017) Molecular taxonomy in 2D: a novel ITS2 rRNA sequence-structure approach guides the description of the oysters' subfamily Saccostreinae and the genus *Magallana* (Bivalvia: Ostreidae). Zool J Linn Soc 179 (2):263-276. https://doi.org/10.1111/zoj.12455

Salvi D, Mariottini P (2021) Revision shock in Pacific oysters taxonomy: The genus *Magallana* (formerly *Crassostrea* in part) is well-founded and necessary. Zool J Linn Soc 192 (1):43-58. https://doi.org/10.1093/zoolinnean/zlaa112

Steinberg CEW (2018) Aquatic Animal Nutrition–A Mechanistic Perspective from Individuals to Generations. Springer Nature Switzerland AG, Cham, Switzerland. https://doi.org/10.1007/978-3-319-91767-2

Steinberg CEW (2022) Aquatic Animal Nutrition – Organic Macro- and Micro-Nutrients. Springer Nature Switzerland AG, Cham, Switzerland. https://doi.org/10.1007/978-3-030-87227-4

Steinberg CEW (2023) A Salmon is A Salmon. SAquaRes 2 (1). https://doi.org/10.5281/zenodo.7820847

Thome P, Wolinska J, Van den Wyngaert S, Reñé A, Ilicic D, Agha R, Grossart H-P, Garcés E, Monaghan M, Strassert J (2023) Phylogenomics including new sequence data of phytoplankton-infecting chytrids reveals multiple independent lifestyle transitions across the phylum. bioRxiv. https://doi.org/10.1101/2023.06.28.546836

Vance DJ, Rothlisberg PC (2020) The biology and ecology of the banana prawns: *Penaeus merguiensis* de Man and *P. indicus* H. Milne Edwards. In: Sheppard C (ed) Advances in Marine Biology, vol 86. Academic Press, pp 1-139. https://doi.org/10.1016/bs.amb.2020.04.001

Willan RC (2021) *Magallana* or mayhem? Molluscan Res 41 (1):75-79. https://doi.org/10.1080/13235818.2020.1865514

SUSTAINABLE AQUATIC RESEARCH
www.saquares.com

Sustainable Aquatic Research (2024) 3(1):5-15
DOI: 10.5281/zenodo.11058366
e-ISSN: 2822-4140

RESEARCH PAPER

Particle selectivity based on pre-ingested phytoplankton composition in Charru Mussel *Mytella strigata* (Hanley, 1843)

Dominique P. Mediodia [1*], Carlos C. Baylon [2], Nathaniel C. Añasco[1], and Sheila Mae S. Santander-de Leon[1]

[1*] *Institute of Marine Fisheries and Oceanology, College of Fisheries and Ocean Sciences University of the Philippines Visayas, Miagao, Western Visayas, Philippines 5023*
[2] *Institute of Aquaculture, College of Fisheries and Ocean Sciences University of the Philippines Visayas, Miagao, Western Visayas, Philippines 5023*

Citation

Mediodia, D.P., Baylon, C.C., Añasco, N.C., Santander-de Leon, S.M.S. (2024). Particle selectivity based on pre-ingested phytoplankton composition in Charru Mussel *Mytella strigata* (Hanley, 1843). *Sustainable Aquatic Research, 3*(1), 5-15.

Article History

Received: 24 November 2023
Received in revised form: 28 December 2023
Accepted: 03 April 2024
Available online: 30 April 2024

Corresponding Author

Dominique P. Mediodia
E-mail: dpmediodia@up.edu.ph
Tel: +63 033 315 83 81

Keywords

Non-native species
Food preference
Plankton
Philippines

Abstract

Identification of phytoplankton trapped in the gills suggests initial selectivity and food preference in marine bivalve molluscs. In this study, pre-ingested phytoplankton in the gills of *Mytella strigata* were determined and compared to the existing phytoplankton in the waters of three sites in the Philippines (Pangasinan, Cavite, and Bataan). Pre-ingested phytoplankton of *M. strigata* varied with size ($p<0.05$) and site ($p<0.05$). *Coscinodiscus* spp. and *Skeletonema* spp. were most abundant across sizes (<20mm, 20–40mm, and >40mm) and sites. Particle selection observed in the gills suggests food preference for centric diatoms such as *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. The ingestion of these diatoms ranged from 26% to 46% of the total phytoplankton composition in the waters of different sites. The results highlight particle selection during filtration and indicate a potential food preference of *M. strigata*.

Introduction

Bivalves filter food particles from the water column using their cilia-lined grooves sorting system, while deposit-feeders use their modified labial palps (Leal 2002). Their gills serve as sieve and the particle retention depends on the movement of the latero-frontal cilia, which varies among species (Dral 1967). When resting on a substrate, water is drawn into the bivalve through the inhalant opening or siphon and the gills. Particles trapped in the gills are considered to be pre-ingested particles (Widdows *et al*. 1979). Particles are then transferred along the ctenidium to the labial palps, which assists in particle selection. After selection, some particles are rejected as pseudo-feces while others are ingested through the mouth (Gosling 2003). Suspension feeders such as *Cerastoderma edule*, *Mya arenaria* and *Mytilus edulis* take their food out of the water column (Purchon 1968). In contrast, others take their food both from the surface of the sediment and out of the water column, like the tellinid clams *Macoma balthica*, *Scrobicularia plana* (Hummel 1985; Thompson and Nichols 1988), and *Placuna placenta* (Stella *et al*. 2010).

Bivalves feed on phytoplankton (Gosling 2003), including species that vary in size and other structural characteristics (Shumway et al. 1990), as well as on bacteria, zooplankton, and detritus organisms (Vakily 1989; Langdon and Newell 1990; and Lehane and Davenport 2002). Previous studies reported that bivalve grazing might influence phytoplankton abundance (Cloern 1982; Noren *et al*. 1999), eutrophication (Officer *et al.* 1982), trophic dynamics (Huang *et al.* 2008), light penetration (King and Mc Neal 2010), energy and nutrient flux of benthopelagic communities (Dame 1996) and hypoxia in estuaries (Konrad 2014). Several studies have reported that species in the family Mytilidae demonstrate selective feeding behavior towards phytoplankton with a higher preference for dinoflagellates (Soon and Ransangan 2014; Sivalingam 1977). Sidari (1998) revealed that *Mytillus galloprovincialis* seemed to feed on dinoflagellates rather than diatoms. *M. galloprovincialis* in the Adriatic Sea ingested Tintinnids (Jasprica 1997). Kreeger and Newell (2001) showed that the mussel *Geukensia demissa* preferred resuspended benthic microalgae over phytoplankton. Cowden *et al*. (1984) observed *Mytilus edulis* ingested larvae of polychaetes, asteroids, gastropods, and echinoids in laboratory settings. Mesozooplankton such as amphipods (size up to 6 mm), nematodes, polychates and *Carcinus maenas* zoea (2 mm) are being ingested by *M. edulis* larvae (Davenport *et al*. 2000) exhibiting particle selection (Kiorboe *et al*. 1980). Riisgard *et al*. (1980) reported that *M. edulis* larvae (5 and 13 days old with lengths 150 to 170 mm) cannot ingest particles with diameters smaller than 1 µm or larger than 9 µm. The same result was found by Sprung (1984) using 112–150 µm mussel larvae. In addition, Bass *et al*. (1990) found that *M. mercenaria* can filter cyanobacteria and picoplankton *Nannochloris atomus* 3 µm in diameter. The size of the mouth and esophagus may have contributed to the size selectivity of the veligers.

In early 2000, *Mytella strigata*, a non-native species, affected wild green mussel spats and other bivalves observed in the Philippines (Rice 2016). *M. strigata* is an epifaunal estuarine species colonizing rocky substrates in the Atlantic and Caribbean coasts of South America (Scarabino *et al*. 1975). Massive colonization of this species in different areas in the country has become a significant problem for the local farmers. This study was conducted to identify the pre-ingested phytoplankton in the gills of *M. strigata* as an indicator of its initial food particle selection. The influence of factors such as the size of *M. strigata* and the phytoplankton composition of sampling areas was also determined. The results of this study may explain the cause of the rapid proliferation of this organism in the waters of the Philippines and may reveal its invasive mechanism.

Materials and Methods

Sampling was conducted during November 2016 when *M. strigata* was abundant in (1) Abucay, Bataan (N 14043.057'; E 120033.862'), (2) Bacoor, Cavite (N14029.087'; E 120056.820') and (3) Lucao, Pangasinan (N 16001.282'; E120018.848) (Fig. 1). Sites were selected based on the abundance of charru mussel reported during a preliminary interview with the mussel farmers. Charru mussels were collected from bamboo, nets, and rubber tire substrate at 2 to 3 m depth. Pooled samples from different substrates were photographed with size reference, and 30 pieces were preserved in 10% buffered formalin and were further processed in the laboratory.

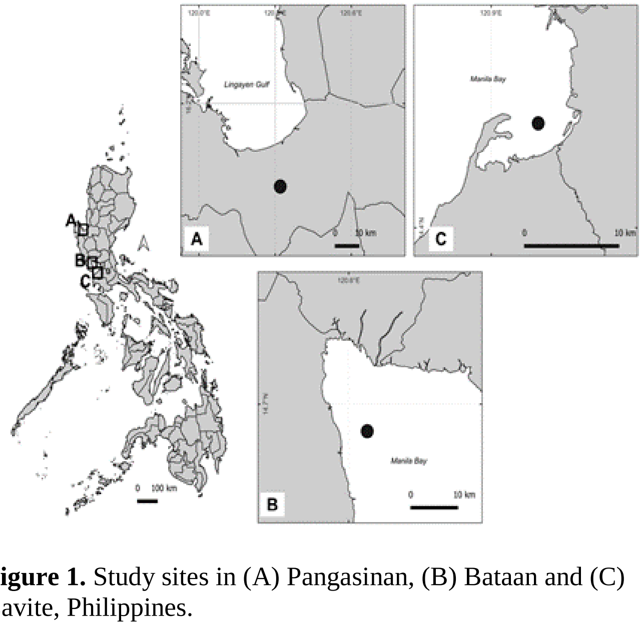


Figure 1. Study sites in (A) Pangasinan, (B) Bataan and (C) Cavite, Philippines.

Triplicate water samples were collected using a 50-L plastic container from approximately 0.5-meter sub-surface depth to determine the phytoplankton abundance and composition of each site. Pooled water samples were filtered in a 28-µm mesh phytoplankton net, preserved in Lugol's solution, and added with distilled water in a beaker to obtain 100 mL. One (1) ml of sub-sample was collected while constantly stirred for homogenization and transferred to Sedgewick rafter or counting under a compound microscope (Motic, USA). For replication, the same steps were followed 5 times. Photos of phytoplankton were captured using Motic software and identified using the guidebook of Isamu Yamaji (1966). All phytoplankton identified were pooled and averaged (n = 5). The diversity index was computed using Shannon and Simpson's diversity index (Table 1). To determine the pre-ingested phytoplankton, thirty (30) whole charru mussels from each size class (<20 mm, 20–40 mm, and >40 mm) were partially opened using a knife and were preserved in 10 % buffered formalin. In the laboratory, meat was separated from the shell. All gills were separated and scraped using a scalpel and transferred to a petri dish. Samples were homogenized and added to distilled water for a 10 mL volume. One mL of well-mixed samples was pipetted and moved into the Sedgewick rafter in triplicate. Phytoplankton was then counted and identified using a compound microscope. Photos were captured, and images of the samples were identified (Yamaji 1966). Pre-ingested phytoplankton abundance and composition were calculated using standard formula (Table 1).

Table 1. Summary of formula used in the study to determine different variables.

Variable (no. of samples)	**Formula**	**Description**	**References**
Phytoplankton Abundance	$PA = (n/N) \times 100\%$	PA = phytoplankton abundance n = total count per taxa N = total count of pooled taxa	Heip, *et al.* (1998)
Phytoplankton Composition	$PC = (n/N) \times 100\%$	PC = Phytoplankton composition n = total no. of species N = total phytoplankton species	
Phytoplankton Diversity Index (n = 30)	Shannon Diversity Index $H' = - \Sigma((n1/N) \ln (n1/N)$	H = Diversity Index n1 = number of samples N = total samples	
	Simpson's Diversity Index $D = N (N-1) / \Sigma [n(n-1)]$	D = diversity N =total no. of organisms n = no. of individual	
	Evenness $E=H/S$	E = Evenness H = Value derived from Shannon Index of Diversity S = No. of taxa identified	

Results

Phytoplankton composition in mussel areas

Results showed diverse phytoplankton species in water samples from Bataan, Cavite, and Pangasinan (Table 2). Out of 1406 total phytoplankton identified in Bataan, 66% were *Skeletonema* spp., 14.01% were *Ceratium* spp., 4.77% were *Tintinnopsis* spp., and 15.22 % of which were species with lower than 3% abundance including *Dytilum* spp., *Chaetoceros* spp., *Thalasionema* spp., *Gonyaulax* spp., *Rhizosolenia* spp., *Chaetoceros* spp., *Nitszchia* spp., *Hemialus* spp., *Biddulphia* spp., *Coscinodiscus* spp., *Hyalodiscus* spp., *Melosira* spp., *Proroceratium* spp., *Mougeotia* spp., *Eucampia* spp., and *Coscinosira* spp. (Fig. 2).

Table 2. Diversity profile of the phytoplankton community in three sampling sites (n = 30).

Diversity Index	**Bataan**	**Cavite**	**Pang**	**p-value ($p<0.05$)**	**Post Hoc (Tukey Test)**
Composition	18	11	14	0.110	(Bataan=Cavite=Pangasinan)
Abundance	469	69	114	0.000	(Bataan>Cavite=Pangasinan)
Shannon (H)	1.33	1.76	1.84	0.076	(Bataan=Cavite=Pangasinan)
Simpson's	2.22	3.92	4.47	0.016	(Bataan>Cavite>Pangasinan)
Evenness (e^H/S)	1.08	1.17	1.16	0.010	(Bataan<Cavite=Pangasinan)

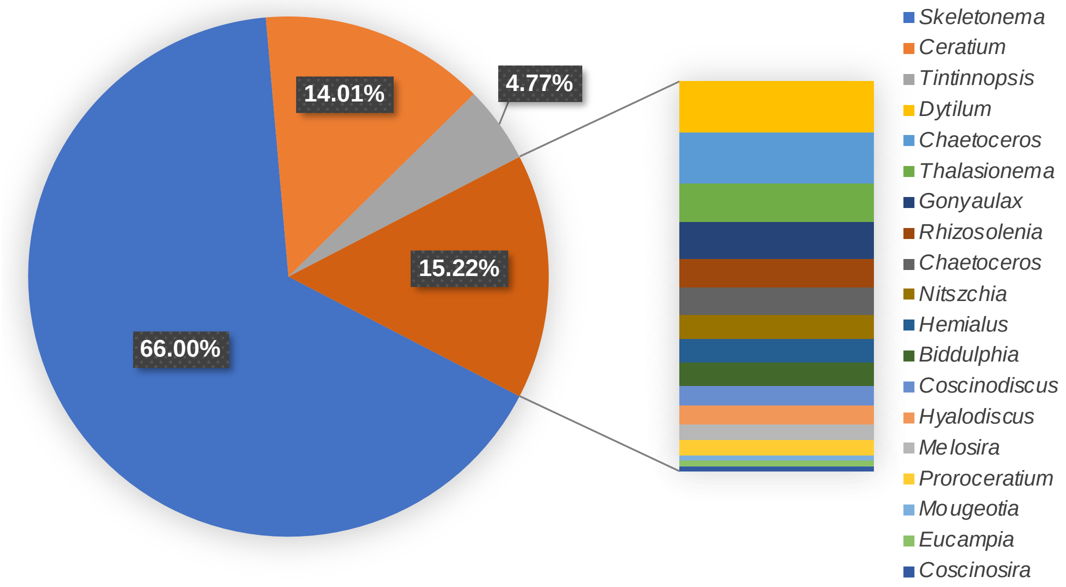


Figure. 2. Relative abundance of phytoplankton species in Abucay, Bataan. The dominant species are *Skeletonema* spp. (66%), *Ceratium* spp. (14.01%), and *Tintinnopsis* spp. (4.77%). The secondary bar graph reveals phytoplankton species with less than 3.0% abundance from a total of 15.22%.

Skeletonema spp. comprising 33.97%, was also found to be the most abundant phytoplankton in Bacoor, Cavite, followed by *Thalasiosera* spp. (19.14%), *Melosira* spp. (10.05%), *Gonyaulax* spp. (6.70%), *Coscinodiscus* spp. (5.74%), *Mougeotia* spp. (5.26%), *Dytilum* spp. (4.31%) and *Cyclotella* spp. (3.83%). Species with lower than 3.0% abundance include *Titinnopsis* spp., *Thalasionema* spp., *Pleurosigma* spp., *Hyalodiscus* spp., *Mougeotia* spp., *Proroceratium* spp., *Biddulphia* spp., *Triceratium* spp., *Peridinium* spp. A total of 17 phytoplankton species were found in the water samples collected (Fig. 3).

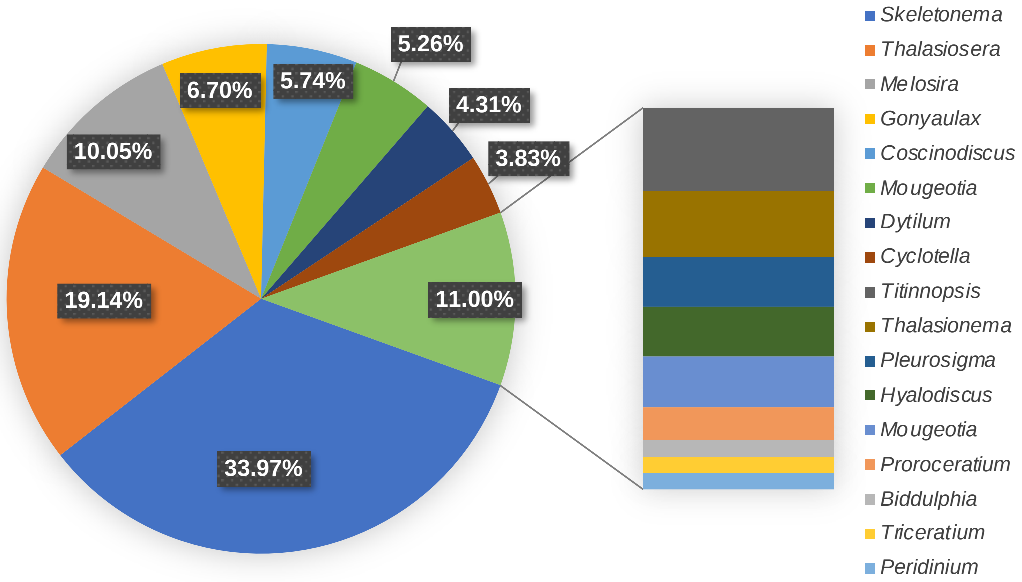


Figure 3. Relative abundance of phytoplankton species in Bacoor, Cavite. Phytoplankton with higher abundance includes *Skeletonema* spp. (33.97%), *Thalasiosera* spp. (19.14%), and *Melosira* spp. (10.05%). The secondary bar graph revealed phytoplankton species with less than 3.0% abundance from a total of 11.0%.

In Lucao, Pangasinan, 22 phytoplankton species were identified. Among them, *Actinoptychus* spp. had the highest percentage of 37.54%, followed by *Coscinodiscus* spp. (17.01%), *Thalasiosera* spp. (16.12%), *Bacteriastrum* spp. (5.87%), *Triceratium* spp. (5.28%) and *Tintinnopsis* spp. (4.11%). Species with lower than 3.0% percentage abundance include, *Pleurosigma* spp., *Leptocylindrus* spp., *Melosira* spp., *Gyrodinium* spp., *Skeletonema* spp., *Rhizosolenia* spp., *Chaetoceros* spp., *Asterolampra* spp., *Biddulphia* spp., *Ceratium* spp., *Dictyocha* spp., *Fragilaria* spp., *Protoperidinium* spp., *Thalasionema* spp., *Gymnodinium* spp., and *Gonyaulax* spp. (Fig. 4).

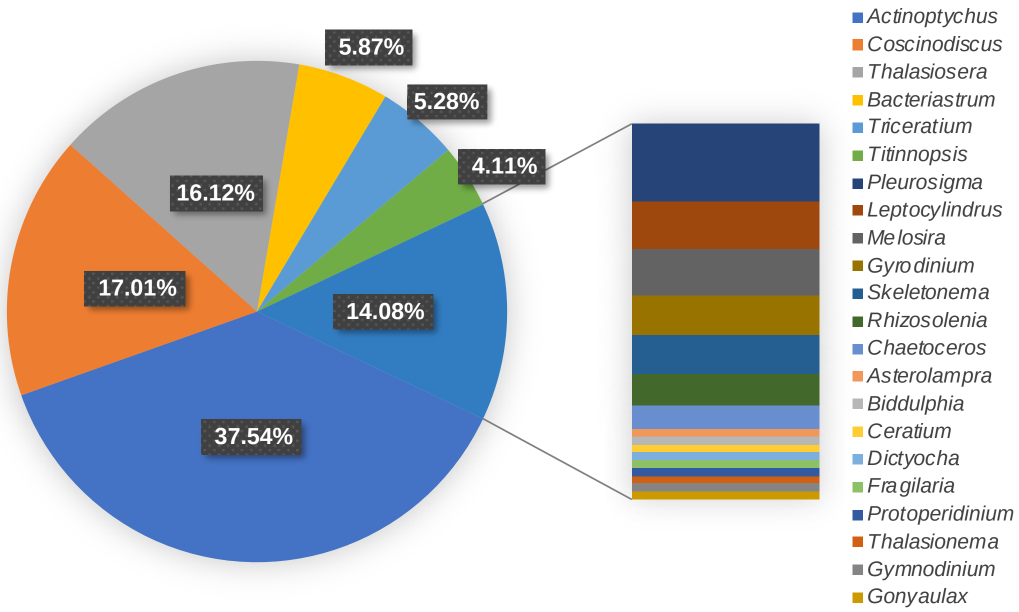


Figure 4. Relative abundance of phytoplankton in Lucao, Pangasinan. Phytoplankton with higher abundance includes *Actinoptychus* spp. (37.54%), *Coscinodiscus* spp. (17.01%), and *Thalasiosera* spp. (16.12%). The secondary bar graph revealed phytoplankton with less than 3.0% abuindance from a total of 14.0%.

The majority of phytoplankton identified were centric diatoms. Diatoms showed significantly higher ($p<0.05$) abundance in the different sites than dinoflagellates and chlorophytes (Fig. 5). Phytoplankton abundance was significantly higher ($p>0.05$) in Bataan (469) than in Pangasinan and Cavite (114). However, Shannon and Simpson's Diversity Index showed significantly lower diversity in Bataan (1.33 and 2.22) than in Pangasinan (1.84 and 4.47) and Cavite (1.76 and 3.92).

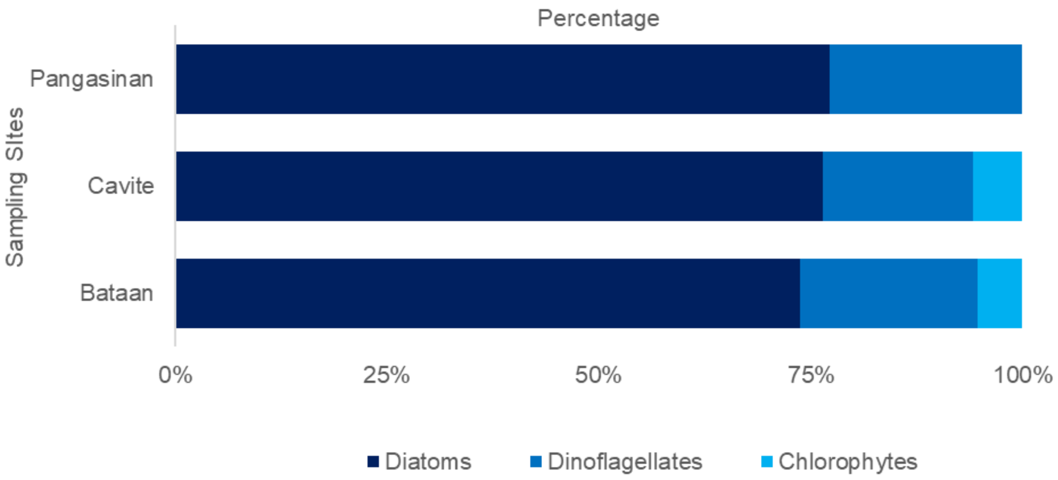


Figure 5. Phytoplankton abundance per group (Diatoms, Dinoflagellates, and Chlorophytes) in three sampling sites (Pangasinan, Cavite, and Bataan) presented as percentage.

Pre-ingested phytoplankton of charru mussel

Pre-ingested phytoplankton of *M. strigata* varied with size ($p<0.05$) and site ($p<0.05$). *Coscinodiscus* spp. and *Skeletonema* spp. had the highest abundance in different sizes and sites (Fig. 6). The same species were abundant in the water samples collected in Bataan and Cavite. *Navicula* spp. was also common but was not found in Pangasinan in size 20-40 mm. Phytoplankton composition was significantly higher in Pangasinan than in Bataan and Cavite ($p>0.05$, Tukey Test: Bataan=Cavite=Pangasinan) (Fig. 7). Size affected phytoplankton abundance in Bataan but not in Cavite and Pangasinan ($p>0.05$, Tukey Test: <20mm = 20–40mm < >40mm). The ingested material in the mussel samples ranged from 26% to 46% of the total phytoplankton composition in the waters of the different sites (Fig. 8).

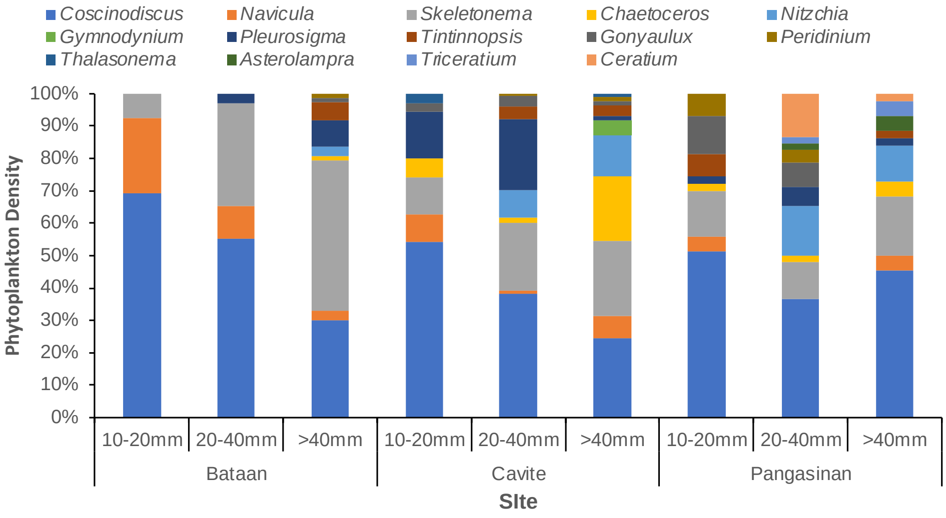


Figure 6. Pre-ingested phytoplankton of *M. strigata* in different sites and size class (n = 10).

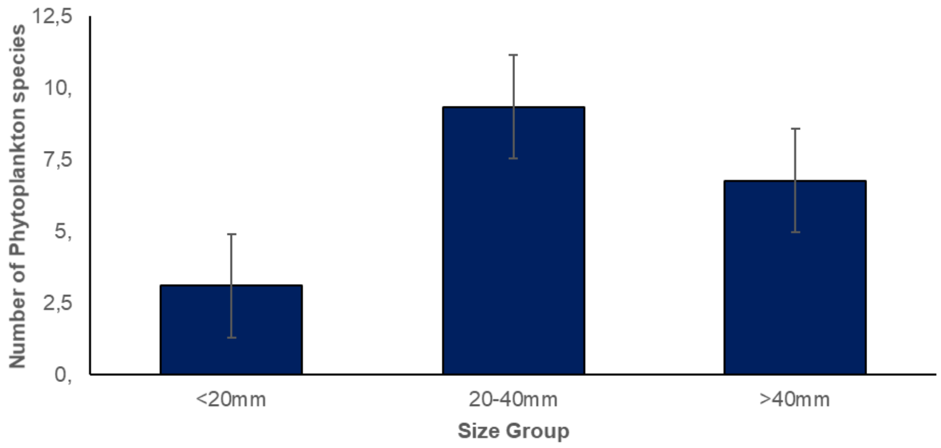


Figure 7. Phytoplankton composition in different size groups shows that 20 – 40mm has more species than the lower size classes (<20mm, >40mm).

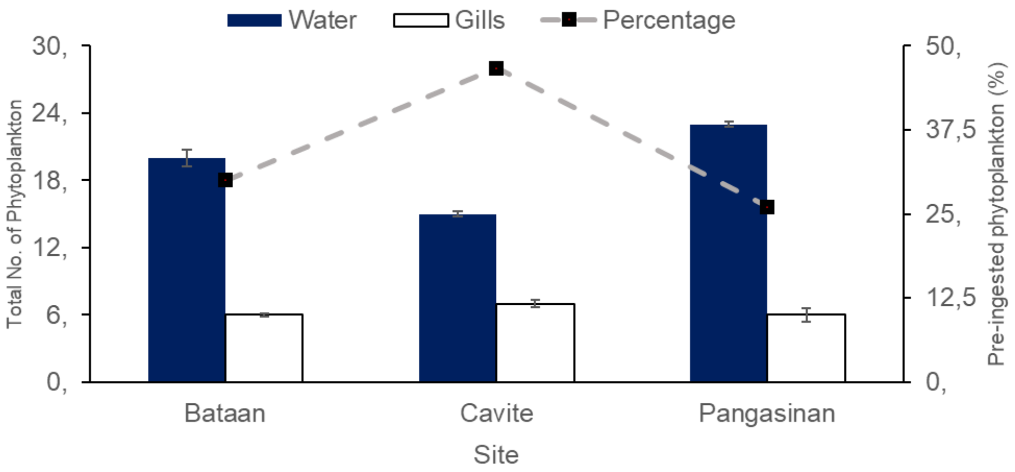


Figure 8. Total number of pre-ingested species in the gills compared to the total number of species in the water from different sites ranged from 25 % – 45 % (n = 30).

Discussion

The present study identified three pre-ingested phytoplankton genera in the gills of *M. strigata* that include *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. Identified pre-ingested particles are phytoplankton, specifically centric diatoms, considered the most preferred food of bivalves (Gosling 2003), together with bacteria, zooplankton, and detritus organisms (Vakily 1989; Langdon and Newell 1990; and Lehane and Davenport 2002). However, several studies have reported that species in the family Mytilidea demonstrate selective feeding behavior towards phytoplankton with a higher preference for dinoflagellates (Soon and Ransangan 2014; Sivalingam 1977). Sidari (1998) revealed that *M. galloprovincialis* seemed to feed on dinoflagellates rather than diatoms. This study also revealed that during water filtration, there is already particle retention in the gills of *M. strigata* that can also be affected by the movement of latero-frontal cilia and gill structure and particle concentration (Dral 1967; Jorgensen 1996). Ward *et a*l. (1998) revealed that ctenidia are responsible for particle sorting in *Crassostrea virginica* and *Crassostrea gigas*, which can also be true for mytilid species. In addition, gills and labial palp have been used to determine the efficiency of particle selection for marine bivalves with no distinct visible gut (Owen 1966).

It was further revealed that body size significantly affects the potential food preference of *M. strigata.* According to Beecham (2008), particles from 1 to 7 µm were preferentially ingested, while particles > 50 µm were rejected because it does not fit in the ctenidium, though they can be ingested and processed. Rijsgard *et al*. (1980) revealed that five and 13-year-old *M. edulis* consume particles smaller than 1 µm or larger than 9µm. Berry and Schleyer (1983) also identified pre-injected particles (0.46 µm in diameter) in *Perna perna*. Further, digestible particles are preferred over inorganic particles. This study found higher phytoplankton compositions in larger shell sizes (>40 mm) of *M. strigata,* suggesting possible food and size selectivity. This linear relationship may be due to the increasing demand for food for mussels as it grows. Bass *et al*. (1990) found that the size selectivity of *M. mercenaria* veligers is linked to the size of the mouth and esophagus, which get larger (5-6 µm in diameter) as they grow. Factors such as chemotactic attractiveness, size, and shape are believed to affect the active selection of phytoplankton in bivalve larvae (Raby 1997).

The present study showed a wide range of phytoplankton species filtered in the gills of *M. strigata,* which varied with site depending on the availability of phytoplankton in the area. *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. are the most dominant species found in the gills of *M. strigata,* which are also the dominant species in the waters of Bataan, Cavite, and Pangasinan. *Coscinodiscus* spp. found most prevalent is a free-living saucer- to petri-dish-shaped discoid with a valve mantle deeper on one side common in marine waters (Ehrenberg 1839). Five common species identified include *C. argus, C. centralis, C. lineatus, C. minor, and C. patina*. Lacuna *et al*. (2012) also considered *Coscinodiscus* spp. one of the four major phytoplankton identified in Panguil Bay, Philippines. In this study, *Coscinodiscus* sp. dominated in the month of November with 53.21% compared to in September and in October with only 11.51% and 13.05%, respectively. The abundance of *Coscinodiscus* sp. in this study is similar to the report of Lacuna *et al*. (2012), showing that this species is most abundant in November. In addition, *Skeletonema* spp. and *Navicula* spp. were also dominant in the different sites and are brackish water diatoms that have long been used as food for aquaculture species. A combination of this dominant species and some other culture microalgae can serve as the primary food for *M. strigata*, if the culture potential of this species is taken into account. There were also harmful algal cells such as *Gymnodynium* spp., *Skeletonema* spp., *Peridinium* spp., and *Navicula* spp. It shows that *M. strigata*, like any other bivalve, may still be able to ingest algal cells, resulting in diuretic or paralytic shellfish poisoning once eaten (Hermes and Villoso 1983; Bajarias *et al*. 2006). Aside from the phytoplankton identified, detritus forming matrix termed as dust fine detritus by Baker (1916) was also observed, which could not be estimated. Filter feeder sediment trapped in the gills may also serve as food for sessile bivalve species like *M. strigata*. The availability of food resources in the area is worth considering to understand further the bioinvasion ecology of *M. strigata* to control its likely further dispersal.

Conclusion

This study provided information on the particle selection of *Mytella strigata* based on the pre-ingested phytoplankton. A potential food preference for centric diatoms such as *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. was observed. These phytoplankton species were dominant in the waters of Bataan, Cavite, and Pangasinan, where there was a rapid proliferation of *M. strigata*. Size influences the pre-ingestion, as shown in the higher species diversity in size 20– 40mm than in <20 mm and >40mm. Further, pre-ingested particles present in the gills can be used to determine the efficiency of particle selection and preference for bivalves with no distinct visible gut, such as that of *M. strigata*.

Acknowledgments

The authors are grateful to John Ray Moleño, Catherine May Erazo-Malones, Leonilo Endoma Jr., and the Local Government Units of Abucay, Bataan, Bacoor, Cavite and Lucao, Pangasinan for their support.

Ethical approval

The authors declare that this study complies with research and publication ethics.

Informed Consent

Not Applicable

Conflict of interest

There is no conflict of interests for publishing this study.

Data availability statement

The authors declare that the data from this study are available upon request.

Funding organizations

This project is supported by the Department of Science and Technology - Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD).

Author contributions

DPMediodia - Conceptualization, Sampling, Analyses, Writing, Editing

NCAñasco - Conceptualization, Editing

CPBaylon - Conceptualization, Editing

SSSantander-de Leon - Conceptualization, Analyses, Writing, Editing

References

Baker, F.C. (1916). The relation of mollusks to fish in Oneida Lake. Tech. Publ. State Univ. Coll. For. Syracuse Univ. No. 4, 366pp. https://doi.org/10.1126/science.45.1154.144.a

Bajarias, F.F.A., Relox J.R., Fukuyo, Y. (2006). PSP in the Philippines: three decades of monitoring a disaster. Coastal Marine Science. 30, 104–106.

Bass, A.E., Malouf R.E., Shumway, S.E. (1990). Growth of northern quahogs (*Mercenaria mercenaria* (Linnaeus, 1758)) fed on picoplankton. Journ. Shellfish Res. 9(2), 299–307.

Cloern, J.E. (1982). Does the benthos control phytoplankton biomass in South San Francisco bay? Marine Ecology Progress Series 9, 191–202.

Cowden, C., Young, C.M., Chia, F.S. (1984). Differential predation on marine invertebrate larvae by two benthic predators. Mar. Eco. Prog. Ser. 14, 145–149.

Dame, R.F. (1996). Ecology of marine bivalves: an ecosystem approach. CRC Press, Boca Raton. 254.

Davenport, J., Smith, R.J.J.W., Packer, M. (2000). Mussels (*Mytilus edulis* L.): significant consumers and destroyers of mesozooplankton. Mar. Eco. Prog. Ser. 198, 131–137.

Descy, J.P., Padisak, J. (1994). Are phytoplankton dynamics in rivers so different from those in shallow lakes? Hydrobiologia. 289,1–7.

Dral, A.D.G. (1967). The movements of the latero-frontal cilia and the mechanism of particle retention in the mussel (*Mytilus edulis* L.). Netherlands J. Sea Res. 3, 391–422.

Ehrenberg, C.G. (1839). Über die Bildung der Kreidefelsen und des Kreidemergels durch unsichtbare Organismen. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin 1838:59–147.

Gosling, E. (2003). Bivalve Molluscs: Biology, Ecology and Culture. Fishing New Books, Oxford. 439.

Hermes, R., Villoso, E.P. (1983). A recent bloom of the toxic dinoflagellate *Pyrodinium bahamnese* var. compressa in the central Philippine waters. Fish. Res. J. Philippines 8(2), 1–8.

Huang, C., Lin, H., Huang, T., Su, H., Hung, J. (2008). Responses of phytoplankton and periphyton to system-scale removal of oyster-culture racks from a eutrophic tropical lagoon. Marine Ecology Progress Series 358, 1–12.

Hummel, H. (1985). Food intake of *Macoma balthica* (Mollusca) in relation to seasonal changes in its potential food on a tidal flat in the Dutch Wadden Sea. Neth. J. Sea Res. 19, 52–76. https://doi.org/10.1007/BF00391544

Jasprica, N., Caric, M., Bolotin, J., Rudenjak-Lukenda, M. (1997). The Mediterranean mussel (*Mytilus galloprovincialis* Lmk.) growth rate response to phytoplankton and microzooplankton population densities in the Mali Ston Bay (Southern Adriatic). Period Biol 99, 255–264.

King, T. Mcneal, J. (2010). Bivalves for clean water. Bivalve Shellfish – Canaries in the Coal

Mine, Grazers of the Sea. Washington Sea Grant Communications. WSG-AS.1001.

Kiorboe, T., Mshlenberg, F., Nahr, O. (1980). Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. Ophelia 19, 193.

Konrad, C. (2014). Approaches for Evaluating the Effects of Bivalve Filter Feeding on Nutrient Dynamics in Puget Sound, Washington. U.S. Geological Survey Scientific Investigations Report 2013. 5237, 22.

Kreeger, D.A., Newell, R.I.E. (2001). Seasonal utilization of different seston carbon sources by the ribbed mussel, *Geukensia demissa* (Dillwyn) in a mid-Atlantic salt marsh. Journal of Experimental Marine Biology and Ecology 260, 71–91.

Lacuna, M.L.D., Esperanza, M.R., Torres, M.A., Orbita, M.L. (2012). Phytoplankton diversity and abundance in Panguil Bay, Northwestern Mindanao, Philippines in relation to some physical and chemical characteristics of the water. AES Bioflux 4(3), 122–133.

Langdon, C.J., Newell, R.I.E. (1990). Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Marine Ecology Progress Series 58, 299–310.

Leal J.H. (2002). Bivalves. In: Carpenter, K. E. (ed). The living marine resources of Western Central Atlantic. FAO Identification Guide for Fishery Purposes. The Food and Agricultural Organization of the United Nations, Rome.

Lehane, C., Davenport, J. (2002). Ingestion of mesozooplankton by three species of bivalve; Mytilus edulis, Cerastoderma edule and Aequipecten opercularis. Jour. of the Mar. Bio. Assoc. of the UK, 82, 615–619.

Noren, F., Haamer, J., Lindahl, O. (1999). Changes in the plankton community passing a *Mytilus edulis* mussel bed. Mar.Eco. Prog. Ser. 191, 187–194.

Nowrouzi, S., Valavi, H. (2011). Effects of Environmental Factors on Phytoplankton Abundance and Diversity in Kaftar Lake. Journal of Fisheries and Aquatic Science, 6, 130–140.

Officer, C.B., Smayda, T.J., Mann, R. 1982. Benthic filter feeding:a natural eutrophication control. Mar. Ecol. Prog. Ser. 9, 203–210.

Purchon, R.D. (1968). The biology of the molluscs. Pergamon Press, Oxford.

Riisgard, H.U., Randlov, A., Kristensen, P.S. (1980). Rates of water processing, oxygen consumption and efficiency of particle retention in veligers and young post-metamorphic *Mytilus edulis*. Ophelia 19(1), 37–47.

Shumway, S.E., Newell, R.C., Crisp, D.J., Cucci, T.L.1990. Particle selection in filter feeding bivalve molluscs: a new technique on an old theme. In The Biualvia Proceedings of a Memorial Symposium in Honour of Sir Charles Maurice Yonge (Edited by Morton B.).152–165. Hong Kong University Press, Hong Kong.

Sidari, L., Nichetto, P., Cok, S., Sosa S Tubaro, A., Honsell, G., Della Loggia, R. (1998). Phytoplankton selection by mussels, and diarrhetic shellfish poisoning. Marine Biology. 131:103–111.

Sivalingam PM. 1977. Aquaculture of the green mussel, *Mytilus viridis* Linnaeus, in Malaysia. Aquaculture 11: 297–312. 10.1016/0044-8486(77)90079-5

Soon, T.K., Ransangan, J. (2014). A Review of Feeding Behavior, Growth, Reproduction and Aquaculture Site Selection for Green-Lipped Mussel, *Perna viridis*. Adv. BioSci. Biotech. 5, 462–469. 1010.4236/abb.2014.55056

Sprung, M. (1984). Physiological energetics of mussel larvae *Mytilus edulis* L. II. Food uptake. Mar Ecol. Prog. Ser .17, 295–305.

Stella, C., Sesh, S.J., Siva, J. (2010). New Distributional Records of *Placuna ephippium* (Reizius 1788) Family: Placunidae from Mandapam Area -Southeast Coast of India. World J. Fish and Mar. Sci. 2, 40–41.

Thompson, J.K. Nichols, F.H. (1988). Food availability controls seasonal cycle of growth in *Macoma balthica* (L.) in San Francisco Bay, California. J. Exp. Mar. Biol. Ecol. 116, 4361. 10.1016/0022-0981(88)90245-6

Vakily, J.M. (1989). The Biology and Culture of Mussels of the Genus *Perna*, International Center for Living Aquatic Resources Management, Manila, Philippines.

Valiela, I. (1984). Marine Ecology Process. Springer-Verlag, New York. 546.

Widdows, J., Donkin, P., Salkeld, P.N., Cleary, J.J., Lowe, D.M., EvaNS, S.V., Thompson, P.E. (1984). Relative importance of environmental factors in determining physiological differences between two populations of mussels (*Mytilus edulis*). Mar. Ecol. Prog. Ser. 17, 33–47.

Sustainable Aquatic Research (2024) 3(1):16-25
DOI: 10.5281/zenodo.10886465
e-ISSN: 2822-4140

SUSTAINABLE AQUATIC RESEARCH
www.saquares.com

RESEARCH PAPER

Investigations on the phytoplankton composition and trophic status of Lake Karagöl (Dikili-İzmir-Türkiye)

Haşim Sömek [1*], Esat Tarık Topkara[2]

[1*] *Department of Basic Sciences, Faculty of Fisheries, Izmir Katip Celebi University, İzmir, Türkiye*
[2] *Department of Marine–Inland Waters Sciences and Technology, Faculty of Fisheries, Ege University, İzmir, Türkiye*

Citation

Sömek, H., Topkara, E.T. (2024). Investigations on the phytoplankton composition and trophic status of Lake Karagöl (Dikili-İzmir-Türkiye). *Sustainable Aquatic Research, 3*(1), 16-25.

Article History

Received: 12 March 2024
Received in revised form: 24 March 2024
Accepted: 27 March 2024
Available online: 30 April 2024

Corresponding Author

Haşim Sömek
E-mail: hasim.somek@ikcu.edu.tr
Tel: +90 505 577 25 91

Keywords

Phytoplankton
Biodiversity
Small lakes
TSI, TN:TP ratio
Eutrophication

Abstract

The phytoplankton composition, TN:TP ratios as the limiting factor on phytoplankton and the trophic Status of Lake Karagöl, a small volcanic-originated lake, were first investigated in this study. Additionally, the physicochemical parameters of the lake were measured. As a result of the investigations, 21 dominant taxa from four divisions among phytoplanktonic organisms were identified in Karagöl. Three taxa belonged to Cyanobacteria, eleven to Chlorophyta, six to Ochrophyta (only Bacillariophycea), and to one to Myzozoa. The average depth of the lake was calculated as 8.7 m. In line with the atmospheric temperatures brought by the Mediterranean climate prevailing in the region, surface water temperatures of the lake fluctuated between 23.0 °C (summer) and 6.5 °C (winter). Conductivity ranged from 780 to 423 µS and dissolved oxygen ranged from 15.3 to 7.3 mg l^{-1}. The average scores of TSI (SD), TSI (Chl-a), TSI (TN) and TSI (TP) were calculated as 68.0, 55.1, 62.1 and 86.4 respectively. These TSI scores indicated that Lake Karagöl was at eutrophic or hypereutrophic levels. TN:TP ratio of the lake ranged from 9.8 to 7.2 and in this case, the Lake Karagöl food chain is nitrogen-limited because of the low TN:TP ratios. *Dolichospermum flosaquae* (N2-fixing heterocystous Cyanobacteria) was the dominant species in Lake Karagöl phytoplankton during the summer months when the high-water temperature. As a result of this study, based on phytoplankton composition, dominant algae groups (especially Cyanobacteria) and TSI scores, it was evaluated that Lake Karagöl (Dikili) has suitable environmental conditions for many algae species found in mesotrophic and eutrophic lakes and is in a very rapidly progressing hypertrophication process.

Introduction

Small lakes, typically stagnant water bodies with surface areas ranging from a few acres to several hectares, host highly diverse floristic and faunistic communities. These lakes serve as vital habitats for a variety of aquatic organisms such as fish, amphibians and invertebrates, and also contribute to the conservation of local biodiversity by supporting populations of waterfowl and other birds (Schafft et al., 2023; Labat et al., 2022; Biggs et al., 2017). In addition, these lakes, which are recreation areas because they offer opportunities such as fishing, boating, swimming and nature observation, contribute to the physical and mental health of local people, as well as providing numerous ecological, social and economic benefits

(Meyerhoff et al.,2022). Although the protection and management of these valuable aquatic ecosystems have the potential to provide sustainable contributions to the well-being of current and future generations, until recently research on the ecology or hydrobiology of small lakes has lagged that of large lakes (Downing, 2010). Due to their limited morphometry, they exhibit complex ecological dynamics affected by factors such as water depth, nutrient availability and surrounding land use, and are highly sensitive to pressures such as anthropogenic pollution, habitat modification and climate change (Koff et al., 2016; Winslow et al., 2015).

Phytoplankton as communities of microscopic algae, are important primary producers in lake ecosystems and the basis of the aquatic food web because of using sunlight and nutrients for photosynthesis (Reynolds, 2006). Their abundance and diversity serve as crucial indicators of water quality and ecosystem health (Padisák et al., 2006). According to the literature search, curiosity about understanding the driving factors of regulating the spatial and temporal phytoplankton distribution and their functional assemblages of small and shallow lakes started to increase in the early 2000s. (O’Farrell et al., 2003; Ortega-Mayagoitia et al., 2003; Padisák et al., 2003; Stoyneva, 2003). In the following years, studies on the phytoplankton communities, other primary producers, trophic conditions, and ecology of these lakes have also risen in our country (Çelekli et al., 2007; Soylu and Gönülol, 2006; Taş et al., 2010; Altınsaçlı et al., 2014; Taş, 2012). When these studies from the past to present are examined, it is evaluated that the most characteristic problem of these valuable and sensitive aquatic habitats is mostly pollution, and the most common result of this is the eutrophication phenomenon. Global warming and its negative effects on lakes also play a booster role in this dramatic process (Kosten et al., 2012). The Total Nitrogen and Total Phosphorus ratio (TN:TP) is a pivotal factor influencing eutrophication dynamics in water bodies (Smith et al., 1999). This ratio serves as a crucial indicator of nutrient availability, with imbalances often leading to excessive algal growth (especially Cyanobacterial bloom) and subsequent oxygen depletion (Jeppesen et al., 2005; Conley et al., 2009; Frenken, 2023). Such nutrient-driven eutrophication can profoundly impact water quality and ecosystem health (Paerl and Paul, 2012), emphasizing the necessity for effective nutrient management strategies to maintain balanced TN:TP ratios and mitigate eutrophication risks (Elser et al. 2007). Moreover, the Trophic Status Index (TSI) is a critical tool for swiftly evaluating the nutrient levels and ecological health of lakes. Through the analysis of parameters such as nutrient concentrations and chlorophyll levels, TSI provides a concise assessment of a lake's trophic status, facilitating informed management decisions to uphold water quality and ecological equilibrium (Carlson, 1977).

The aims of this study were to reveal preliminary knowledge of the phytoplankton composition, to determine the limiting factor on phytoplankton by calculating TN:TP ratios, and to evaluate the Trophic Status of this small lake (Lake Karagöl).

Materials and Methods

Study area and sampling

Lake Karagöl (38°57'29"N 26°50'55"E) is located at an altitude of 430 m above sea level and 2.5 km away from the Aegean Sea shores as the crow flies' distance within the borders of the Dikili District of Izmir in Western Anatolia. (Figure 1). Karagöl is a small lake with a surface area of approximately 3.5 ha and it is known that the lake was formed as a result of volcanic activities. Four different eruption phases were distinguished within the volcanic piles on the Kardağ Mountain, where the lake is located, and it was reported that due to the spread of lava flows away from the center of the lake, the origin of the explosion was the lake pit, and this stagnant water structure was a volcanic lake (Karacık et al., 2007). Agricultural and livestock activities were observed near the lake. Biological and water sampling were made seasonally from a single point determined in the lake between 2012 and 2013 years. Phytoplankton sampling was carried out using a Hydrobios plankton net with a 55 µm mesh size and drawing circles for 15 minutes. The collected phytoplankton samples were fixed with formaldehyde to a final concentration of 4%. Prefiltered (60 µm) surface water samples were stored in polyethylene jars (1 L volume) and then taken to the laboratory in freezers to protect them from atmospheric effects. The water samples for chlorophyll *a* analysis were collected into 200-mL amber-glass bottles.

http://www.gooogle.com/earth/index.html [Accesed 05 March 2024]

Figure 1. Study location and sampling point

Identification and physicochemical analysis

Monograph-level key books of various researchers were used in the identification of phytoplankton species (Huber-Pestalozzi, 1941, 1942; Philipose, 1967; Sims, 1996; Komárek & Anagnostidis, 1999; John et al., 2003; Komárek & Zapomelova, 2007). The current positions of the identified taxa in the systematic hierarchy were checked on the algaebase.org website (Guiry & Guiry, 2014). All the species identifications were made using an Olympus BX53 upright microscope (Olympus Corporation, Japan).

Measurements of temperature, light penetration (with 30 cm Ø black/white Secchi disk), dissolved oxygen (with WTW Oxi 330) and electrical conductivity (with YSI 30 model SCT meter) from the physicochemical properties of water were carried out in situ. HACH LANGE spectrometric test kits [Total Nitrogen (Koroleff digestion+ 2,6-dimethylphenol method with LCK 138 range: 1-16 mg/L TN), Total Phosphorus (Phosphomolybdenum blue methods with LCK 348 range: 0.5-5.0 mg/L PO_4-P)] were used to analyze the chemical properties of water samples. Analyzes were made with a HACH LANGE DR 2800 model Spectrophotometer and HACH LANGE BRB 200 Thermoreactor (for Total Nitrogen). The fluorometric method (Madden & Day, 1992) was used to determine chlorophyll-*a* concentrations (10 AU Model Turner Designs fluorometer). The Trophic State Index (TSI) method was used to determine the trophic level of the lake. Index results were calculated using the values of Secchi depth, Chlorophyll-*a*, TP and TN concentrations to the formulas given respectively (Kratzer & Brezonik, 1981; Carlson and Simpson, 1996): TSI (SD) = 60-14.41 ln(SD) (SD :Secchi Depth ,m); TSI (Chl-*a*) = 9,81 ln(Chl-*a*) + 30,6 (Chl-*a* : Chlorophyll-*a*, µg l^{-1}); TSI (TP) = 14.42 ln TP+ 4.1 (TP: Total Phosphorus, µg l^{-1}); TSI(TN) = 54.45 + 14.43 ln(TN: Total Nitrogen mg l^{-1}).

Results and Discussion

As a result of the algological examinations, 21 dominant taxa from four divisions among phytoplanktonic organisms were identified in Karagöl. Three taxa belonged to Cyanobacteria, eleven to Chlorophyta, six to Ochrophyta (only Bacillariophycea), and to one to Myzozoa. (Figure 2).

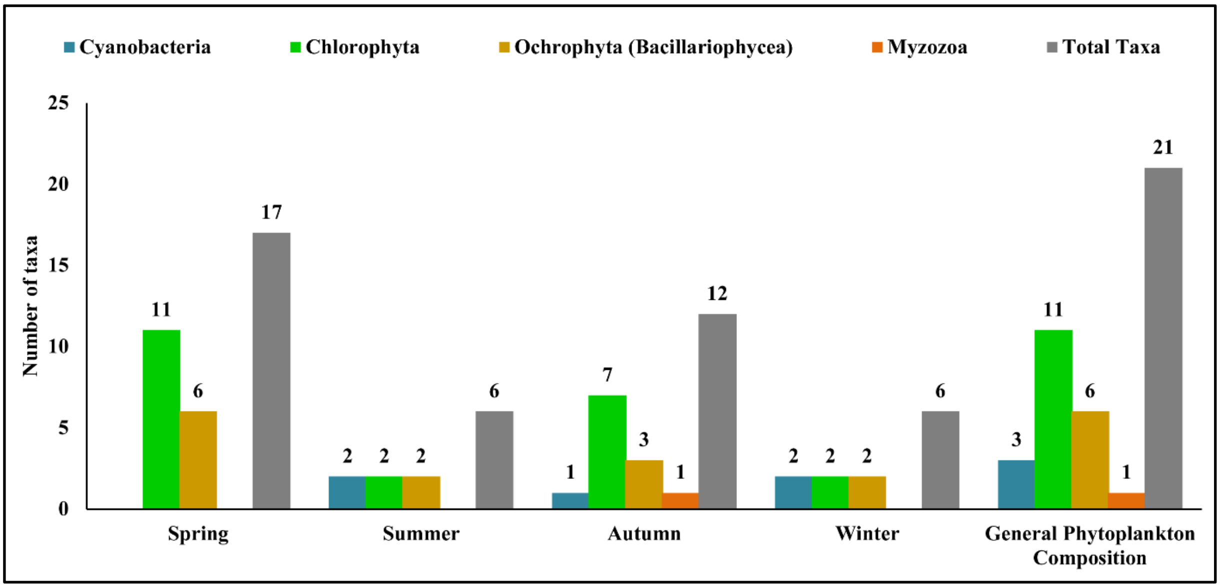


Figure 2. The distribution of phytoplankton divisions and number of taxa by seasons

Dominantly observed phytoplankton species and their seasonal compositions in Lake Karagöl are listed in Table 1. Cyanobacteria (blue-green algae) members were detected more commonly in eutrophic lakes in our country (Cirik-Altındağ, 1982; Gönülol & Çomak, 1992; Albay et al., 2003**;** Sömek & Balık, 2009), and the members of this section were found to be abundant in nutrient-rich and eutrophic lakes in the summer months (Vaitomaa, 2006). During this research, very few taxa from Cyanobacteria were identified, but when their density was evaluated, they were found to be relatively dominant in phytoplankton in summer. In a study conducted on the processes regulating the dominance of planktonic diatoms and cyanobacteria in several eutrophic lakes, it was found that diatoms and, to a lesser extent, Chlorophyta species were dominant in periods when water temperature (<15 °C) and water column stability were low, and in periods when water temperature and water column stability were high. It has been reported that cyanobacteria are dominant (Zhang and Prepas, 1996). When the seasonal change of phytoplankton of Karagöl (Dikili) was examined, an analogous phytoplankton dynamic was observed.

The Chlorophyta (green algae) division was the phytoplankton group represented by the highest number of taxa in our research area. The entire Chlorophyta division consisted of Chlorococcales members. This pattern is like the phytoplankton composition of many mesotrophic and eutrophic lakes in our country. (Gönülol & Obalı, 1998; İşbakan-Taş et al., 2002; Ongun-Sevindik, 2010). The presence of Chlorococcales members was reported as a transition from the oligotrophic level to the eutrophic level (Hutchinson, 1957). It is known that species belonging to the *Monoraphidium* genus are dominant in oligotrophic and mesotrophic lakes (Legnerova, 1965). *Scenedesmus* and *Pediastrum* species, which are other Chlorococcales members frequently found in current study area, were frequently encountered in oligomesotrophic reservoirs and eutrophic lakes in our country (İşbakan-Taş et al., 2002; Kıvrak & Gürbüz, 2005; Ongun-Sevindik, 2010). Desmids, another group of green algae whose species are found in many oligotrophic and mesotrophic lakes in our country (Akgöz & Güler, 2004; Baykal et al., 2004; Karacaoğlu et al., 2004), were not observed in Lake Karagöl (Dikili). Palmer (1980) reported that most Desmidiaceae species can be found in oligotrophic water bodies, and a few in eutrophic water bodies.

As a result of the examinations, diatoms (Bacillariophyceae) in phytoplankton were the second most dominant group after the members of the Chlorophyta division. It has been reported that the pelagic species, *Ulnaria ulna* (Nitzsch) Compère, which was frequently detected in our research area, is one of the characteristic species of eutrophic lakes (Hustedt, 1930, 1945; Reynolds et al., 2002). Centric diatoms are algal groups that are best adapted to systems that are rich in nutritious

mineral substances and have high turbidity (Izaguirre et al., 2001). Species of the *Aulacoseira* and *Cyclotella* species were observed in Lake Karagöl (Dikili) in the autumn phytoplankton, where water column stability was disrupted and mixing increased. Species originating from benthic were also identified among the diatom members in the lake (*Gomphonema olivaceum* (Hornemann) Ehrenberg, *Pinnularia maior* (Kützing) Rabenhorst). It is known that pennate diatoms of benthic origin are transported to the pelagic region due to various water movements in relatively shallow, small-area lakes and ponds (Round, 1973). Similar situations were reported in the lakes of our country (Şen et al., 2001; Akgöz & Güler, 2004). Most of the Bacillariophyceae members identified in this research have a high tolerance to environmental variables and are widely distributed in many lakes and other water bodies at different trophic levels in our country (Aysel, 2005). In our research area, only *Peridiniopsis cunningtonii Lemmermann* species from the Myzozoa (Dinoflagellates) was identified. *P. cunningtonii* is also found in mesotrophic or eutrophic inland water ecosystems in Türkiye (Sömek et al., 2005; Ongun-Sevindik, 2010).

Table 1. Dominant phytoplankton species and seasonal composition of Lake Karagöl

	Spring	**Summer**	**Autumn**	**Winter**
Cyanobacteria				
Dolichospermum flosaquae (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek		+	+	
Merismopedia minima G.Beck				+
Limnothrix sp.		+		+
Chlorophyta				
Actinastrum hantzschii Lagerheim	+			
Botryococcus braunii Kützing	+	+	+	
Desmodesmus communis (E.Hegewald) E.Hegewald	+		+	
Desmodesmus opoliensis (P.G.Richter) E.Hegewald	+		+	
Golenkinia radiata Chodat	+			
Micractinium pusillum Fresenius	+			+
Monoraphidium griffithii (Berkeley) Komárková-Legnerová	+			
Monoraphidium irregulare (G.M.Smith) Komárková-Legnerová	+		+	
Pseudopediastrum boryanum (Turpin) E.Hegewald	+	+	+	+
Scenedesmus obliquus (Turpin) Kützing	+		+	
Tetraëdron minimum (A.Braun) Hansgirg	+		+	
Ochrophyta (Bacillariophycea)				
Aulacoseira italica (Ehrenberg) Simonsen	+			
Cyclotella meneghiniana Kützing	+			
Gomphonema olivaceum (Hornemann) Brébisson	+			+
Nitzschia acicularis (Kützing) W.Smith	+	+	+	
Pinnularia maior (Kützing) Cleve	+		+	+
Ulnaria ulna (Nitzsch) P.Compère	+	+	+	
Myzozoa				
Peridiniopsis cunningtonii Lemmermann			+	

Physicochemical parameters measured in Karagöl are given in detail in Table 2. During the study, no dramatic changes were observed in the depth of the lake and the average depth was calculated as 8.7 m. In line with the atmospheric temperatures brought by the Mediterranean climate prevailing in the region, surface water temperatures of the lake fluctuated between 23.0 °C (summer) and 6.5 °C (winter). Conductivity ranged from 780 to 423 µS and dissolved oxygen ranged from 15.3 to 7.3 mg l^{-1}, according to, Turkish Surface Water Quality Regulation (SWQR, 2016), the quality of Lake Karagöl surface water was class II (good) and class I (very good), respectively. Carlson's Trophic

State Index (TSI), developed by Robert G. Carlson, is a method used to evaluate and classify the trophic state of freshwater bodies such as lakes and reservoirs, based on measurements of chlorophyll-a concentration, total phosphorus and Secchi disk transparency (Carlson, 1977). The classification of freshwater bodies according to TSI values ranges from oligotrophic (TSI < 40) to hypereutrophic (TSI > 70), with mesotrophic (TSI 40-50) and eutrophic (TSI 50-70) classifications in between. Secchi depth (SD), Chlorophyll *a* (Chl-a), Total Nitrogen (TN) and Total Phosphorus (TP) values were used to generate the Carlson Trophic State Index scores. The average scores of TSI (SD), TSI (Chl-*a*), TSI (TN) and TSI (TP) were calculated as 68.0, 55.1, 62.1 and 86.4 respectively. These TSI scores indicated that Lake Karagöl was at eutrophic or hypereutrophic levels.

The TN:TP ratio is a key approach frequently used to analyze the dynamics of lake ecosystems (van Wijk et al., 2024). It has been reported that nitrogen is limiting when the TN:TP ratio is <10, phosphorus is limiting when the TN:TP ratio is >17, and freshwater ecosystems are balanced when the TN:TP ratio is between 10-17 (Smith, 1983). TN:TP ratio of the lake ranged from 9.8 to 7.2 and in this case, the Lake Karagöl food chain is nitrogen-limited because of the low TN:TP ratios. It has been observed in the studies that the blooms of N_2-fixing (heterocystous blue-green algae) cyanobacteria can be supported by low TN:TP ratios or N-limited conditions (Moisander et al, 2012; González-Madina et al., 2019). This succession dynamic was confirmed by the presence of *Dolichospermum flosaquae* (Bornet & Flahault) P.Wacklin, L.Hoffmann & Komárek as the dominant species in Lake Karagöl phytoplankton during the summer months when the high-water temperature.

Table 2. Physicochemical parameters and Carlson's TSI values of Lake Karagöl

PARAMETERS	Mean	Maximum	Minimum	TSI_Mean	TSI _Max	TSI _Min
Depth (m)	8.7	10.0	8.1	-	-	-
Temperature (°C)	14.8	23.0	6.5	-	-	-
Conductivity (25°C) µS	497.2	780	423	-	-	-
Dissolved Oxygen (mg l^{-1})	9.6	15.3	7.3	-	-	-
Secchi Depth (m)	0.58	0.70	0.35	** 68.0	*** 75.1	** 65.1
Chlorophyll *a* (µg l^{-1})	12.2	21.2	6.8	** 55.1	** 60.5	* 49.4
TN (µg l^{-1})	2846	4580	1400	** 62.1	** 66.7	** 58.2
TP (µg l^{-1})	300	466	195	*** 86.4	*** 92.7	*** 80.2
TN:TP Ratio	9.5	9.8	7.2	N limitation	N limitation	N limitation

(*mesotrophic, **eutrophic, ***hypertrophic)

Conclusions

As a result of the study, it was evaluated that Lake Karagöl (Dikili) is eutrophic or hypereutrophic based on phytoplankton composition, dominant algae groups (especially Cyanobacteria), and TSI scores and so environmental conditions of the lake were suitable for many algae species commonly observed in nutrient-rich inland waters of the world. For this reason, the authors have predicted that Lake Karagöl (Dikili), which is the drainage area of the land it's around, was partially affected by the agricultural and livestock activities nearby.

Acknowledgements

The authors are grateful for the help of M. Ruşen Ustaoğlu, Murat Özbek, Seray Yıldız, Cem Aygen, Didem Özdemir Mis and Ayşe Taşdemir. This study was financially supported by the Scientific Research Unit of Ege University (İzmir-Türkiye) under Grant number 2011/SÜF/037.

Ethical approval

The author declares that this study complies with research and publication ethics.

Informed consent

Not available.

Conflicts of interest

There is no conflict of interests for publishing their study.

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Funding organizations

This study was financially supported by the Scientific Research Unit of Ege University (İzmir-Türkiye) under Grant number 2011/SÜF/037.

Author contribution

Haşim Sömek: Writing original draft, Conceptualization, Sampling, Species identification.

Esat Tarık Topkara: Project administration, Investigation, Analysis, Review, Editing.

References

Akgöz, C. & Güler, S. (2004) Topçu Göleti (Yozgat) Alg Florası I: Epilitik ve Epifitik Algler. *S.Ü. Fen Ed. Fak. Fen Derg., 23:* 7-14.

Albay, M., Akcaalan, R., Aykulu, G., Tufekci, H., Beattie. K.A. & Codd, G.A. (2003). Occurrence of toxic cyanobacteria before and after copper sulphate treatment in a water reservoir, Istanbul, Turkey. *Arch Hydrobiol Supp Algol Stud, 109*:67–78.

Altınsaçlı, S., Altınsaçlı, S., & Paçal, F. P. (2014). Species composition and qualitative distribution of the macrophytes in three Turkish lakes (Kandira, Kocaeli, Turkey). *Phytologia Balcanica, 20*(1), 89-98.

Aysel, V.(2005) Check-List of The Freshwater Algae of Turkey. *J. Black Sea/Mediterranean Environment, 11* (1): 5-128.

Baykal. T., Açıkgöz, İ., Yıldız, K. and Bekleyen, A. (2004) A Study on Algae in Devegeçidi Dam Lake. *Turkish Journal of Botany, 28*: 457-472.

Biggs, J., Von Fumetti, S., & Kelly-Quinn, M. (2017). The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia, 793*, 3-39. https://doi.org/10.1007/s10750-016-3007-0

Carlson, R.E. (1977). A trophic state index for lakes. *Limnology and Oceanography. 22*:361-369.

Carlson, R.E. & Simpson, J. (1996). *A Coordinator's Guide to Volunteer Lake Monitoring Methods.* North American Lake Management Society. 96 pp.

Çelekli, A., Albay, M., & Dügel, M. (2007). Phytoplankton (except Bacillariophyceae) flora of lake Gölköy (Bolu). *Turkish Journal of Botany, 31*(1), 49-65.

Cirik-Altındağ, S. (1982). Manisa - Marmara Gölü Fitoplanktonu. I -Cyanophyta, *Doğa Bilim Dergisi, Temel Bilimler, 6* (3) : 67-81.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., ... & Likens, G. E. (2009). Controlling eutrophication: nitrogen and phosphorus. *Science, 323*(5917), https://doi.org/10.1126/science.1167755

Downing, J. A. (2010). Emerging global role of small lakes and ponds: little things mean a lot. *Limnetica, 29*(1), 0009-24.

Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., ... & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology letters, 10*(12), 1135-1142.

Frenken, T., Brandenburg, K. M., & Van de Waal, D. B. (2023). Long-term nutrient load reductions and increasing lake TN: TP stoichiometry decrease phytoplankton biomass and diversity in a large shallow lake. *Limnology and Oceanography, 68*(10), 2389-2401. https://doi.org/10.1002/lno.12428

Gönülol, A. & Obalı, O. (1998). Seasonal Variations of Phytoplankton Blooms in Suat Uğurlu (Samsun-Turkey). *Turkish Journal of Botany, 22*: 93-97.

Gönülol, A. & Çomak, O., 1992, Bafra Balık Gölleri (Balık Gölü, Uzun Göl) Fitoplanktonu Üzerinde Floristik Araştırmalar. I -Cyanophyta, Doğa Tr. J. of Botany 16 : 223-245

González-Madina, L., Pacheco, J. P., Yema, L., de Tezanos, P., Levrini, P., Clemente, J., ... & Mazzeo, N. (2019). Drivers of cyanobacteria dominance, composition and nitrogen fixing behavior in a shallow lake with alternative regimes in time and space, Laguna del Sauce (Maldonado, Uruguay). *Hydrobiologia, 829*, 61-76.

Guiry, M.D. & Guiry, G.M. (2014) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 29 April 2014.

Huber-Pestalozzi, G. (1941). *Das Phytoplankton des Süßwassers. (Die Binnengewässer, Band XVI). Teil 2. (i) Chrysophyceen, Farblose Flagellaten Heterokonten.* E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Huber-Pestalozzi, G. (1942). *Das Phytoplankton des Süßwassers. (Die Binnengewässer, Band XVI). Teil 2. (ii). Diatomeen. E.* Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Hustedt, F. (1930) *Bacillariophyta Diatome* Heft: 10 A Pascher DieSusswasser Flore Mitteleuropas. Ed. Gustav Fischer Pub., Jena, Germany,466pp.

Hustedt, F. (1945). *Die Diatomeenflore Norddeutscher Seen Mit Besonderer Berücksichtigung Des Holsteinischen Seen-Gebiets*. *Arch. Hydro. 41*:392-414.

Hutchinson, G.E. A. (1957) *Treatise on Limnology* Volum I: Geopraphy, Physics an Chemistry,.John Wiley, Newyork.

İşbakan-Taş, B., Gönülol, A. & Taş, E. (2002) A Study on the Seasonal Variation of the Phytoplankton of Lake Cernek (Samsun-Turkey). *Turkish Journal of Fisheries and Aquatic Sciences*, *2*: 121-128.

Izaguirre, I., O'Farrell, I., & Tell, G. (2001). Variation in phytoplankton composition and limnological features in a water–water ecotone of the Lower Paraná Basin (Argentina). *Freshwater Biology*, *46*(1), 63-74.

Jeppesen, E., Søndergaard, M., Jensen, J. P., Havens, K. E., Anneville, O., Carvalho, L., ... & Winder, M. (2005). Lake responses to reduced nutrient loading–an analysis of contemporary long-term data from 35 case studies. *Freshwater biology*, *50*(10), 1747-1771. https://doi.org/10.1111/j.1365-2427.2005.01415.x

John, D.M., Whitton, B.A. & Brook, A.J. (2003). *The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae*. Cambridge University Press. New York. 701 pages.

Karacaoğlu, D., Şükran D. & Dalkıran, N. (2004) A Taxonomic Study on the Phytoplankton of Lake Uluabat (Bursa). *Turkish Journal of Botany, 28*: 473-485.

Karacık, Z., Yılmaz, Y. & Pearce, J. A. (2007) The Dikili-Çandarl› Volcanics, Western Turkey: Magmatic Interactions as Recorded by Petrographic and Geochemical Features. *Turkish Journal of Earth Sciences, 16*: 493-522.

Kıvrak, E. & Gürbüz, H. (2005) Seasonal variations in phytoplankton composition and physical-chemical features of Demirdöven Dam Reservoir, Erzurum, Turkey, *Biologia, Bratislava, 60*/1: 1-8.

Koff, T., Vandel, E., Marzecová, A., Avi, E., & Mikomägi, A. (2016). Assessment of the effect of anthropogenic pollution on the ecology of small shallow lakes using the palaeolimnological approach. *Estonian Journal of Earth Sciences*, *65*(4), 221–233. https://doi.org/10.3176/earth.2016.19

Komárek J. & Anagnostidis K. (1999). *Cyanoprokaryota. Chroococcales*. In: E. Ettl, G. Gärtnerand D. Mollenhauer (eds) Süsswasserflora von Mitteleuropa. Bd. 19/1. Gustav Fischer JenaStuttgart, Lübeck Ulm: 548 pp.

Komárek, J. & Zapomelova E. (2007). Planktic morphospecies of the cyanobacterial genus Anabaena_subg. Dolichospermum—1. part: coiled types. *Fottea 7*: 1–31.

Kosten, S., Huszar, V. L., Bécares, E., Costa, L. S., van Donk, E., Hansson, L. A., ... & Scheffer, M. (2012). Warmer climates boost cyanobacterial dominance in shallow lakes. *Global Change Biology*, *18*(1), 118-126. https://doi.org/10.1111/j.1365-2486.2011.02488.x

Kratzer, C.R. and Brezonik, P.L. (1981) A Carlson-Type Trophic State Index for Nitrogen in Florida Lakes. *Water Resources Bulletin, 17*, 713-

715. https://doi.org/10.1111/j.1752-1688.1981.tb01282.x

Labat, F., Piscart, C., & Thiebaut, G. (2022). Invertebrates in small shallow lakes and ponds: a new sampling method to study the influence of environmental factors on their communities. *Aquatic Ecology*, *56*(3), 585-603. https://doi.org/10.1007/s10452-021-09939-1

Legnerova, J. (1965) The Genera *Ankistrodesmus* Corda and *Raphidium* Kützing and their Position In the Family Ankistrodesmusmaceae, *Preslia 37*: 1-8.

Madden, C.J. & Day, Jr. J.W. (1992).An instrument system for the high-speed mapping of chlorophyll-a and physicochemical variables in surface waters. *Eustaries.*, *15*(3): 421-427

Meyerhoff, J., Klefoth, T., & Arlinghaus, R. (2022). Ecosystem service trade-offs at small lakes: Preferences of the public and anglers. *Aquatic Ecosystem Health & Management*, *25*(3), 1-11. https://doi.org/10.14321/aehm.025.03.01

Moisander, P. H., Cheshire, L. A., Braddy, J., Calandrino, E. S., Hoffman, M., Piehler, M. F., & Paerl, H. W. (2012). Facultative diazotrophy increases *Cylindrospermopsis raciborskii* competitiveness under fluctuating nitrogen availability. *FEMS Microbiology Ecology*, *79*(3), 800-811. https://doi.org/10.1111/j.1574-6941.2011.01264.x

O’Farrell, I., Sinistro, R., Izaguirre, I., & Unrein, F. (2003). Do steady state assemblages occur in shallow lentic environments from wetlands?. In *Phytoplankton and Equilibrium Concept: The Ecology of Steady-State Assemblages:* Proceedings of the 13th Workshop of the International Association of Phytoplankton Taxonomy and Ecology (IAP), held in Castelbuono, Italy, 1–8 September 2002 (pp. 197-209). Springer Netherlands.

Ortega-Mayagoitia, E., Rojo, C., & Rodrigo, M. A. (2003). Controlling factors of phytoplankton assemblages in wetlands: an experimental approach. In *Phytoplankton and Equilibrium Concept:* The Ecology of Steady-State Assemblages: Proceedings of the 13th Workshop of the International Association of Phytoplankton Taxonomy and Ecology (IAP), held in Castelbuono, Italy, 1–8 September 2002 (pp. 177-186). Springer Netherlands.

Ongun-Sevindik, T. (2010) Phytoplankton Composition of Çaygören Reservoir, Balikesir-Turkey. *Turkish Journal of Fisheries and Aquatic Sciences 10*: 295-304.

Padisák, J., Borics, G., Fehér, G., Grigorszky, I., Oldal, I., Schmidt, A., & Zámbóné-Doma, Z. (2003). Dominant species, functional assemblages and frequency of equilibrium phases in late summer phytoplankton assemblages in Hungarian small shallow lakes. *Hydrobiologia*, *502*, 157-168. https://doi.org/10.1023/B:HYDR.0000004278.10887.40

Padisák, J., Borics, G., Grigorszky, I., & Soróczki-Pintér, É. (2006). Use of phytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index. *Hydrobiologia*, *553*, 1-14. https://doi.org/10.1007/s10750-005-1393-9

Palmer, C.M. (1980). *Algae and Water Pollution*, Castle House Pub., London.

Paerl, H. W., & Paul, V. J. (2012). Climate change: links to global expansion of harmful cyanobacteria. *Water research*, *46*(5), 1349-1363. https://doi.org/10.1016/j.watres.2011.08.002

Philipose, M. T. (1967) *Chlorococcales*. I. C. A. R., New Delhi, 365 p.

Reynolds, C. S., Huszar, V., Kruk, C., Naselli-Flores, L., & Melo, S. (2002). Towards a functional classification of the freshwater phytoplankton. *Journal of plankton research*, *24*(5), 417-428.

Reynolds, C. S. (2006). *The ecology of phytoplankton*. Cambridge University Press. Cambridge, UK, 535 pp.

Round, F. E. (1973) The Biology of The Algae, Edward Arnold, London,

Schafft, M., Nikolaus, R., Matern, S., Radinger, J., Maday, A., Klefoth, T., ... & Arlinghaus, R. (2023). Impact of water-based recreation on aquatic and riparian biodiversity of small lakes. *Journal for Nature Conservation*, 126545. https://doi.org/10.1016/j.jnc.2023.126545

Şen, B., Çağlar, M. ve Toprak Pala, G. (2001). Tadım Göleti (Elazığ) Diyatomeleri ve Yıl İçindeki Dağılımları. *F.Ü. Fen ve Müh. Bilimleri Dergisi 13*, 2, 255-261.

Sims, P.A. (1996). *An Atlas of British Diatoms.* Illustrated by Horace G. Barber, & John R. Carter, arranged by Bernard Hartley. Biopress Ltd., Bristol, United Kingdom. 601 pp.

Smith, V. H. (1983). Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science, 221*(4611), 669-671.

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental pollution, 100*(1-3), 179-196. https://doi.org/10.1016/S0269-7491(99)00091-3

Sömek, H. & Balık, S. (2009). Karagöl'ün (Dağ Gölü, İzmir-Türkiye) Alg Florası ve Çevresel Koşullarının Mevsimsel Değişimi. *E.Ü. Su Ürünleri Dergisi, 26* (2) : 121-128.

Sömek,H., Balık, S. & Ustaoğlu M.R., 2005, Topçam Baraj Gölü (Çine-Aydın) Fitoplanktonu ve Mevsimsel Değişimleri. *S.D.Ü.Eğirdir Su Ürünleri Fakültesi Dergisi, 1*(1): 26-32.

Soylu, E. N., & Gonulol, A. (2006). Seasonal variation in the diversity, species richness and composition of the phytoplankton assemblages in a shallow lake. *Cryptogamie-Algologie*, *27*(1), 85-102.

Stoyneva, M. P. (2003). Steady-state phytoplankton assemblages in shallow Bulgarian wetlands. *Hydrobiologia*, *502*, 169-176.

SWQR (2016). Regulation on amending the surface water quality regulation; [Access Date 2017 July 03]. Access Address http://www.resmigazete.gov.tr/eskiler/2015/04/2015 0415-18.htm (in Turkish)

Taş, B., Candan, A. Y., Can, Ö., & Topkara, S. (2010). Ulugöl (Ordu)'ün bazı fiziko-kimyasal özellikleri. *Journal of FisheriesSciences. com, 4*(3), 254-263. https://doi.org/10.3153/jfscom.2010027

Tas, B. (2012). Diversity of phytoplankton and trophic status in the Gaga Lake, Turkey. *EES Technology Part A: Energy Sci Res, 30*(1), 33-44.

Vaitomaa, J. (2006) *The effects of environmental factors on biomass and microcystin production by the freshwater cyanobacterial genera Microcystis and Anabaena*. Edita , Helsinki, Finland, 56 p.

van Wijk, D., Janse, J. H., Wang, M., Kroeze, C., Mooij, W. M., & Janssen, A. B. (2024). How nutrient retention and TN: TP ratios depend on ecosystem state in thousands of Chinese lakes. *Science of The Total Environment*, 170690. https://doi.org/10.1016/j.scitotenv.2024.170690

Winslow, L. A., Read, J. S., Hansen, G. J., & Hanson, P. C. (2015). Small lakes show muted climate change signal in deepwater temperatures. *Geophysical Research Letters, 42*(2), 355-361. https://doi.org/10.1002/2014GL062325

Zhang, Y., & Prepas, E. E. (1996). Regulation of the dominance of planktonic diatoms and cyanobacteria in four eutrophic hardwater lakes by nutrients, water column stability, and temperature. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(3), 621-633.

SUSTAINABLE AQUATIC RESEARCH
www.saquares.com

Sustainable Aquatic Research (2024) 3(1):26-43
DOI: 10.5281/zenodo.11061456
e-ISSN: 2822-4140

RESEARCH PAPER

Integrated multitrophic aquaculture of sandfish (*Holothuria scabra*) with marine water acclimatized Nile tilapia (*Oreochromis niloticus*) and oyster (*Sacostrea cucullata*) for increased production in Kenya

Esther Wairimu Magondu[1,2]*, Jonathan Mbonge Munguti[3], Bernerd Mulwa Fulanda[1], Chrisestom Mwatete Mlewa[1]

[1]Department of biological sciences, Pwani University P.O. Box 195-80100, Kilifi, Kenya*
[2]Mariculture department, Kenya Marine and Fisheries Research Institute, Mombasa P.O. Box 81651-80100 Mombasa, Kenya
[3]Aquaculture department, Kenya Marine and Fisheries Research Institute, National Aquaculture Research Development and Training Center P.O. Box 451, Sagana, Kenya.

Citation

Magondu, E.W., Munguti, J.M., Fulanda, B.M., & Mlewa, C.M. (2024). Integrated multitrophic aquaculture of sandfish (*Holothuria scabra*) with marine water acclimatized Nile tilapia (*Oreochromis niloticus*) and oyster (*Sacostrea cucullata*) for increased production in Kenya. *Sustainable Aquatic Research, 3*(1), 26-43.

Article History

Received: 07 March 2024
Received in revised form: 22 April 2024
Accepted: 24 April 2024
Available online: 30 April 2024

Corresponding Author

Esther Wairimu Magondu
E-mail: estherwairimu82@gmail.com
Tel: +254 705 17 51 77

Keywords

IMTA
Monoculture
sea cucumber
oysters
Nile tilapia

Abstract

This study compared the growth performance, production, and quality of culture environment of integrated multitrophic aquaculture (IMTA) systems with different species combinations in earthen ponds. The experiment was designed to assess the viability of using marine water acclimatized Nile tilapia (*Oreochromis niloticus*) as the fed component of the IMTA system in combination with sea cucumbers (*Holothuria scabra)* and Oysters *(Sacostrea cucullata*) as extractive species. The control (C) experiment and a monoculture of *O. niloticus*. Treatment 1 (T1) had a combination of *O. niloticus* and *H. scabra*. Treatment 2 (T2) had a combination of *O. niloticus*, *H. scabra* and *S. cucullata* and Treatment 3 (T3) had a combination of *O. niloticus* and *S. cucullata*. Stocking was 2 ind./m^2, 1.9 ind./m^2 and 2.1 ind./m^2 for tilapia, sea cucumber and oysters respectively and replicated in all the treatments. During the 150 days culture period, *S. cucullata* attained weight gain of 26.55 ± 0.26 g and 23.74 ± 2.6 g in T2 and T3 respectively. Weight gain of *H. scabra* in T1 and T2 was 146.7 ± 6 g and 153.39 ± 2.04 g respectively. The final average body weight (ABW) of the fed species *O. niloticus* was significantly higher ($p < 0.05$) in T2 at 218.82 ± 1.55 which had all the three species combinations. The findings of the study show that the IMTA that had a combination of all three species outperformed those that had two species.

Introduction

Global fish consumption has been on the increase at an annual rate of 3.1 % which is nearly twice that of yearly world population growth of 1.6 % and more than all other animal protein food like meat and milk which increase at 2.1 % per year (FAO, 2020). This has been as a result of world aquaculture production which has constantly been on the increase reaching 46 % in the year 2018 up from 25.7 % in the year 2000 (FAO, 2020). However, the rapid expansion in aquaculture has raised several concerns mainly on environmental and biosecurity issues resulting from accumulation of nutrients and pathogens in aquatic ecosystems as a result of external inputs in form of fertilizers, feeds and effluents directed to open culture systems (Skriptsova and Miroshnikova, 2011; Dong et al., 2018). A possible remedy is in situ removal of organic and inorganic nutrients which could be attained through biological means by introduction of filter feeders and deposit feeders (Neori et al., 2004, Samocha et al., 2015, Irisarri et al., 2015). This approach comprises the concept of integrated multi trophic aquaculture (IMTA) (Neori et al., 2004). In IMTA systems the waste of the main cultured species (the fed component) is utilized by the introduced extractive species which depend on it for energy and growth while at the same time cleaning the culture environment (Chopin, 2013). In addition to supporting sustainable and resilient aquaculture, integration has resulted in benefits like higher productivity, improved resource–use efficiency, and reduced environmental impacts (FAO, 2020).

In Kenya, marine aquaculture has mainly been practiced as a single species (monoculture) venture in most of the farms with most farmers growing either shrimps (*Penaeus monondon* or *Penaeus indicus),* milkfish (*Chanos chanos*), mud crab (*Sylla seratta*) or recently Nile tilapia (*Oreochromis niloticus*) in marine environment (Mirera et al., 2022). There have also been trials of polyculture of shrimp and milkfish in intertidal ponds (Roonback et al., 2002; Mirera, 2011). The main difference between polyculture and IMTA is that whereas in polyculture the cultured organisms are both fed species sharing the same chemical and biological processes, in IMTA system the cultured organisms usually feed at different trophic levels provided by the system. The IMTA system thus, in addition, has gained of higher nutrient utilization efficiency and environmental mitigation as compared to polyculture system (Biswas et al., 2020).

Practice of IMTA has advantages of boosting production and achieving environmental sustainability, moreover the introduction of other species enhances diversification of culture species which is key towards resilience of marine aquaculture sector. Previous studies by Largo et al. (2016); Cunha et al. (2019) and Chang et al. (2020) have demonstrated viability of IMTA systems established within the intertidal zone and as well as those within sheltered bays. The studies used different species combinations of fed and extractive species. A successful IMTA system therefore requires identification of suitable combinations of species which will yield better returns on investment and mitigate some negative environmental impacts of aquaculture.

The benefits derived from integrated multitrophic aquaculture systems are both ecological and economic in nature (Chopin, 2021) and involves recovery and reuse of wastes through trophic interactions by growing valuable complementary species (Barrington et al., 2009). The farmer must therefore understand the ecological dynamics of the culture system and the organisms under culture for better performance of the enterprise. In Kenya, there has not been any studies on semi-intensive mariculture in earthen ponds where IMTA technologies have been applied. However, promising results were reported in the first IMTA trial in Kenya where shrimps (*P. indicus*) was the fed organisms with sea cucumber and cockles (*Anadara antiquate)* as extractive components (Magondu et al. (2021). The present study was designed to compare the performance of different species combinations; a herbivorous fish, a filter feeder, and a deposit feeder in earthen ponds in an IMTA setup. The overall objective was to evaluate the growth performance of three species: Nile tilapia (*Oreochromis niloticus),* Sea cucumber (*Holothuria scabra* and oysters (*Sacostrea cucullata)*; in different species combination in an IMTA experimental setup.

Materials and Methods

Experimental design and study organisms

The study was conducted over a period of 150 days using eight rectangular earthen ponds each measuring 12 x 10 m and 1m depth hired from Umoja self-help group located in Kilifi County, Kenya (Figure 1). The study employed an IMTA design with four treatments designated as C (control), T1, T2, and T3 with different species combinations. The first treatment (i) C (control) had a monoculture of Nile tilapia, (ii) T1 had a combination of Nile tilapia and Sea cucumbers (iii) T2 had a combination of Nile tilapia, Sea cucumbers, and oysters and (iv) T3 had a combination of Nile tilapia and oysters. Each treatment had two randomly assigned replicate ponds.

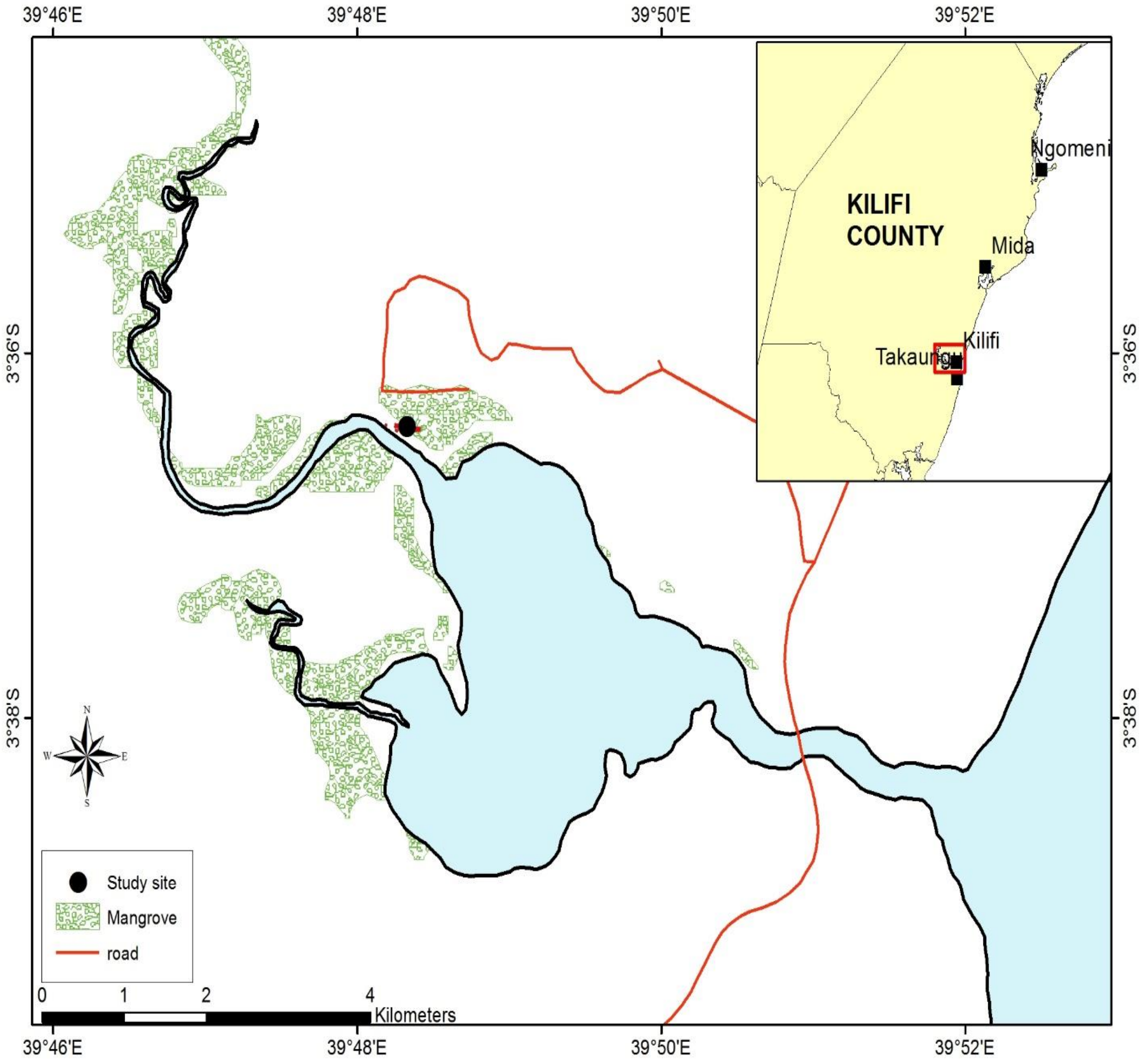


Figure 1. Site location for Umoja self-help group mariculture ponds in Kibokoni, Kilifi County, Kenya.

Preparation of experimental ponds

The experimental ponds were drained completely and sundried for a period of two weeks to eradicate predators and other living organisms. Sand was added to ponds in treatment T1 and T2 so as to make their bottom substrate allow easy burrowing by sea cucumbers as in their natural habitat environment. Lime ($CaCO_3$) was then applied to each pond bottom at 300 g/m^2 and the ponds allowed dry for 7 days. The ponds were then filled with water during high tide coming in through dug in channels from the creek. Screens were put at the inlet to prevent predators getting into the ponds. Three days after filling ponds with water, the ponds were fertilized using a combination of Urea and Diammonium Phosphate fertilizers at 3 g/m^2 and 2 g/m^2 respectively. The ponds were left for 7 days to allow growth of natural food organisms.

Collection and transportation of culture organisms

Sea cucumbers and oysters used in this study were collected from the wild while Nile tilapia were obtained from the hatchery facility at KMFRI, Mombasa. A total of 1000 juvenile sea cucumbers were collected from Vanga (4°39´S, 39°13´E) South Coast, Kenya. Collection was done by hired fishermen who hand-picked the organisms during low tide and placed them into inert polythene bags for transportation to a holding tank at KMFRI, Mombasa. A total of 1050 oyster spats settled on mangrove plant trunks were gathered by hired experienced oyster collectors during low tide from Kilifi creek (3°36´S, 39°50E) North Coast, Kenya. Collected oyster spats were placed in open basins filled with sea water for transportation to the culture baskets mounted in the experimental ponds. A total of 1950 juvenile Nile tilapia obtained from the hatchery at KMFRI Mombasa together with the acclimatized sea cucumbers were transported in separate containers to the experimental site for stocking into the culture ponds.

Stocking and management of ponds

Each pond was stocked with the culture organisms according to the stocking numbers as illustrated in Table 1 and according to the species combinations in the four treatments. Among the stocked species, Nile tilapia (*O. niloticus*) was the fed species, whereas oyster (*S. cucullata*) and sea cucumber *(H. scabra)* were the extractive species. Oysters were put in perforated plastic baskets used for shell fish culture and suspended in water column from horizontally fixed poles in respective experimental ponds following Higgins et al. (2011).

A low cost formulated feed of 30% crude protein was given as supplementary feed to the Nile tilapia which were the fed species in the IMTA setup for this study. Fish were fed at 5% body weight of the formulated feed twice daily at 9:00 AM and 3:00 PM by casting the feed over the pond surface to achieve even distribution. The quantity of feed was adjusted at 20 days interval based on the body weight of fish calculated from periodic sampling. An assumption of 90 % survival was made during the culture period, which might have occurred due to unavoidable mortality during sampling and undetected predation. Net screens were placed at the pond inlets and outlets and strings on top across the ponds to prevent bird predation. Desirable alkalinity levels, water quality, and primary productivity were maintained by monthly application of lime and inorganic fertilizer at 300 g/m^2 and 2 g/m^2 respectively. Pond water level was maintained at 1 m depth during high spring tide after compensating for evaporation and seepage.

Table 1. Number of individuals of the cultured species stocked in control (C) and IMTA treatments (T1, T2 and T3)

Species	C	T1	T2	T3
Oreochromis niloticus	240	240	240	240
Holothuria scabra		228	228	
Sacostrea cucullata			252	252

Sampling and measurement of cultured organisms

Determination of length and weight of cultured organisms was done during sampling which took place after every 20 days subsequent to the next high tide. A 20 % sample size of the stocking numbers of the different organisms was measured. The weight of 50 individuals of Nile tilapia from each replicate were measured using a digital scale (Aslor model) to the nearest 0.01g. Total length was measured to the nearest 0.01 cm on a ruler scale. A sample of 45 sea cucumbers from each replicate were handpicked, placed in small buckets to reduce shock and allow water come out of their body, where necessary paper towels were used to dry out excess water. The organisms were further measured for body length (to the nearest 0.01 cm) using a meter ruler and weighed (to the nearest 0.01 g) using a digital balance (Watanabe et al., 2014). Sampling of oysters involved hand picking of 50 pieces from each replicate pond and placing them in a basin with clean water. Shell length of the oyster was determined to the nearest 0.1 mm using a vernier caliper and later converted to cm during calculations. Individual weight of the oysters was determined on a digital weighing balance to the nearest 0.01 g (Comeau, 2013).

Determination of growth parameters such feed conversion ratio (FCR) and specific growth rate (SGR) were calculated from the data obtained. FCR is the ratio of the quantity of food distributed (g) to the weight gain of fish (g), over the culture period. This was used to judge the efficiency of feed utilization by fish for both diets. It was calculated by dividing the total amount of feed used (dry matter basis) by the weight gain. Specific growth rate (%body weight/day) was calculated using the formula, SGR = ($\ln WT_F$-$\ln WT_I$)*100/T where WT_F= average final fish weight (g), WT_I = average initial fish weight (g), T= duration of the experiment (days).

Harvesting of cultured organisms

At the end of the experiment all organisms were harvested; measured for total length and weighed. Each experimental pond was first drained to 0.25 m level by opening the outlets to allow the water out while retaining the screens to prevent the organisms from escaping. Nile tilapia in the control were first partially harvested using a drag net. A scoop net was then used to harvest the remaining fish. In the IMTA ponds, harvesting of the oysters was first conducted by emptying the oyster culture baskets and placing them in labeled basins with water, Sea cucumber were then harvested by handpicking from the pond bottom and placing them in separate labeled basins for later measurement and weighing. All harvested organisms were counted and recorded. Harvested organisms were sold at prevailing market rates for respective species and data recorded for economic analysis.

Determination of water quality parameters

Sampling for water quality parameters was done on monthly basis preceding the next high or low tide. The main parameters monitored were; salinity, water temperature, transparency, pH, dissolved oxygen, total dissolved solids which were done in-situ using a multi-parameter kit Hanna instruments model. Light penetration as a measure of water transparency from each sampling station was determined using a white Secchi disk. Water samples from each pond are collected for determination of Nitrite-nitrogen (NO_2-N), Nitrate-nitrogen (NO_3-N), Nitrogen-ammonia (NH_3-N) (TAN) and phosphate-phosphorous (PO_4-P) using a horizontal water sampler from three sampling points in each pond and preserving samples at 10:00hrs at each sampling day. In the laboratory, the water samples were filtered through microfiber glass filter paper (Whatman GF/C) using a vacuum pressure air pump (Shemer et al., 2017). The samples were then subjected to an auto analyser for nutrient analysis using the Continuous Flow Analysis technique following standard procedure outlined in APHA (2005).

Evaluation of economic performance

Data on expenditure and income from sale of harvested organisms were used in an economic analysis based on the developed IMTA system in comparison to the marine tilapia monoculture system. The economic performance of the treatments and their comparison were analyzed with estimation of gross returns, net income, and Cost Benefit Ratio (CBR) as shown below and according to (Biswas et al., 2012).

Net income = Total income – total expenditure

CBR was determined as CBR=Total income/total expenditure

Expenditure comprised of costs of inputs including the cost the different seeds (*O. niloticus*, *S. cucullata*, and *H. scabra*), feeds administered, labour, fertilizers, lime, netting material, and other operational costs like purchase of tools for pond maintenance, payment for security hire and pond attendant. The cost of production was estimated based on local market value price in the current US Dollar equivalence (1USD =105 Kenya currency). Produced crop of marine tilapia was sold at the local market outlet and in hotel, oysters were harvested for local consumptions at farm level while taking into consideration their market price while the sea cucumbers were sold to a local dealer for export. Total return from the crop produced was estimated by price of organisms sold. Gross margin was estimated by subtracting the total production cost from the total return. The costs did not include the initial investments but rather the routine farm operations. The outcome of the economic analysis was used to determine the viability of both systems.

Statistical analyses

Comparisons of the different IMTA treatments and the control were analyzed by calculating the mean and standard error of the mean of length and weights of replicated biomass using the descriptive statistics tool in Microsoft Excel 2013. Comparisons for water quality parameters among the four treatments was done by one-way ANOVA and repeated measures ANOVA respectively followed by post hoc Tukey HSD test (Zar, 1998). Data were checked for normality and homogeneity of variance using Shapiro-wilk's and Levene's tests respectively. Statistical analyses were done using Excel version 2013 and SPSS statistical software IBM version 22. In all statistical testing, differences were considered to be statistically significant at $p < 0.05$.

Results

Growth performance of the cultured organisms among the different treatments

The final average body weight (ABW) of the fed species (Nile tilapia) was significantly higher ($p < 0.05$) in T2 which had a full integration of both fed and extractive species. The other two IMTA treatments (TI and T3) showed higher final ABW of the fed species as compared to the control which had a mean of 160.75 ± 3.75 g (Table 2). There were no any significant differences among the treatment groups ($p > 0.05$). During the 150 days culture period, there was a weight gain of oyster of 26.55 ± 0.26 g and 23.74 ± 2.6 g in T2 and T3 respectively. Over the same period sea cucumber in T1 and T2 had respective weight gain of 146.7 ± 6 g and 153.39 ± 2.04 g. Results in the graphical plot indicate that harvesting weight gain of the fed species (Nile tilapia) was highest in T2 and lowest in the control (Figure 2).

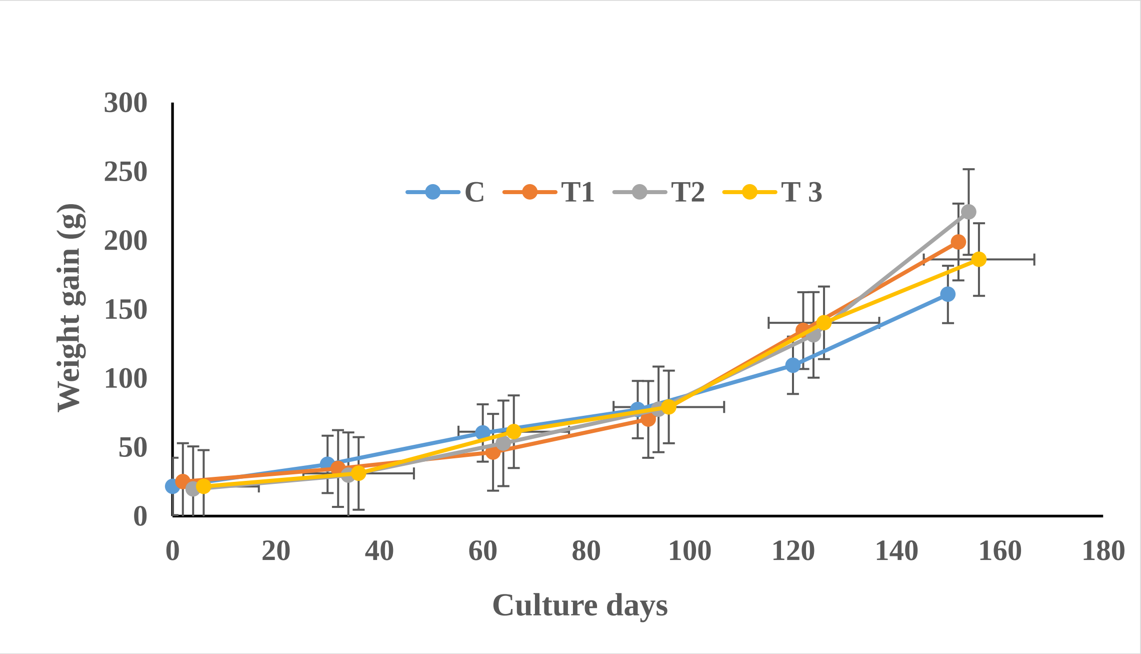


Figure 2. Weight gain in (g) of the fed species (*O. niloticus*) in the four treatments. Values are means (± SEM) of pooled data of replicates in each pond per sampling.

The highest survival was recorded among the Nile tilapia in T2 at 92.9 % compared to those in other treatments which ranged from 79.16 % to 85.40 % in T3 and the control respectively. Survival of oyster was 87.5 % and 79.2 % for the T2 and T3 treatments respectively. Lower survival was recorded for sea cucumber at 53.2 % and 45.6 % for T1 and T2 treatments respectively. Food conversion ratio determined for the fed species, Nile tilapia, was lowest in T2 at 1.78 ± 0.04 and highest in T3 at 2.32 ± 0.3. Mean food conversion ratio was significantly different ($t = -13.67$, $p = 0.046$) for Nile tilapia in the control but not for those in the other treatments.

Results in Table 2 show that the growth of experimental organisms was highest in IMTA treatment T2 which had a combination of Nile tilapia, sea cucumber, and oysters. The fed component, *O. niloticus* in T2 had a weight gain of 182.11 ± 16.6 g, which was followed by T1 that had 173.85 ± 2.05 g and then T3 that had 164.62 ± 6.9 g. The fed species (*O. niloticus*) in the control had the lowest mean weight gain of 136.5 ± 2.8 g. Among the extractive organisms; *H. scabra* in T2 attained higher mean weight gain of 153.39 ± 2.04 g than those in T1 which had 146.7 ± 6 g. *S. cucullata* in T3 had slightly higher weight gain of 26.55 ± 0.26 g as compared to T2 that had a weight gain of 23.74 ± 2.6 g. The daily growth rate was high for *H. scabra* and *O. niloticus* in T2 as compared to those in T1 and control. Specific growth rate (SGR) was lowest for oysters, 4.1 % in both T2 and T3. Figure 3 shows the growth of the organisms in treatment 2 from stocking to harvesting that had full IMTA combination. *O. niloticus* and *H. scabra* performed better as compared to *S. cucullata.*

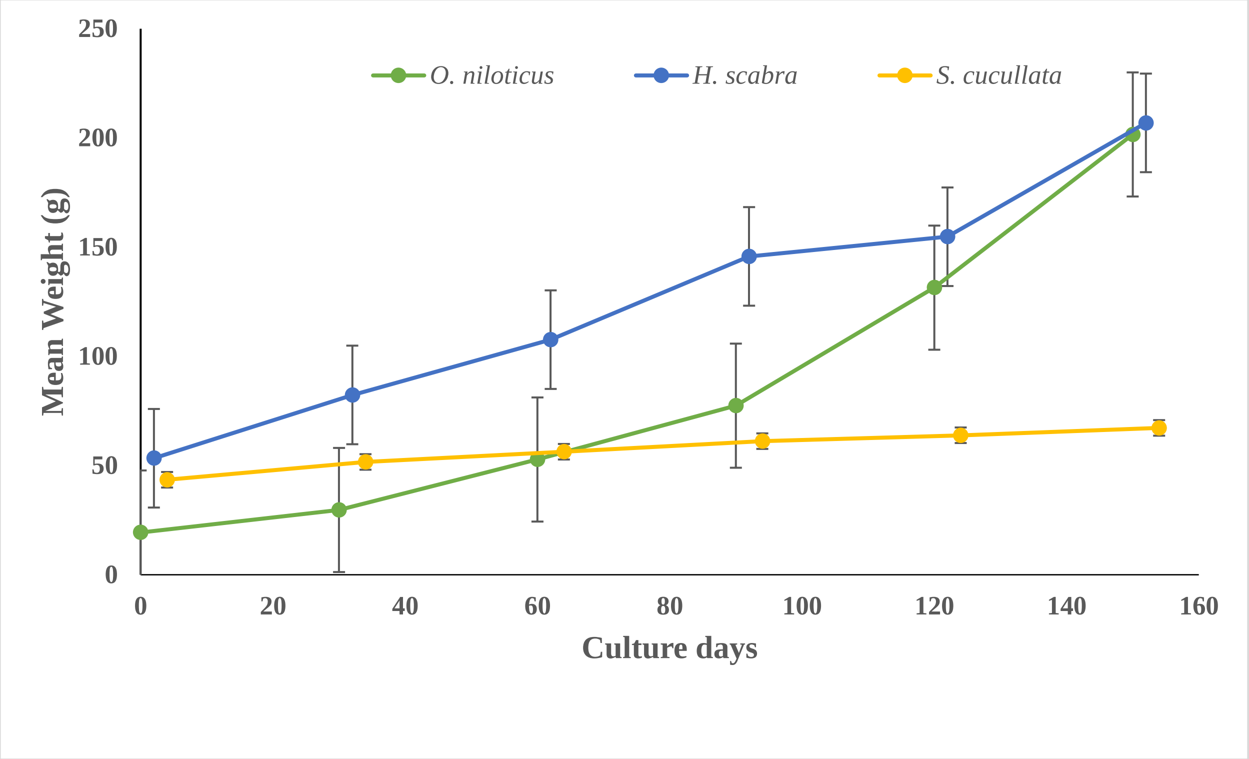


Figure 3. Growth of organisms in full IMTA treatment (T2) from stocking to harvesting.

Water quality parameters

Among the physico-chemical parameters of culture water from the experimental ponds, transparency differed significantly ($p < 0.05$) between treatments being higher (32.72 ± 0.25) in T2 than in the control that had a transparency of 24.84 ± 0.2 (Table 3). Over the 150 days study period, water temperature in the culture facilities varied between 21.4 and 32.6 °C, while salinity ranged from 28.5 to 40.5 ppt throughout the experimental period.

Dissolved oxygen levels were within suitable ranges for culture and was significantly higher ($p < 0.05$) in the IMTA treatments as compared to the control. T2 had the highest dissolved oxygen at 3.901 ± 0.01 mg/l^{-1} whereas the control had the lowest at 3.25 ± 0.01 mg/l^{-1}. The pH values did not vary much between the treatments, ranging from 7.6 in T2 to 8.0 in T3. Both T1 and the control had a pH 7.9. The lowest value of TAN (ammonia nitrogen) was observed in T2 0.024 ± 0.007 mg/l^{-1} which was significantly different ($p < 0.05$) from that of T3 0.076 ± 0.007 mg/l^{-1} but not from T1 and control ($p > 0.05$). The Nitrate–nitrogen differed significantly ($p < 0.05$) being lower in the IMTA treatment T2 (0.025 ± 0.007) mg/l^{-1} which had the three species than in the control (0.057 ± 0.007 mg/l^{-1}) that had tilapia monoculture. Mean value of nitrite- nitrogen was lowest in T2 (0.023 ± 0.004 mg/l^{-1}). There were no any significant differences on phosphate phosphorous concentrations among the treatments.

Economic performance evaluation

Results of the economic analysis showed that, net income, and CBR were significantly better ($p < 0.05$) in T2, T1, and C as shown in Table 4. T1 and T2 had similar CBR which was higher than the control ($p < 0.05$). Net income was highest in the IMTA T2 with a value of USD 77.5 followed by IMTA T1 that had a value of USD 33.9. Higher production and better selling price of the harvested organisms in T2 and T1 contributed to higher profit in IMTA system.

Table 2. Results of analysis of growth parameters of *O. niloticus*, *H. scabra* and *S. cucullata* in the control (C) and the IMTA treatments (T1, T2, and T3).

Treatments	**C**	**T1**		**T2**			**T3**	
Experimental organisms	***O.niloticus***	***O.niloticus***	***H.scabra***	***O.niloticus***	***H.scabra***	***S.cucullata***	***O.niloticus***	***S.cucullata***
Growth and yield parameters								
Stocking density (Ind/m2)	2	2	1.9	2	1.9	2.1	2	2.1
Total Stock (n)	240	240	228	240	228	252	240	252
Initial ABW (g)	21.5 ± 1	25±0.5	53.5 ± 2.5	19.5 ± 1	53.5 ± 1	43.5 ± 1.5	21.5 ± 1	41.5 ± 1
Final ABW (g)	160.8 ± 3.8	198.85 ± 2.5	199.7 ± 3.5	218.81 ± 1.54	206.86 ± 3.04	68.4 ± 0.07	186.12 ±7.9	65.96 ±0.75
Initial stocking length (cm)	13.97 ± 1.5	12.66 ± 0.85	14.41 ± 1.66	13.08 ± 0.63	7.93 ± 0.42	8.94 ± 0.11	8.26 ± 0.41	7.69 ± 0.15
Final stocking Length (cm)	21.07 ± 0.48	20.35 ± 0.14	21.22 ± 1.01	22.19 ± 0.72	15.44 ± 0.78	14.55 ± 0.13	14.32 ± 0.35	14.07 ± 0.11
Individual net weight gain (g)	136.5 ± 2.8	173.85 ± 2.05	146.7 ± 6	182.11 ± 16.6	153.39 ± 2.04	26.55 ± 0.26	164.62 ± 6.9	23.74 ± 2.6
AFCR	2.09 ± 0.3	1.97 ± 0.09		1.78 ± 0.04			2.32 ± 0.3	
Survival % day	85.4	81.25	53.26	92.91	45.6	87.5	79.16	79.2
Daily growth rate (g day-1)	0.91 ± 0.18	1.15 ± 0.01	0.97 ± 0.04	1.21 ± 0.11	1.02 ± 0.01	0.15 ± 0.01	1.09 ± 0.05	0.17 ±0.001
Specific growth rate (% day-1)	5.04 ± 0.23	5.28 ± 0.012	5.28 ±0.017	5.29 ± 0.78	5.31 ± 0.01	4.17 ±0.02	5.21 ± 0.043	4.18 ±0.011
Production (Kg/treatment 150 days-1)	33.78 ± 1.6	38.80 ±2.4	24.47 ± 0.9	45.03 ±4.5	21.75 ± 2.3	14.63 ± 1.08	35.40 ± 2.4	13.44 ± 0.32

Table 3. Mean (±SEM) values of various water quality parameters of pond water collected at 30 day interval from the monoculture (C) and IMTA treatment ponds (T1, T2, and T3).

Water quality parameters

Parameter	**Treatments**				**Sampling time**				
	C	**T1**	**T2**	**T3**	**2/20/2021**	**3/26/2021**	**4/25/2021**	**5/24/2021**	**6/25/2021**
Dissolved oxygen (mgl^{-1})	3.25± 0.011	3.32 ± 0.01^{c}	3.901 ± 0.01^{a}	3.51± 0.01^{b}	3.53 ± 0.01	3.26 ± 0.25^{a}	3.32 ± 0.11	3.59 ± 0.067	3.38 ± 0.06
Temperature (°C)	29.37 ± 0.309	28.28 ± 0.3	29.22 ± 0.31	28.91 ± 0.31	27.32 ± 0.27^{c}	29.22 ± 0.16^{b}	32.15 ± 0.56^{a}	32.62 ± 0.23^{a}	21.39 ± 0.34^{d}
Turbidity (NTU)	28.2 ± 1.7^{a}	28.4 ± 1.78^{a}	22.3 ±1.7^{b}	17.9 ± 1.78^{c}	20.11 ± 1.14	20.55 ± 1.21	21.91 ± 0.58	22.73 ± 0.76^{b}	23.23 ± 0.95 ^{a}
Salinity (ppt)	36.95 ± 0.3	35.81 ± 0.31	36.57 ± 0.32	34.81 ± 0.3	36.53 ± 0.55	40.47 ± 0.49^{a}	37.86 ± 0.51	36.8 ± 0.27	28.51 ± 0.45 ^{b}
pH	7.9 ± 0.12	7.93 ± 0.1	7.76 ± 0.13	8.06 ± 0.12	8.02 ± 0.26^{a}	7.6 ± 0.034^{b}	7.64 ±0.16^{b}	8.2 ± 0.11^{a}	8.1 ± 0.75^{a}
Transparency	24.84 ± 0.2	25.34± 0.25^{b}	32.71 ± 0.25^{a}	30.56 ± 0.2	22.35 ± 0.39	29.78 ± 0.79^{a}	25.49 ± 0.58	25.49 ± 0.58	25.2 ± 0.36
Phosphate phosphorous (mgl^{-1})	0.157 ± 0.04	0.142±0.047	0.053 ± 0.05	0.057 ± 0.04	0.076 ± 0.005	0.277 ± 0.15	0.057± 0.016	0.056± 0.013	0.045± 0.013
Nitrate-nitrogen (mgl^{-1})	0.057± .007^{a}	0.052±0.007	0.025 ± 0.007^{b}	0.048± 0.007	0.059± 0.005^{a}	0.051 ± 0.003	0.042± 0.004	0.045 ± 0.01	0.031±0.005^{b}
Ammonia nitrogen (mgl^{-1})	0.067 ± 0.007	0.043±0.007	0.024 ± 0.007^{b}	0.076± .007^{a}	0.052 ± 0.002	0.06 ± 0.005	0.053± 0.004	0.048± 0.004	0.05 ± 0.007
Nitrite nitogen (mgl^{-1})	0.041 ± 0.004	0.036± .004	0.023 ± 0.004^{a}	0.034± 0.004	0.029±0.006^{b}	0.028 ± 0.004	0.037±0.003^{a}	0.041± 0.003	0.033± 0.002

Means with different superscripts in a row differ significantly ($p < 0.05$).

Table 4. Operational costs and economic returns among monoculture (C) and IMTA treatments (T1, T2, and T3). Calculations were for 150 days experimental duration. Currency indicated in USD (1USD=Ksh 105). (TI is Total Income, TE is Total Expenditure, and NI is Net Income)

Items	Quantity	Price Rate (USD)	C	T1	T2	T3
Operational costs						
Oreochromis niloticus seed	240	0.19	45.7	45.7	45.7	45.7
Holothuria scabra seed	228	0.28		63.84	63.84	
Sacostrea cucullata seed	252	0.14			35.28	35.28
Lime (CaCO3) (kg)	120	0.21	25.1	25.1	25.1	25.1
Fertilizer (kg)	DAP 4.8	0.62	3.01	3	3	3
	UREA 7.2	0.51	3.7	3.7	3.7	3.7
Feeds (kg)	117.5, 88.8, 106, 107	1	118	88.8	106	107
Pond hire	2	28.5	57	57	57	57
Casual labour	5	19.04	95	95	95	95
Total operational Cost			347.5	382.14	434.7	372
Economic returns						
O. niloticus advanced sale (fingerling)	300,220,180,200	0.19	57	42	34.2	38
Sale of harvested *O. niloticus* (kg)	34,38.8,45,35.4	2.4	81	93	108	84.96
H. scabra sale (kg)	24.4, 21.7	19.04		465	413.16	
S. cucullata sale	13.4,14.6	3.8			50.92	55.6
Gross return			138	600	606.28	177
Net income (TI-TE)			-209	217.8^{b}	171.3^{a}	-193
Cost Benefit Ratio (CBR) TI/TE			0.4^{b}	1.57^{a}	1.39^{a}	0.47
Return on Investment (%)	NI/TE*100		-60.2	56.7	39.4	-52.1

Means with different superscripts in a row differ significantly ($p < 0.05$).

Treatment T3 and the control had the lowest income which did not breakeven with USD values of (193) and (209) respectively. The calculated CBR was 1 for both IMTA T1 and T2, a zero value for T3 and 0.4 for the control. The determined return on investment was highest in T2 at 17.6 % while IMTA T1 had 8.6 %. IMTA T3 and the control showed negative rates.

Discussion

Growth performance of the cultured organisms

The comparison of IMTA system and the control made in this study tested the feasibility of culturing fin fish, oysters, and sea cucumbers together in an intertidal earthen pond ecosystem. The results showed that organisms cultured in the IMTA set up realized better growth and production than those in the control monoculture system. Individual weight gain and total production differed among cultured species in the different treatments which concurs with studies by Cunha et al, (2019). Nile tilapia which was the fed component of the IMTA set up in the study showed good performance during the 5 months culture period compared to the co-cultured extractive species; sea cucumbers and oysters.

The low production of sea cucumber observed among the different treatments could be attributed to predation from the Nile tilapia, the fed component in the IMTA set up. Sea cucumbers are deposit feeders that consume mud particles (Tresnati et al., 2019), a feeding habit that helps reduce the organic load from the bottom of earthen ponds while being integrated with other organisms (Funge-Smith and Briggs, 1998). This attribute, coupled with the species high market value makes sea cucumber a good candidate for multitrophic aquaculture systems (Magondu et al., 2021).

In the present study, the fed component of the IMTA T2 showed significantly ($p > 0.05$) high individual body weight of Nile tilapia at 218.82 ± 1.6 g, which was followed by T1 and T3 that had an integration of tilapia and sea cucumber at 198.9 ± 2.6 g and tilapia and oysters at 186 ± 7.9 g respectively. Nile tilapia in the control achieved relatively lower individual weight of 160.75 ± 3.8 g. The better growth of Nile tilapia in IMTA could be attributed to efficient removal of nutrient wastes from the culture environment as nutrient waste, fecal waste and uneaten feeds were extracted from the system and utilized by the extractive organisms such as filter feeders and deposit feeders (Samocha et al., 2015).

The specific growth rate of cultured organism ranged from 4.18 % day^{-1} among oysters to 5.3 % day^{-1} among sea cucumbers. Given the water quality parameters were within optimal range, these findings indicate the conditions for growth and species combinations tested the three IMTA treatments in this trial were favorable. In aquaculture, feed conversion ratio (FCR) is used to determine efficiency of utilization of fed feed that is converted to flesh. Nile tilapia in IMTA treatment T2 in this study had an FCR of 1.78 ± 0.04 compared to T1, T3 and the control which had slightly higher levels Table 2. This might have been as a result of better feed utilization by the cultured organisms. Waite et al. (2014) showed that FCR is related to environmental performance as it provides indications of nutrients loss to the environment and any undesirable output. A lower FCR shows an efficient system (Hasan and Soto, 2017). A similar study reported that ‘species combination in an IMTA reduced FCR by 12-15% compared to monoculture’ (Shpigel et al., 2016).

Bivalves have been used in IMTA systems based on their potential to directly filter organic particulates from the culture site and also nutrient extraction from the system (Chopin, 2013). Being suspension feeders, bivalves alter the plankton structure which promotes nutrient cycling in the culture environment (Smyth et al., 2013; Murphy et al., 2016). In this study oysters were used as filter feeders and contributed to increased biomass and efficiency of the system. In the current study, integration of oysters was found to be feasible in the co-culture IMTA pond system with Nile tilapia through improving water quality and increasing production in tilapia and sea cucumbers integration. Several studies have shown that extractive species in IMTA systems grow faster than in monoculture which implies that IMTA farms can provide greater economic gains and environmental benefits (Whitmarsh, et al., 2006; Sara et al., 2009; Sanderson et al., 2012).

Survival of culture organisms in different treatments

IMTA treatment (T2) achieved the highest survival for the fed species at 92.2%. Results of water quality and the culture environment in T2 were suitable for culture of the different organisms. Integration led to pond ecological balance that ensured high survival of tilapia as the fed organisms and sufficient nutrients that were captured by the oyster filter feeders which also showed high survival rates of 87.5 % and 79.2 % in T2 and T3 respectively Table 2. Sea cucumber was used as one of the extractive organisms due to its bio-mitigation ability and potential to reduce excess accumulation of waste from the pond bottom (Purcell, 2015; Cubillo et al., 2016). In this study survival of sea cucumber was lowest in treatment T2 that had 45.6 % and T1 that had 53.26 %. Some sea cucumbers were observed with injuries which were likely caused by predatory attacks by Nile tilapia, and it is possible that severe wounds may as well explain the higher overall mortality of this species reported in this study. Incidences of predator attack on *H. scabra* has earlier been reported in studies by Lavitra et al. (2009) and Tresnati et al. (2019). In sea cucumber farming, challenges related to biotic and abiotic parameters are normally experienced (Purcell, 2004; Wang et al., 2004). These include; salinity drops against the optimum of 28 to 31 ppt, Xilin (2004) that occurs in seasons of heavy rainfall (Chen, 2004) and especially during low tides a situation that makes the organisms to burrow underground even when they are supposed to be active and foraging for food. Abnormal occurrence of high numbers of parasites such as Isopods in ponds during hot season has been a cause for sea cucumber mortality (Pitt and Duy, 2004). In addition, abundance of crabs near the culture environments has been blamed for attack on both juveniles and adult sea cucumbers and cause of high mortality (Magondu et al., 2021). Skin ulcerations were also evident which were followed by mucus secretions on the body, skin discoloration and behavior changes as reported by Purcell and Eeckhaut (2005).

The observed mortality of sea cucumber in T1 and T2 could be attributed to the presence of Nile tilapia. These attacks were more serious during foraging for feeds from the pond environment. Nile Tilapia has been categorized as herbivorous fishes (Getachew and Fernando, 1989), which mainly feed on algae, while other workers have classified them as omnivores with food composition dominated by zooplanktons like copepods and rotifers (Bwanika et al., 2004). Such feeding habits may also have contributed to the observed attacks on sea cucumber as the tilapia forage for food at the pond bottom where the sea cucumbers dwell. Dorsal and ventral injuries and skin ulcerations were observed on most injured sea cucumbers. `Other possible predators were mud crabs and mangrove snails which were found to be present in some of the experimental ponds concurring with findings by Tresnati et al. (2019). However, these were removed physically during the pond preparation before stocking and by use of lime to kill any predator eggs and larvae present. Another plausible explanation for the low number of sea cucumber harvested is that some individuals burrowed into the substrate to avoid predator attack by tilapia (Pitt and Duy, 2005). Such burrowing caused substrate disturbance (bioturbation) leading to better nutrient recycling and utilization benefiting the overall culture environment.

Water quality

The water quality parameters in this study showed better results in the IMTA system as compared to the control monoculture. The parameters were stable throughout the experimental period and within acceptable range of finfish mariculture (Biswas et al., 2019). The dissolved oxygen levels shown in the three IMTA treatments could have been attributed to the species combinations that filtered the culture water by consuming the algae available making the culture water less turbid. Presence of fish, oysters, sea cucumber, and phytoplankton contributed to pond ecological balance due to the synergies created. The enhanced water quality led to better fish performance and higher biomass production. Further, less amount of energy was needed to maintain pond oxygen levels which improved the system efficiency. The photosynthetic activity within the systems was dependent on the type of treatment whereby ponds without oysters (T1) and (C) showed significantly lower dissolved oxygen production in comparison with the treatments that had oysters (T2 and T3). The treatments without oysters had lower Secchi disk readings which implied higher turbidity due to suspended particulate matter in the water column which prevented light penetration that hampered photosynthetic activity and consequently feed accessibility. In other studies, poor water quality has often been blamed for lack of sustainability and profitability in aquaculture enterprises (Biao, 2007; Cao et al., 2007). Studies by Newell (1988) showed that oysters filter water at a rate of $0.12m^3g^{-1}$ dry weight per day which has shown potential to extract large amounts of organic particles from the culture water. There were lower nutrient levels in terms of nitrates, nitrites and ammonia in the treatments that had sea cucumber as they prevented formation of anaerobic conditions by consuming high amounts of organic matter in the sediment that caused reduction in ammonia levels hence accelerating nitrification and denitrification processes which improved water quality in the ponds (Uthicke, 2001; İşgören-Emiroğlu and Günay, 2007).

Economic performance

The current study found that IMTA had a higher net income, cost benefit ratio and return on investment than either of the monoculture operations. Despite these encouraging results, IMTA is likely to be justifiable for investors only if there is additional profitability (Whitmarsh *et al.*, 2006; Ridler and Ridler, 2011). Whitmarsh *et al.* (2006), Ridler *et al. (*2007), and Shi *et al.* (2013) all suggested that higher profitability is possible with IMTA than with monoculture aquaculture farms, ascribing it to higher growth rates of co-cultured extractive IMTA species, the ability to spread some of the IMTA's administrative and operational expenses over a wider range of products (e.g. marketing and sales costs, salaries and wages, utilities), and access to additional income streams.

Clearly, economic sustainability is a key objective to be considered before venturing into commercial aquaculture practices. Hishamunda *et al*. (2014) showed that without economic viability, aquaculture ventures can only continue if subsidized. Higher profitability in IMTA farms has contributed to its more preference to monoculture practices by farmers attributing it to higher growth rates of the co-cultured extractive species, possibilities of spreading costs over a wide range of products in addition to access to different income streams (Whitmarsh *et al*., 2006; Ridler *et al*., 2007b; Shi *et al*., 2013). Presence of co-cultured items in an aquaculture enterprise can contribute to water quality improvement, additional productivity, and profitability of the farm operation (Fantini-Hoag *et al*., 2022).

A comparative economic performance analysis between three IMTA treatments (TI, T2, T3) and a control monoculture (C) made in this study indicated the possibilities of farming a fin fish, oysters and sea cucumber together in brackish water intertidal ponds. One complete (T2) and two partial IMTA treatments (T1 and T3) were tested in this study in comparison to fin fish monoculture. The full IMTA (T2) performed better in terms of production and economic returns as compared to the other treatments. Gross returns, net income, and cost benefit ratio (CBR) showed significant differences with highest values in T2 followed by T1. This was as a result of increased total production and higher selling price of harvested fin fish and sea cucumbers that had better weight gain in T2. In addition, the estimated market price of oysters increased the net income further for T3. The results of economic analysis show the viability of the IMTA system as a suitable farming option. Under the two scenarios, the analysis revealed that the IMTA system was more profitable, looking at the benefits of product diversification, reduction of production risk and reduced cost of inputs. Review studies by Knowler *et al*. (2020) showed that economics of IMTA has been mainly focusing on financial analysis on profitability, economics of environmental externalities and market analysis on consumer perceptions and acceptability of IMTA systems and products.

Conclusions

The IMTA set up involves maintaining a clean culture environment at reduced costs of inputs such as feed and fertilizers to facilitate improved efficiency while supporting sustainable production. Growth performance of Nile tilapia (the fed species) was better in the IMTA treatments than in the monoculture (control). The co-cultured species sea cucumber and oysters also exhibited good growth in the IMTA ponds. In the present study, Nile tilapia as the fed component of the system contributed to the overall production of the system in addition to providing food and energy that the co-cultured organisms depended upon. The production costs, net incomes, CBR and ROI were higher in IMTA setups. This indicated IMTA production system is a profitable business. The species combination and pond environment were important factors for increasing production suggesting more attention should be paid to these parameters for more economic gains. Sea cucumbers experienced predation attacks from the tilapia leading to their low survival rates. This finding thus recommends further analysis of the Nile tilapia stomach contents. Therefore, the findings of this study revealed that use of Nile tilapia as fed species in an IMTA system with sea cucumbers as an extractive component is not viable due to predation attacks. Possibilities of using other fed species for IMTA studies need to be explored and provision of appropriate substrates that the sea cucumbers can burrow in to escape predation.

Acknowledgments

The authors are grateful to the field staff of Umoja self-help group and technicians of Kenya Marine and Fisheries Research Institute Mombasa for their kind help during collection of samples and laboratory analysis and overall contribution to this research. This work contributed to the award of the degree of Doctor of Philosophy in Fisheries of Pwani University by the first author.

Ethical approval

The author declares that this study complies with research and publication ethics

Informed consent

Not available

Conflicts of interest

There is no conflict of interests for publishing their study

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Funding organizations

This research was financially supported by the Kenya Climate Smart Agriculture Productivity Project (KCSAP) under the Student Research Grant Award Number: KCSAP/2019/064

Author contribution

The experiment was conceived by Author 1 - as part of the requirements for a PhD in Fisheries degree of Pwani University.

Author 1: Writing original draft, Conceptualization, Data curation, Formal analysis, sample preparations, chemical analyses, data analysis and drafted the manuscript.

Author 2 Supervision, Validation, Visualization, Project administration, Review, Editing and approved the final version of the manuscript for submission.

Author 3 Supervision, Visualization, Review, Editing, and approved the final version of the manuscript for submission.

Author 4 Supervision, Validation, Visualization, Project administration, Review, Editing critical examination, and approved the final version of the manuscript for submission.

References

APHA. (2005). Standard Methods for the Examination of water and wastewater, 21st ed. *American Public Health Association*, Washington, D.C., USA.

Barrington, K., Chopin, T., & Robinson, S. (2009). Integrated multi-trophic aquaculture (IMTA) in marine temperature waters. In: Integrated mariculture: a global review (ed. Soto, D.) FAO Fisheries and Aquaculture Technical Paper, 529: 7-46.

Biswas, G., Kumar, P., Ghoshal, T.K., Lailasam, M., De, D., Bera, A., Mandal, B., Sukumaran, K., & Vijayan, K.K. (2020). Integrated Multi-trophic aquaculture (IMTA) outperforms conventional polyculture with respect to environmental remediation, productivity, and economic return in brackish water ponds. *Aquaculture* 516, 734626. https://doi.org/10.1016/j.aquaculture.2019.734626

Biswas, G., Kumar, P., Kailasam, K., Ghoshal, T.K., Bera, A., & Vijayan, K.K. (2019). Application of integrated multi-trophic aquaculture (IMTA) concept in brackish water eco-system: the first exploratory trial in the Sundarban, India. J. Coast. Res., Special Issue 86, 50–56. https://doi.org/10.2112/SI86-007.1

Biswas, G., Ananda Raja, R., De, D., Sundaray, J.K, Ghoshal, T.K., Anand, S., Kumar, S., Panigrahi, A., Thirunavukkarasu, A.R., & Ponniah, A.G. (2012). Evaluation of productions and economic returns from two brackish water polyculture systems in tide-fed ponds. *Journal of Applied Ichthyology*, 28 (1), 116-122. https://doi.org/10.1111/j.1439-0426.2011.01909.x

Bwanika, G., Makanga, B., Kizito, Y., Chapman, L.J., & Balirwa, J. (2004). Observations on the biology of Nile tilapia, *Oreochromis niloticus* L., in two Ugandan crater lakes. *African Journal of Ecology*. 42: p. 93-101. https://doi.org/10.1111/j.1365-2028.2004.00468.x

Biao, X., & Kaijin, Y. (2007). Shrimp farming in China: operating characteristics, environmental impact, and perspectives. *Ocean and Coastal Management* 50: 538–550. https://doi.org/10.1016/j.ocecoaman.2007.02.006

Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., & Xiong, S. et al. (2007). Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Environmental Science and Pollution Research* 14: 452–462. https://doi.org/10.1065/espr2007.05.426

Chang, Z., Neori, A., He, Y., Li, J., Qiao, L., Pretson, S.I, Liu, P., & Li, J. (2020). Development and current state of seawater shrimp farming with emphasis on Integrated Multitrophic pond aquaculture farms, in China-a review. *Reviews in*

Aquaculture, 1-15. https://doi.org 10.1111/raq.12457.

Chen J. (2004). Present status and prospects of sea cucumber industry in China. p. 25–38. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: *Food and Agriculture Organization of the United Nations*.

Chopin, T. (2013). Aquaculture, Integrated Multi-trophic Aquaculture (IMTA). http://www.springerreference.com/index/chapterdbid/226358©*Springer-Verlag* Berlin Heidelberg 2013.

Cunha, M.E., Quental-Ferreira, H., Parejo, A., Gamito, S., Ribeiro, L., Moreira, M., Monteiro, I., Soares, F., & Pousao-Ferreira, P. (2019). Understanding the individual role of fish, oyster, phytoplankton, and macro algae in the ecology of integrated production in earthen ponds. *Aquaculture* 512 (2019) 734297. https://doi.org/10.1016/j.aquaculture.2019.734297

Cubillo, A.M., Ferreira, J.G., Robinson, S.M.C., Pearce, C.M., Corner, R.A., & Johansen, J. (2016). Role of deposit feeders in integrated multi-trophic aquaculture — a model analysis. *Aquaculture* 453, 54–66. https://doi.org/10.1016/j.aquaculture.2015.11.031

Comeau, L.A. (2013). Suspended versus bottom oyster culture in eastern Canada: comparing stocking densities and clearance rates. *Aquaculture.* 2013:410–411. 57–65. https://doi.org/10.1016/j.aquaculture.2013.06.017

Dong, X., Linlan, L.V., Zhao, W., Yu, Y., & Liu, Q. (2018). Optimization of integrated multi-trophic aquaculture systems for the giant freshwater prawn *Macrobrachium rosenbergii Aquaculture Environmental Interaction* 10: 547-556. https://doi.org/10.3354/aei00287

FAO. (2020). The State of World Fisheries and Aquaculture (2020). Sustainability in action. Rome. *FAO* https://doi.org/10.4060/ca9229en.

Fantini-Hong, L., Hanson, T., Kubitza, F., Povh, J. A., Alberto, R., Filho, C.C., & Chappell, J. (2022). Growth performance and economic analysis of hybrid Catfish (Channel Catfish *Ictalurus punctatus* ♀ × Blue Catfish, *I. furcatus* ♂) and Channel Catfish (*I. punctatus*) produced in floating In-Pond Raceway System. *Aquaculture Reports,* Vol 23, 101065. https://doi.org/10.1016/j.aqrep.2022.101065

Funge-Smith, S.J. & Briggs, M. R. (1998). Nutrient budgets in intensive shrimp ponds: Implications for sustainability. *Aquaculture.* 164 (1-4): p. 117-133. http://dx.doi.org/10.1016/S0044-8486(98)00181-1

Getachew, T. & Fernando, C. (1989). The food habits of an herbivorous fish (*Oreochromis niloticus* Linn.) in Lake Awasa, Ethiopia. *Hydrobiologia*. 174(3): p. 195-200.

Hasan, M.R., & Soto, S. (2017) Improving Feed Conversion Ratio and its Impact on Reducing

Greenhouse Gas Emissions in Aquaculture. FAO Non-Serial Publication, Rome, *FAO,* pp. 33. http://www.fao.org/3/a-i7688e

Higgins, C.B., Stephenson, K., & Brown, B.L. (2011). Nutrient bio-assimilation capacity of aqua cultured oysters: Quantification pf an Ecosystem Service. *Journal of Environmental quality volume* 40: 271-277 https://doi.org10.2134/jeq2010.0203.

Hishamunda, N., Ridler, N., & Martone. E. (2014). Policy and governance in aquaculture: Lessons learned and way forward. *FAO* Fisheries and Aquaculture Technical Paper No. Hickey, J.P. (2009). Carbon sequestration potential of shellfish. Available from: URL. www.thefishsite.com/articles/615/carbon-sequestationpotential-of-shellfish. Accessed 09 August 2019. 577, 68. *Rome: FAO*. www.fao.org/3/a-i3156e.pdf.

İşgören-Emiroğlu, D., & Günay, D. (2007). The effect of sea cucumber Holothuria tubulosa (G., 1788) on nutrient and organic matter contents of bottom sediment of oligotrophic and hypereutrophic shores. *Fresenius Environmental Bulletin, 16*(3), 290-294. https:/doi pjbs.2007.586.589

Knowler, D., Chopin, T., Martinez-Espineira, R., Neori, A., Nobre, A., Noce, A., & Reid, G. (2020). The economics of Integrated Multitrophic Aquaculture: where are we now and where do we need to go? *Reviews in Aquaculture* 12, 1579-1594 doi: 10.1111/raq.12399

Largo, D.B., Diola, A.G., & Marababol, M, S. (2016). Development of an integrated multi-trophic aquaculture (IMTA) system for tropical marine species in Southern cebu, Central Philippines. *Aquaculture reports* 3 (2016) 67-76. https://doi.org/10.1016/j.aqrep.2015.12.006

Lavitra, T., Rosolofornirina, R., Jangoux, M., & Eeckhaut, I. (2009). Problems related to the farming of *Holothuria scabra* (Jaeger, 1833). *SPC Beche-de-mer Information Bulletin*. 29: p. 20-30.

Murphy, A.E., Anderson, I.C., Smyth, A.R., Song, B., & Luckenbach, M.W. (2016). Microbial nitrogen processing in hard clam (*Mercenaria mercenaria*) aquaculture sediments: the relative importance of denitrification and dissimilatory nitrate reduction to ammonium (DNRA): Nitrogen cycling in clam aquaculture sediment. *Limnology and Oceanography* 61: 1589–1604. https://doi.org/10.1002/lno.10305

Newell, R.I.E. (1988) Ecological changes in Chesapeake Bay: are they the result of overharvesting the American oyster. In: *Crassostrea virginica*? Understanding the Estuary: Advances in Chesapeake Bay Research. vol. 129. *Chesapeake Research Consortium Publication*, pp. 536–54619. https://doi.org/10.1111/raq.12645

Irisarri, J., Fernández-Reiriz, M.J., Labarta, U., Cranford, P.J., & Robinson, S.M.C. (2015). Availability and utilization of waste fish feed by mussels *Mytilus edulis* in a commercial Integrated multi-trophic aquaculture (IMTA) system: a multi-indicator assessment approach. *Ecological Indicators* 48, 673–686. https://doi.org/10.1016/j.ecolind. 2014.09.030.

Magondu, E. W., Fulanda, B. M., Munguti, J. M., & Mlewa, C. M. (2021). Towards integration of sea cucumber and cockles with culture of shrimps in earthen ponds in Kenya. *Journal of the World Aquaculture Society*, 1–15. https://doi.org/10.1111/jwas.12861

Mirera, D.O., & Okemwa, D. (2023). Salinity tolerance of Nile tilapia (*Oreochromis niloticus)* to seawater and growth response to different feeds. *Western Indian Ocean Journal of Marine Sciences.* https//doi/*10.4314/wiojms.v22i2.6*

Mirera, D. O. (2011). Experimental polyculture of milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) using earthen ponds in Kenya. *Western Indian Ocean J. Mar. Sci*., 10, 59-71.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, P., Halling, C., Shpigel, M., & Yarish, C. (2004). Integrated aquaculture: rationale, evolution, and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* 231, 361–391. https://doi.org/10.1016/j.aquaculture.2003.11.015 .

Purcell, S.W. (2015). Value, market preferences, and trade of beche-de-mer from pacific island sea cucumbers. *PLoS One* 9 (4), e95075.https://doi.org/10.1371/journal.pone.0095075.

Purcell S.W. & Eeckhaut, I. (2005). An external check for disease and health of hatchery-produced sea cucumbers. *SPC Beche-de-mer Information Bulletin* 22:34–38.

Purcell, S.W. (2004). Rapid growth and bioturbation activity of the sea cucumber *Holothuria scabra* in earthen ponds. *Proceedings of Australasian Aquaculture* 2004, 244.

Pitt, R. & Duy, N.D.Q. (2005) Breeding and rearing of the sea cucumber *Holothuria scabra* in Viet Nam. FAO Fisheries Technical Paper. p. 333-346.

Ridler, C. B., & Ridler, N. (2011). A potential conflict between economic and environmental sustainability: A case study (pp. 1131–1142). In MIC 2011: managing sustainability? Proceedings of the 12th International Conference, [Selected Papers] Portoroz, University of Primorska, *Faculty of Management* Koper, 23–26 November 2011.

Ridler, N., Barrington, K., Robinson, B., Wowchuk, M., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J., & Boyne-Travis, S. (2007a). Integrated multitrophic aquaculture. Canadian project combines salmon, mussels, kelps. *Global Aquaculture Advocate* 10, 52–55.

Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S. et al (2007b). Integrated multi-trophic aquaculture

(IMTA): A potential strategic choice for farmers. *Aquaculture Economics and Management*. 11(1):99-110. https://doi.org/10.1080/13657300701202767

Samocha, T.M., Fricker, J., Ali, A.M., Shpigel, M., & Neori, A. (2015). Growth and nutrient uptake of the macroalga *Gracilaria tikvahiae* cultured with the shrimp *Litopenaeus vannamei* in an integrated multi-trophic aquaculture (IMTA) system. *Aquaculture* 446: 263–271. https://doi.org/10.1016/*j.aquaculture*.2015.05.008

Shpigel, M.; Ben Ari, T.; Shauli, L.; Odintsov, V., & Ben-Ezra, D. (2016). Nutrient recovery and sludge management in seabream and grey mullet co-culture in Integrated Multi-Trophic Aquaculture (IMTA). *Aquaculture*, 464, 316-322. https://doi.org/10.1016/j.aquaculture.2016.07.007 .

Skriptsova, A.V., & Miroshnikova, N.V. (2011). Laboratory experiment to determine the potential of two macroalgae from the Russian Far-East as biofilters for integrated multi-trophic aquaculture (IMTA*). Bio resource Technology*. 102, 3149–3154. https://doi.org/10.1016/j.biortech.2010.10.093.

Smyth, A.R., Geraldi, N.R., & Piehler, M.F. (2013). Oyster-mediated benthic-pelagic coupling modifies nitrogen pools and processes. *Marine Ecology Progress Series* 493: 23–30. https://doi.org/10.3354/meps10516

Samocha, T.M., Fricker, J., Ali, A.M., Shpigel, M., & Neori, A. (2015). Growth and nutrient uptake of the macroalga *Gracilaria tikvahiae* cultured with the shrimp *Litopenaeus vannamei* in an Integrated Multi-Trophic Aquaculture (IMTA) system. *Aquaculture* 446, 263–271. https://doi.org/10.1016/*j.aquaculture*.2015.05.008

Sara, G, Zenone, A., & Tomasello, A. (2009). Growth of *Mytilus galloprovincialis* (mollusca, bivalvia) close to fish farms: a case of integrated multi-trophic aquaculture within the Tyrrhenian Sea. *Hydrobiologia* 636:129-136. https://doi.org 10.1007/s10750-009-9942-2

Sanderson, J., Dring, M., Davidson, K., & Kelly, M., (2012). Culture, yield and bioremediation potential of *Palmaria palmata* (Linnaeus) Weber and Mohr and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayers, Druehl and G.W. Saunders adjacent to fish farm cages in northwest Scotland. *Aquaculture* 354–355: 128– 135. https://doi.org/10.1016/j.aquaculture.2012.03.019

Shemer, H., Sagiv, A., Holenberg, M., & Maor, A.Z. (2017). Filtration characteristics of threaded microfiber water filters. *Desalination*, 431. https://doi.org 10.1016/j.desal.2017.07.009

Shi, H., Zheng, W., Zhang, X., Zhu, M., & Ding, D. (2013). Ecological-economic assessment of monoculture and integrated multi-trophic aquaculture in Sanggou Bay of China. *Aquaculture*; 410-411, 172–178. doi:10.1016/j.aquaculture.2013.06.033

Tresnati, J., Yasir, I., Syafiuddin., Aprianto, R., Mutmainnah, Yanti, A., Bestari, A.D., & Tuwo, A. (2019). Predators effect on mortality of sandfish *Holothuria scabra* cultured in multitrophic system. *Journal of Physics*: Conference Series1341 022026 doi:10.1088/1742-6596/1341/2/022026.

Uthicke S. (2001) Nutrient regeneration by abundant coral reef holothurian. Journal of Exp. Mar. Biol. and Ecol. 265, 153-170. https://doi.org/10.1016/S0022-0981(01)00329-X

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S., Mungkung, R., Nawapakpilai, S., & Phillips, M. (2014). Improving productivity and environmental Performance of Aquaculture. In: Working Paper, Installment 5 of Creating a Sustainable Food Future. *World Resources Institute, Washington, DC*. http://www.worldresourcesreport.org.

Wang, Y.G., Zhang, C., Rong, X., Chen, J., & Shi C. (2004). Disease of cultured sea cucumber, *Apostichopus japonicus*, in China. p. 297–310. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. *Fisheries Technical Paper* No. 463. Rome: Food and Agriculture Organization of the United Nations.

Watanabe, S., Sumbing, J.G., Hazel, M.J., & Ramos, L. (2014). Growth pattern of the tropical sea cucumber *Holothuria scabra*, under captivity. *Japan Agricultural Research Quarterly*, Vol 48 (4), 457-464 https://doi.org/10.6090/jarq.48.457

Whitmarsh, D., Cook, E., & Black, K. (2006). Searching for sustainability in aquaculture: an investigation into the economic prospects for an integrated salmon mussel production system. *Marine Policy* 30(3): 293. https://doi.org/10.1016/j.marpol.2005.01.004

Xilin S. (2004). The progress and prospects of studies on artificial propagation and culture of the sea cucumber, *Apostichopus japonicus*. p. 273–276. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. *Fisheries Technical Paper* No. 463. Rome: Food and Agriculture Organization of the United Nations.

Zar, J.H. (1998). Biostatistical Analysis (4th Edition). *Prentice Hall*, Englewood Clifs, New Jersey, USA.

.

SUSTAINABLE AQUATIC RESEARCH
www.saquares.com

Sustainable Aquatic Research (2024) 3(1):44-57
DOI: 10.5281/zenodo.11061708
e-ISSN: 2822-4140

RESEARCH PAPER

Phytoplankton-periphyton orientation influences feeding behavior of cultivable species: A case study on rohu, *Labeo rohita* (Hamilton, 1822)

Sandip Majumder [1,2], **Surjya K Saikia** [1*]

[1*]*Aquatic Ecology and Fish Biology Laboratory, Department of Zoology, Visva-Bharati University, West Bengal, India, Pin-73125*
[2]*Currently at PG Department of Conservation Biology, Durgapur Government College, Durgapur-713214, West Bengal*

Citation

Majumder, S., Saikia, S.K. (2024). Phytoplankton-periphyton orientation influences feeding behavior of cultivable species: A case study on rohu, *Labeo rohita* (Hamilton, 1822). *Sustainable Aquatic Research, 3*(1), 44-57.

Article History

Received: 19 February 2024
Received in revised form: 22 April 2024
Accepted: 22 April 2024
Available online: 30 April 2024

Corresponding Author

Surjya K Saikia
E-mail: surjyasurjya@gmail.com
Tel: +91 7432030340

Keywords

Indian major carp
Periphyton-based aquaculture
Substrate
Sustainable aquaculture
Aquatic resource

Abstract

The Indian Major Carp, *Labeo rohita* was stocked in four different resource environments namely, Periphyton monoculture, Periphyton polyculture, Plankton monoculture, and Plankton polyculture. Bamboo substrates were used as periphyton colonizers in culture ponds. In polyculture stocks, the *Catla catla* and *Cirhinus mrigala* were used as companion crop. The on-farm experiment was conducted for 210 days, and gut contents of rohu were collected and analyzed throughout the stocking period. The gut abundances of rohu showed an inclination towards algal food organisms, especially Cyanophyceae, Chlorophyceae, Baccillariophyceae, and Eugelophyceae, towards the later period of stocking when the fish attains a considerable total length. Results from the Shanon Diversity index supported higher algal diversity in the fish gut in periphytic conditions. The Diet breadth index also clearly indicated that rohu has a strong preferences towards periphyton than plankton. Such preferences are more prevalent in polyculture than in monoculture conditions. In conclusion, it can be accepted that rohu has clear preference towards periphyton over plankton when it is available in the environment. Stocking of other planktonic feeders in polyculture conditions may have no impact on the feeding pattern of rohu if periphytic resources are made available in the environment.

Introduction

Labeo rohita (rohu) is an important freshwater fish species normally cultured in Asia particularly in the Indian subcontinent (Khan et al., 2004). This Indo-Gangetic riverine species is distributed throughout South Asia, South-East Asia, Sri Lanka, the former USSR, Japan, China, Philippines, Malaysia, Nepal and some countries of Africa. Its compatibility for resource utilization with other freshwater carps, mainly catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) made it an ideal candidate for polyculture (Jhingran, 1991).

During the last decade, voluminous studies have been done on the feeding habit of rohu with regard to aquaculture management (Rahman et al., 2006; Majumder et al., 2018; Saikia et al., 2013; Mishra, 2020; Biswas and Mandal, 2021). Few of these studies on its feeding habit has forwarded interesting outcomes in the field of aquaculture research in rohu. For example, Majumder et al.

(2018) has reviewed that it follows ontogenic shifting of food habits from zooplanktivorous to phytoplanktivorous during its growth. Further, Saikia et al. (2013) reported its periphytophagous feeding habit under the availability of periphytic resources. These outcomes are important since fish meal nowadays have become the most expensive protein ingredient in aquaculture research. Several animal protein sources were evaluated to formulate the diets for fish including rohu to accomodate the objective of low input and high produce in aquaculture (De Silva and Gunasekera, 1991; Rangacharyulu et al. 2003; Asimi et al., 2017).

As an alternative way, a concern of growing fish in periphyton-based conditions has been popularized by a group of aquaculturists (Wahab et al., 1999; Azim et al., 2001a; Gangadhara et al., 2004; Azim et al., 2004a). Their studies have shown that rohu attained a profitable growth in periphytic condition when stocked in combination with other cultivable major carps. Subsequently, similar fish culture practices of common carp (*Cyprinus carpio*) has also been reported in India (Saikia and Das, 2009; 2014). Very recently, Biswas et al. (2022) proposed utilization of the periphytic biomass as a replacement of artificial feed in brackishwater polyculture and suggested that periphyton grown on 75% surface area provides a cost-effective production in polyculture. In view of the available reports of feeding habit of rohu and its potential use as a candidate in periphyton based aquaculture conditions, this is necessary to evaluate its comparative performance in feeding under periphyton-free and periphyton-based conditions. The present study has been design to understand such choices by rohu. In most of the periphyton-based fish culture experiments with carps, rohu constituted major part of the composition of fishes stocked (Wahab, 1999; Azim et al., 2001a; Gangadhara et al., 2004; Azim et al., 2004a). Being planktonic feeder, the synergistic effect that helps rohu grow under periphytic condition is not known. These studies performed in periphytic condition concluded that rohu feeds on periphyton under substrate-based condition and hence growth is accelerated. However, such shifting of food from plankton to periphyton by rohu were not based on direct evidence from the gut content and other feeding ecological tools. The present study, therefore, is based on gut content analysis as direct evidence of feeding on available resources under the periphytic monoculture as well as in polyculture conditions.

Materials and Methods

Pond preparation and experimental set up

The present study has been performed in two conventional fish ponds for 210 days separated by an earthen embankment at Jaydeb, Birbhum, West Bengal (23° 38' 0" N, 87° 26' 0" E, Fig 1).

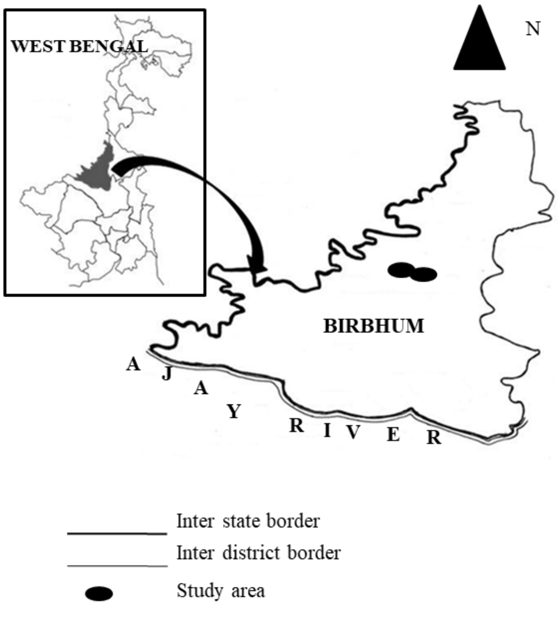
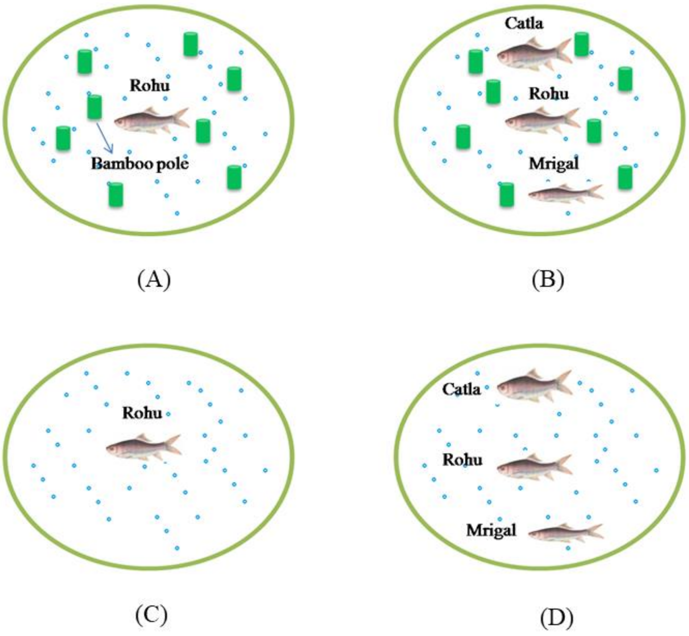


Figure 1. Study area in Birbhum, West Bengal, India. (Not to scale)

Each pond was divided into two areas with the help of fine nylon nets (mesh size <10mm). Along with the rohu, two other fishes (Catla, *Catla catla* (Hamilton, 1822), Mrigal, *Cirrhinus mrigala* (Hamilton, 1822)) were selected because they are compatible for polyculture with rohu (FAO, 2009). For the growth of periphytic organism, bamboo poles (lengths 2.53±0.21 m and diameters 5.2±0.37 cm) were implanted in the two areas of the first pond (depth 1.87 ± 0.25m) vertically at a distance of 1m from each other (4 poles/ m^2) (Fig. 2A and B). Both the areas partitioned in the second pond (depth 1.89 ± 0.28m) remained free i.e. without any bamboo substrates. In one of the bamboo substrate implanted areas of the first (Size: 903 sq. ft.) pond, fingerling of rohu (average wt. 8.15 ±1.75 g, average length 7.3 ± 0.81 cm) were introduced and this area was considered as the periphytic monoculture (**PR-M**) area. In the second area (Size: 1058 sq. ft.) of the first pond, along with rohu, fingerlings of catla (average wt. 8.91 ±1.89 g, average length 7.6 ± 0.75 cm) and mrigal (average wt. 4.53 ± 0.76 g, average length 5.2 ± 0.62 cm) were introduced and this area was considered as the periphytic polyculture (**PR-P**) area. In the second pond, the first area (Size: 923 sq. ft.) was stocked with fingerlings of rohu (average wt. 8.15 ±1.75 g, average length 7.3 ± 0.81 cm) and was considered as the planktonic monoculture (**PL-M**) area. In its second area (Size: 1025 sq. ft.), along with rohu, *Catla catla* (average wt. 8.91 ±1.89 g, average length 7.6 ± 0.75 cm) and *Cirrhinus mrigala* (average wt. 4.53 ± 0.76 g, average length 5.2 ± 0.62 cm) were introduced and this area was considered as the planktonic polyculture (**PL-P**) area (Fig 2C and D). All fishes were released in May 2014. Stocking density in both the monoculture areas was 400 fingerlings of rohu and in polyculture area, 200 fingerlings of rohu, 150 fingerlings of catla and 100 fingerlings of mrigal. Fishes were released 15 days before first sampling.



(A) (B)

(C) (D)

Figure 2. Experimental design. (A) Periphytic monoculture (PR-M) in first pond, (B)) Periphytic polyculture (PR-P) in first pond, (C)) Planktonic monoculture (PL-M) in second pond, (D) Planktonic ployculture (PL-P) in second pond. (Figures are not to scale)

Fish capture technique and collection of gut content

In every sampling, 15-20 fishes (rohu) were captured using fish net from the four experimental plots in 30-day intervals starting from June 2014 to November 2014. All fishes were collected before 9:00 AM Ethical procedures for experiment with animals were maintained throughout the period of research work.

The fishes (n=15-20) were sacrificed and guts were cut from the oesophagus region to first major constriction of the alimentary canal to obtain identifiable food items ingested by the fish (Haroon and Pittman, 1998). This length is around 4.5-12.6 cm (proportionately to TL 11.7-36.5 cm). Before gut collection, every fish was weighed and the total length was recorded. Immediately after collection, guts were transferred to 10% formalin. In the laboratory, each gut along with the content was blotted uniformly with tissue paper and weighed accordingly. In spite of measuring gut weight, guts were cut longitudinally and the fullness index was measured (Haroon 1998). Gut contents from samples with considerable food (Gut fullness index > 0.5) were removed with the help of a fine scalpel visible to the naked eyes. These were then preserved in 4% formalin in 10 ml glass vials (Borosil) for further analysis. After that, empty guts were again weighed to obtain gut content weight.

Identification, quantification, abundance, diversity and dominance of gut samples

Gut-content organisms were identified up to generic level using standard manuals (Pentecost, 1984; Edmondson, 1992; Perumal and Anand, 2009) and online resource (https://www.algaebase.com) and wherever possible, identified up to species level. The whole gut content collected from each gut were analyzed. These were estimated following Lackey's (1938) drop count methods under an inverted microscope (Victory plus, Dewinter, Italy). Abundance was expressed as L/gut sampled for analysis. Shanon's Species diversity measure was performed for documenting taxonomic diversity of gut contents. In addition to relative abundance and Shanon diversity, dominance measure was computed as follows:

Dominance Index (Odum 1971) $C = (ni / N)^2$

Where, ni is the total number of individuals of species.

N is the total number of individuals of all species in hand.

Diet breadth analysis

Three indices of diet breadths were considered. The selection of diet breadth indices was made to obtain a clear understanding of the resource use and mode of selection of resources. The first index was the popularly used Levin's (1968) diet breadth measure. It is calculated as-

$$B_A = \frac{\left(\frac{1}{\Sigma P_i{}^2}\right) - 1}{n - 1}$$

where, B_A is Levin's diet breadth, p_i is the fraction of items in the diet that are of food category j and n is the number of resource states. As Levin's diet measure does not take resource availability in the environment into consideration while determining diet breadth, the second index i.e. Hulbert (1978) diet measure was calculated. It is-

$$B_A = \frac{1}{(\Sigma P^{i2/aj})}$$

Where B′ is Hulbert's standardized diet breadth, pi is the fraction of items in the diet that are of food category i (∑(pi =1.0), aj is the proportion of total available resources consisting of resource j (∑aj=1.0). B′ ranges from 1/n to 1.0.

The third index considered was $Db(\chi^2)$ (Saikia, 2012). Statistically, this measure not only considers resource utilization along with diet breadth, but also sensitive to variation within resources and resource utilized by the organism. As Levin's and Hulbert diet measures are based on proportional availability of food item to the total foods and Hulbert is a product of fraction of resource and gut content, they are insensitive to variations within the sample. The $Db(\chi^2)$ is computed as follows-

$$DB(\chi 2) = \sum_{i=1}^{n} \frac{(\log \mathrm{Oj} - \log \mathrm{Ej})^2}{\log \mathrm{Ej}}$$

Here, the $DB(\chi^2)$ is the diet breadth, $\log O_i$ and $\log E_i$ are the log value of observed and expected food abundances of i^th^ category respectively. The expected food abundance in $DB(\chi^2)$ is constituted of available food resources in the environment. The $DB(\chi^2)$ value '0' indicates a complete overlap of gut content abundance on resource abundance from environment. This value is termed as Resource Db(x) or $R_{DB(\chi 2)}$. Theoretically, $R_{DB(\chi 2)}$ =0.0.

Determination of factors influencing food preference of rohu

To examine which factor is responsible for such preference of food organisms by rohu, three primary factors viz. resource type (Plankton and Periphyton), season (across months) and culture type (monoculture and polyculture) and two secondary factors viz. mono-poly resource and mono-poly season were analyzed.

Statistical analysis

One-way ANOVA was computed to analyze differences among means of samples. Homogeneity measure was tested before computing ANOVA. If ANOVA showed any difference among the means, Tukey's post hoc test was used to identify means with difference. The p value of 0.05 or smaller is considered as significant. The Software **Minitab, version 17.0** was used for all statistical analysis.

Results

Community composition of food organisms in the gut of rohu

In the periphytic culture condition of rohu, a total 51 genera of food organisms including 17 genera of Chlorophyceae followed by 13 genera of Bacillariophyceae, 6 genera of Cyanophyceae, 3 genera of Euglenophyceae, 5 genera of and 7 genera of Cladocera-Copepoda and other zooplanktonic organisms (CCO) were recorded (Table 1). From the planktonic culture condition, a total of 48 genera of food organisms which includes 16 genera of Chlorophyceae followed by 12 genera of Bacillariophyceae, 5 genera of Cyanophyceae, 3 genera of Euglenophyceae, 5 genera of Rotifera and 7 genera of CCO were recorded. The fraction of these food items used by the fish showed great variation with the progress of the season.

Abundance of food organisms in the gut content of rohu from the culture pond

The abundances of Cyanophyceae organisms in PR-M conditions are shown in Fig 3a. In the initial months i.e. in June and July, Cyanophycean abundance did not show any significant difference ($p>0.05$). But with the progression of time starting from September onwards the abundance of Cyanophyceae was significantly increased($p<0.05$) in the gut of rohu as compared to the initial months. In the case of PR-P conditions there was a significant increase in abundance during the later period of months compared to June and July. In planktonic culture conditions (PL-M, PL-P) also there was no significant difference ($p>0.05$) in abundance during the initial months, but from August onwards there was a sharp increase in number. When the abundance of Cyanophyceae was analyzed among the four culture conditions, in the initial months starting from June to August, the abundance did not show any significant difference ($p>0.05$). among all the four culture conditions. But from September onwards, both the PR-M and PR-P conditions showed significant difference($p<0.05$) with the PL-M and PL-P conditions showing significantly higher abundances in the periphytic culture conditions in comparison to the planktonic culture conditions. Although statistically not significant ($p>0.05$), Cyanophyceae showed higher abundances in the PR-P condition compared to the PR-M condition with the progression of time.

Abundance studies of Chlorophyceae showed great variations of periphytic and planktonic food organisms in the gut of rohu (Fig 3b). In the month of June and July in PR-M conditions, the abundance of Chlorophyceae organisms did not show significant difference ($p>0.05$). But from August onwards, significantly ($p<0.05$) higher abundance of Chlorophyceae organisms were observed compared to the initial months. Similar abundance pattern of Chlorophyceae was observed in the other three culture conditions viz. PR-P, PL-M and PL-P. From August onwards, guts of rohu from all these three areas showed significantly higher ($p<0.05$). abundance of Chlorophyceae organisms. When abundances of Chlorophyceae were analyzed among the four culture conditions, in the initial months of June to July, the abundances did not show any significant difference ($p>0.05$) among the four culture conditions. But from August onwards the PR-M and PR-P showed significant difference ($p<0.05$) with the PL-M and PL-P conditions, indicating significantly highest ($p<0.05$) abundances of gut Chlorophyceae from the periphytic culture conditions in comparison to the planktonic culture conditions. Here too, although statistically not significant ($p>0.05$), the abundance of Chlorophyceae organisms from the gut of rohu were higher in the PR-P conditions compared to the PR-M conditions with the progression of time. Similar pattern was observed for the abundances of gut Bacillariophyceae too (Fig 3c) and Euglenophyceae (Fig 3d). Like the gut abundances of Cyanophyceae and Chlorophyceae, the

Bacillophyceae and Euglenophyceae also showed significantly higher abundances in periphytic in comparison to planktonic conditions.

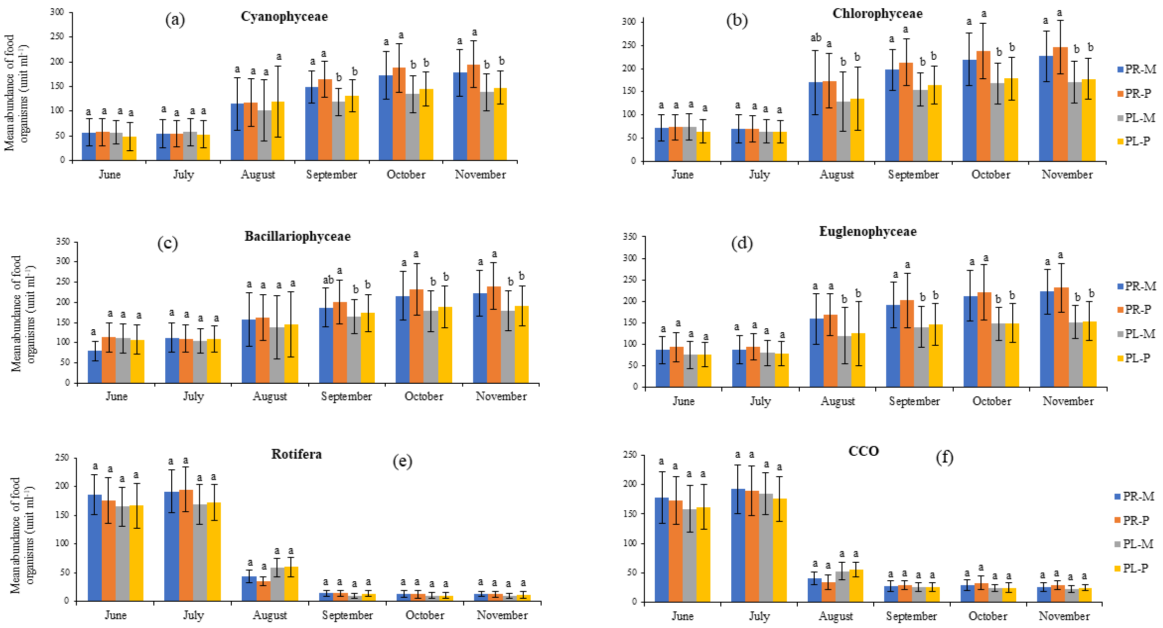


Figure 3. Mean abundance of gut contents of *Labeo rohita*. Abundances of Cyanophyceae (a), Chlorophyceae (b), Bacillariophyceae (c), Euglenophyceae (d), Rotifer (e) and Cladocera, Copepoda and other zooplanktonic organisms (CCO) (f) in Periphytic Monoculture (PR-M), Planktonic Monoculture (PL-M), Periphytic Polyculture (PL-P) and Planktonic culture (PL-P) conditions. Mean±SD, one way ANOVA followed by Tukey's test ($p<0.05$), n=9, Means that do not share any letter are significantly different.

In case of Rotifera, in the initial months i.e. in June and July there was no significant difference ($p>0.05$) in abundance in all the four culture conditions (Fig 3e). But with the progression of time starting from August onwards the abundance of Rotifera significantly decreased ($p<0.05$) in the gut of rohu as compared to the initial months in all the four culture conditions (PR-M, PR-P, PL-M, PL-P). When the abundance of Rotifera was analyzed among the four culture conditions, the abundance did not show any significant difference ($p>0.05$) among all the four culture conditions throughout the months, although there was a sharp decrease in abundance during the later period of months.

Similar to Rotifera, abundances of Cladocera, Copepoda and other zooplanktonic organisms (CCO) showed insignificant differences ($p>0.05$) among all the four culture conditions during June and July (Fig. 3f). But with the progression of time starting from August onwards the abundance of CCO was significantly decreased ($p<0.05$) in the gut of rohu as compared to the initial months in all the four culture conditions. Like Rotifera, when abundance of CCO was analyzed among the four culture conditions, the abundance did not show any significant difference among all the four culture conditions throughout the months, although there was a sharp decrease in abundance starting from August onwards.

Dominance index of food organisms from gut content of rohu

As found in Table 1, a total of 51 species in the gut content of rohu from the periphytic culture conditions and a total of 48 species of gut content organisms of rohu from the planktonic culture conditions were recorded. Among all food organism, only the most dominant species from both periphytic as well as planktonic conditions were identified through species dominance index for analysis and presented in Fig 4. From the dominance analysis in periphytic culture conditions, 12 genera viz. *Anabaena*, *Aphanocapsa*, and *Chroococcus* from the

Cyanophyceae, *Chlorella*, *Closterium*, *Scenedesmus* and *Oedogonium* from Chlorophyceae, *Navicula* and *Diatoma* from Bacillariophyceae, *Phacus* and *Euglena* from Euglenophyceae and *Arcella*, the only representative from the zooplanktonic organisms were identified which showed highest abundance among all the gut content organisms of rohu throughout the study period. Except *Oedogonium* all the other 11 dominant species from the periphytic conditions also showed highest dominance in case of planktonic culture conditions throughout the months.

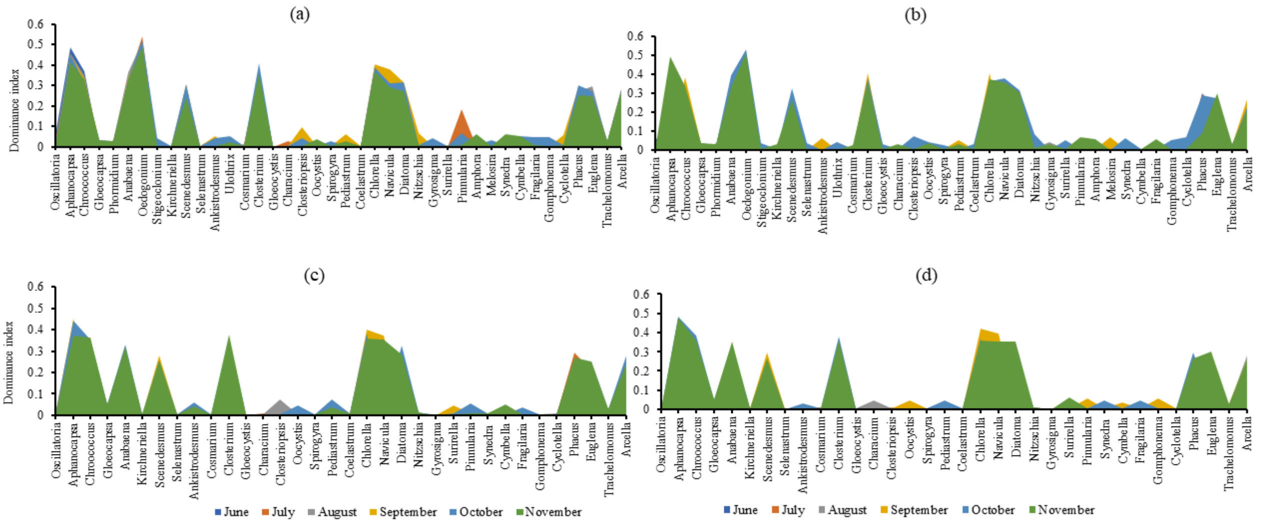


Figure 4. Dominance index of gut content of *Labeo rohita* from Periphytic Monoculture (PR-M) (a), Periphytic Polyculture (PL-P) (b), Planktonic Monoculture (PL-M) (c), and Planktonic Monoculture (PL-P) (d) conditions.

Factors influencing the food preference of rohu

When resource was considered as a factor (Table 2), most of the dominant organisms except *Diatoma* and *Arcella* showed statistically insignificant (p>0.05) results. Similarly, when season was considered as a factor, all of the dominant organisms except *Arcella* showed statistically significant (p<0.05) results. Whereas, when culture condition (monoculture/polyculture) was considered as a factor, none of the dominant organisms showed statistically significant results. But interestingly, when resource was considered as the combined factor with culture conditions, most of the dominant organism showed statistically significant (p<0.05) results. When season was considered as the combined factor with culture type, all of the dominant organisms except *Arcella* showed statistically significant results.

Diversity of food organisms and diet breadths in the gut of rohu

Shanon-Wiener diversity

The diversity (H´) values were low during the initial months but increased gradually with the progression of time in the gut of fish (Fig 5a). In the month of June and July, the diversity (H´) of the food organisms showed no significant difference(p>0.05) among all the four culture conditions. With the progression of time, starting from August onwards, the diversity of the food organisms in the gut content of rohu showed significantly higher (p<0.05) values in both the periphytic monoculture and polyculture conditions over planktonic culture conditions. The diversity of the gut food organisms in the gut of rohu from periphytic polyculture condition was significantly higher (p<0.05) than the periphytic monoculture condition during the later period of months. However, for planktonic conditions, the diversity of food organisms in the fish gut was significantly higher (p<0.05) in polyculture compared to the monoculture condition during the month of August and September only. In all other months there was no significant difference (p>0.05) in diversity of gut content organisms between planktonic monoculture and polyculture condition.

Diet breadth

The Levin's diet breadths (B_A) are presented in Fig 5b. The B_A values were low during the initial months but increased gradually with the progression of time in the gut of fish. In the month of June and July, the B_A values of the food organisms showed no significant difference ($p>0.05$) among all the four culture condition. Whereas, with the progression of time, starting from August onwards, the increment of diet breadth of rohu was significantly higher ($p<0.05$) in both the periphytic monoculture and polyculture condition in comparison to both the planktonic culture condition. The Hulbert diet breadths (B′) are also presented in Fig 5c. The B′ values also were low during the initial months just like the Levin's diet breadth, but increased gradually with the progression of time in the gut of rohu. The Hulbert's diet breadth behaves more or less similar to the Levin's diet breadth throughout the period of culture of the fish. In general, both the Levin's and Hulbert diet breadth showed higher values of diet breadth in periphytic polyculture condition over periphytic monoculture condition during the later part of the study (in months).

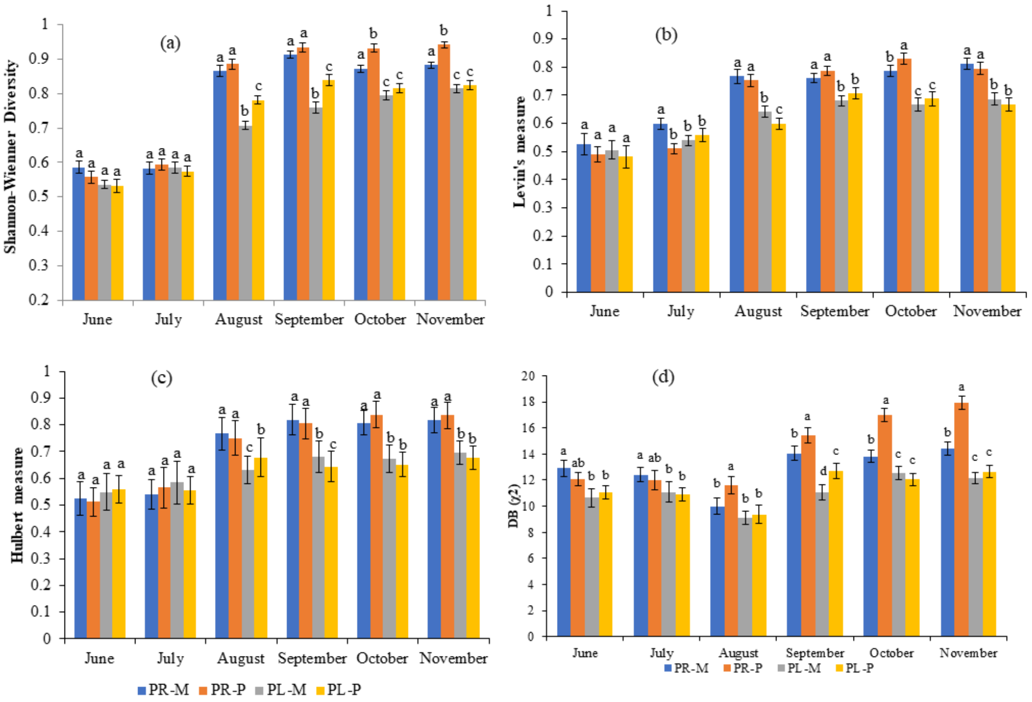


Figure 5. Diversity and diet breadth measures of gut contents of *Labeo rohita*under different culture conditions, (a) Shanon-Wiener diversity measure, (b) Levin's diet breadth , (c) Hulbert's diet breadth and (d) DB (χ2). Mean±SD, one way ANOVA followed by Tukey's test ($p<0.05$), n=9, Means that do not share any letter are significantly different.

Fig 5d represents DB(χ^2) values from all the culture condition. When plankton were considered as food resource, the DB(χ^2) values of PR-M and PR-P were significantly higher ($p>0.05$) in comparison with the PL-M and PL-P during the later months. In the month of June and July, the DB(χ^2) values in the periphytic PR-M showed indifference with PR-P condition but was significantly higher($p<0.05$) compared to the PL-M and PL-P condition. However, from August onward the DB(χ^2) value in PR-P condition was significantly higher ($p<0.05$) compared to the PR-M, PL-M and PL-P area. The PR-M area again showed significantly higher ($p<0.05$) DB(χ^2) value compared to the PL-M and PL-P condition from September onward. In case of the planktonic culture condition, there was no significant difference ($p>0.05$) among the monoculture and polyculture condition throughout the months except in the month of September.

Discussion

The earlier reports with rohu as an active periphyton feeder (Gangadhara et al., 2004; Azim et al., 2004a) did not consider gut analysis to confirm the diet shift of rohu from plankton to periphyton. The present report is the first of its kind to understand resource utilization by rohu under a substrate-based condition. In June and July rohu fed more zooplankton irrespective of monoculture and polyculture conditions, however, in the later months, it inclined mostly towards plankton of plant origin (four major algal groups). This situation happened in both periphytic and planktonic resources. From August onward, abundances of food organisms of plant origin in the gut of rohu increased in PR-M and PR-P conditions. Although statistically not significant, the abundance of food organism in the gut of rohu in PR-P condition is relatively higher compared to PR-M condition. Also, in comparison to the other two plankton-only condition, the gut content of rohu showed significantly higher ($p<0.05$) plant-origin food in the periphyton based condition. These results indicate that rohu has the preference for periphytic resources in substrate-based condition.

During the experiment, two resource types, i.e. plankton and periphyton in the pond conditions, were available for the fish. Even, as discussed for the ontogenic shifting of food habit of rohu, its feeding nature was repeated from the zooplanktivorous to phytoplanktivorous nature all the culture conditions.

Rohu showed an overall narrow diet breadth in the case of plankton throughout the months. The greater the diet breadth, the greater the possibility for fish to access the resource types. A smaller diet breadth is the indication of either resource partitioning (Haroon and Pittman, 2000) or less affinity of the fish towards the resource type on which diet breadth was measured. Small-sized fishes are generally less opportunistic and occasional feeder of different resource types (Haroon and Pittman, 2000), exhibiting feeding activity in a limited zone of the environment, resulting in insignificant narrow diet breadth. Less periphytic productivity during the early months on the bamboo substrates might have some effect on such values of diet breadth. Moreover, it is also evident that the smaller forms of the carp mainly fed on zooplankton.

The Levin's diet breadth was compared for two different resource types, namely, plankton and periphyton in the fish culture condition to draw a more meaningful conclusion about the food accessibility by rohu. The lower diet breadth resulting from plankton compared to periphyton indicated the fish's grazing nature considerably on periphyton biomass. From August onward, Levin's diet breadth of rohu showed a greater value when periphyton was ingested as a food source. This difference increased with the progression of the season showing a maximum diet breadth during October-November.

With the progression of time, Levin's and Hulbert diet breadth increased for the periphytic food organisms, indicating its nature of accessibility to brows on the attached organisms (Fig 4A, B). Simultaneously, the shift towards periphyton resource caused a gradual decline in feeding activity on plankton. The increasing resource availability in the form of periphyton might have positive effect for such findings. Therefore, during later period of months the fish consumed maximum number of available food items through random selection.

Under substrate-based conditions, it was repeatedly reported that the rohu shows faster growth compared to substrate-free conditions. Azim et al. (2001a) reported that its growth was 77% higher in substrate-based conditions than substrate-free conditions. Azim et al. (2001b) observed that periphyton biomass significantly decreased with increasing biomass of rohu in a substrate-based condition. Although rohu has been reported as an exclusive plankton feeder, these observations suggest that rohu is an opportunistic periphyton feeder because when rohu was subjected to a periphytic condition, it preferred periphyton over plankton. In periphytic conditions, from August onwards, Levin's diet breadths for rohu were significantly higher ($p<0.05$) compared to planktonic conditions, indicating rohu as a successful feeder on both resources i.e., periphytic and planktonic. Thus, there is an condition-specific effect on the feeding behavior of rohu when gut contents were considered. However, Levin's measure of diet breadth has been criticized for excluding resources in the environment while enumerating the diet breadth of an organism (Saikia, 2012). Significantly higher ($p<0.05$) Hulbert diet breadth under periphytic condition than plankton condition indicates its exclusive preference for periphyton.

Such result concurs with the findings of Azim et al. (2004b). Earlier, Das and Moitra (1955) reported it as a phytoplankton feeder. Probably for the same reason, in the following stage of growth (during later months) the Hulbert values from the periphytic area for both resources (plankton and periphyton) remained higher, suggesting increased accessibility of the fish on periphytic and phytoplanktonic resources when substrates are installed. From Hulbert's values, it is difficult to conclude that the fish exploited only one resource or both since plankton and periphyton have common algal members in them (Saikia and Das, 2009). Though Hulbert diet breadth suggests increased feeding preference of rohu in substrate-based condition, its specific selection towards any of the both resources could hardly be ascertained from this index. Considering such variations existed within resource data to gut content data, the newly reported diet breadth Db(χ^2) is discussed to understand the real situation.

Similar to Hulbert, Db(χ^2) values also reflected that, initially, there was no difference in diet breadth in both periphytic and planktonic conditions. In August, the fish successfully fed on plankton and periphyton from the periphytic polyculture condition, and as a result it showed significantly higher ($p<0.05$) Db(χ^2) value than other culture conditions. However, the preference was more from periphytic than planktonic resources as evident from the abundance study. One reason behind such preference may be the rate of colonization of periphyton on the substrates which increased during the later period of months. Alikunhi (1958) observed that the structure of gill rakers in rohu is such that they are not adapted to filter minute planktonic organisms. For this reason, the early periphytic colonizers, which are mainly bacteria and blue-green algae, might have escaped from the mouth cavity and could not be retained in the fish gut. Therefore, initially, the Db(χ^2) values were more or less similar in the periphyton and plankton based condition. With the precedence of the stocking period, the successional progression of colonizing communities was occupied with algal forms like diatoms, filamentous algae, etc. This has enhanced its preference towards periphytic food rather than plankton. The fish, being basically column feeders, favored maximally to brows probably on substrate at this period and hence received maximum food items from the substrate than an actual planktonic resource. When periphyton arrives at late successional stage, the colonization rate reduces with the occurrence of self-shading in periphytic layers. This might have enhanced rohu's preference on periphytic communities leading to significantly higher ($p<0.05$) Db(χ2) values in substrate-based condition. Thus, it is evident that the fish actually prefers periphyton in substrate-based condition. This could be the reason why Azim et al. (2001a, 2001b) and Keshavanath et al. (2001) observed a correlation of increased biomass of rohu with periphytic condition. Azim et al. (2004a) also reported that lower periphyton biomass in ponds with lower amounts of substrate indicated a higher grazing pressure on the substrates. This is probably because rohu, being opportunistic in nature, exploits the periphytic resource available on the substrate. Hence, rohu's feeding rate increases, affecting reduced colonization of algae on the substrate.

Rahman et al. (2008) observed that rohu spends 65-85% of swimming time grazing in water column. Such a longer grazing time could be explained by the dependency of the rohu on planktonic food (Rahman et al., 2006). Compared to plankton, periphyton is a static type of community on substrate, enhancing feeding rate of fish through two-dimensional exploration of food (Horne and Goldman, 1994). It is reported that the feeding of rohu is deliberate and selective in nature (Alikunhi, 1958).

Studies on the feeding habit of rohu always suggests it to be an opportunistic feeder. It was reported as a plankton feeder (Das and Moitra, 1955), phytoplankton and zooplankton feeder (Khan and Siddique, 1973), zooplankton feeder under fed and fertilized ponds at fry stage (Miah et al. 1984) and detritus feeder in shallow ponds (Das and Chakrabarty, 2006). Ramesh et al. (1999), Wahab et al. (1999) and Azim et al. (2001a) reported that under fed and polyculture condition, rohu is a very active periphyton grazer. In the present study, all diet breadths measures suggest that in most of the cases the fish explored resources common in both the water column and substrate in periphyton based condition thereby increasing in diet breadth measure in periphytic based condition as compared to the plankton based conditions where only one resource i.e. only plankton is present. As observed in Table 2, the seasonal influence on the significance abundances of resources (plankton and periphyton) in periphytic and planktonic culture conditions may also have led to wider diet breadth of rohu. The probability of occurring common food items in periphytic condition could be possible if planktonic organisms with periphytic intensity appear in close association with the substrate. When colonization of periphyton was initiated on substrate, the fish started feeding on such colonizing resources. Earlier, Saikia et al. (2013) proposed a 'sub-periphytic' zone from which rohu explores the periphytic resources. With precedence of colonizing event, the successional stages of periphyton varied and algae with different colonizing ability started to attach on the substrates that facilitates the increased feeding of rohu on periphytic organisms.

Conclusions

The present study confirmed that the rohu is a potential candidate for culture in planktonic and periphytic ambiances. When both resources are available, it was observed to be inclined towards periphytic biomass. Although exploration from such a hypothetical periphytic zone has not been confirmed physically, the present study on feeding ecology through diet breadth measures has reaffirmed its choice of periphytic resources. The fish can be, therefore, featured as a generalist feeder in terms of plankton and periphyton resources and the best fit to such a cultivable system. The area of research needing further attention is the probability of maximum assimilation of nutrients and growth of rohu under such ambiances. Feeding on periphytic and planktonic organisms might induce assimilation of food-dependent nutrients to fish. Since the fish is mainly cultured as a source of animal protein, a confirmation on protein gain compared to the conventional practice through periphyton based rohu culture would additionally credit the fish for polyculture in rice-fish or pond conditions.

Acknowledgements

Authors acknowledge the financial support in the form of UGC-BSR fellowship to the first author to conduct the present research work. Authors also acknowledge the UGC-CAS and DST-FIST programmes of the Department of Zoology, Visva-Bharati University for providing instrumental support to complete the work.

Ethical approval

Although ethical clearance is not mandatory, the author declares that this study complies with research and publication ethics.

Informed consent

The corresponding author on behalf of all authors is responsible for obtaining and providing written consent of any third party, or their legal representatives.

Conflicts of interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper."

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Funding organizations

UGC, New Delhi

Author contribution

Author1: Writing original draft, Data collection and processing, Review

Author2: Supervision, Validation, Visualization, Project administration, Resources, Review, Editing.

References

Alikunhi, K.H. (1958). Observations on the Feeding Habits of Young Carp Fry. *Indian Journal of Fisheries, 5(1),* 95-106. https://epubs.icar.org.in/index.php/IJF/article/view/47578.

Asimi, O.A., Bhat, T.H., Nair, H., Irfan, K. (2017). Alternative source of protein "silkwarm pupae" (*Bombyx mori*) in cold water aquaculture. *International journal of Poultry and Fisheries Science*, 1(1), 1-4. http://dx.doi.org/10.15226/2578-1898/1/1/00102

Azim, M.E., van Dam, A.A., Beveridge, M.C.M., and Verdegem, M.C.J. (2001a). The potential of periphyton based culture of two Indian major carps, rohu *Labeo rohita* (Hamilton) and gonia *Labeo gonius* (Linnaeus). *Aquaculture Research*, *32*, 209-216. https://doi.org/10.1046/j.1365-2109.2001.00549.x

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Milstein, A., and Verdegem, M.C.J. (2001b). Optimization of fertilization rate for maximizing periphyton production on artificial substrates and their implications for periphyton-based aquaculture. *Aquaculture Research, 32*, 749-760. https://doi.org/10.1016/S0044-8486(01)00602-0

Azim, M.E., Wahab, M.A., Biswas, P.K., Asaeda, T., Fujino, T., Verdegem, and M.C.J. (2004a). The effect of periphyton substrate density on production in freshwater polyculture ponds. *Aquaculture, 232,* 441- 453. https://doi.org/10.1016/j.aquaculture.2003.08.010

Azim, M.E., Wahab, M.A., Kamal, A.H.M., Ahmed, Z.F., and Verdegem, M.C.J. (2004b). Feeding relations of silver barb, *Barbodes gonionotus* (Bleeker) with major Indian and common carp and its effect on fish production in a polyculture area. *Journal of World. Aquaculture Society, 35(1),* 100-108. http://dx.doi.org/10.1111/j.1749-7345.2004.tb01065.x

Biswas, A. and Mandal, S. (2021) Comparative growth performance of rohu (Labeo rohita) fed with low cost feeds using locally available animal proteins in Purulia district of West Bengal. *Journal of Crop and Weed*, 17(1), 252-256. https://doi.org/10.22271/09746315.2021.v17.i1.1433

Das, S.K., and Chakrabarty, D. (2006). Utilization and Flow of Energy in Tropical Ecoarea: A Test Case for Two Ox-bow Lakes. *International Journal of Environmetal Science and Technology, 3(2)*, 141-146. https://doi.org/10.1007/BF03325917

De Silva, S.S. and Gunasekera, R.M. (1991). An evaluation of the growth of Indian and Chinese major carps in relation to dietary protein content. *Aquaculture*, 92, 237-241. https://doi.org/10.1016/0044-8486(91)90025-3

Das, S.K., and Moitra, S.K. (1955). Studies on the food of some common fishes of Uttar Pradesh, India. *Proceedings of National Academy of Science India, 25b*, 1-6. https://eurekamag.com/research/025/579/025579204.php

Edmondson, W.T. (1992). *Freshwater biology*. International books and periodicals supply services, New Delhi, India.

F.A.O. (2009) *Labeo rohita*. In Cultured aquatic species fact sheets. Text by Jena, J.K. Edited and compiled by Valerio Crespi and Michael New. CD-ROM (multilingual).

Gangadhara, B., Keshavanath, P., Ramesh, T.J., and Priyadarshini, M. (2004). Digestibility of Bamboo-grown Periphyton by Carps (*Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Tor khudree*) and Hybrid Red Tilapia (*Oreochromis mossambicus* × *O. niloticus*). *Journal of Applied Aquaculture, 15(3-4)*, 151-162. https://doi.org/10.1300/J028v15n03_12

Haroon, A.K.Y. (1998). Diet and feeding ecology of two sizes of *Barbodes gonionotus* (*Puntius gonionotus*) and *Oreochromis spp*. in Rice fields in Bangladesh. *NAGA, 21(3),* 13-19. https://digitalarchive.worldfishcenter.org/bitstream/handle/20.500.12348/2569/na_2278.pdf?sequence1=

Haroon, A.K.Y., and Pittman, K.A. (1998). Diel feeding pattern and ration of two sizes of tilapia *Oreochromis* spp. In pond and paddy field. *Asian Fisheries of Science, 10,* 281-301. http://dx.doi.org/10.1046/j.1365-2109.1997.00890.x

Haroon, A.K.Y., and Pittman, K.A. (2000). Niche measurement and feeding strategies of *Barbodes gonionotus* and *Oreochromis spp*. from rice-field in Bangladesh. *Bangladesh Journal of Fisheries Research, 4(1)*, 13-26. https://core.ac.uk/download/pdf/33720172.pdf

Horne, A.J., and Goldman, C.R. (1994). *Limnology*. 2nd ed. New York, NY: McGraw-Hill Book

Hulbert, S.H. (1978). The measurement of niche overlap and some relatives. *Ecology*, *59*, 67-77. https://doi.org/10.2307/1936632

Keshavanath, P., Ramesh, T.J., Gangadhara, B., Beveridge, M.C.M., van Dam, A.A., and Verdegem, M.C.M. (2001). On-farm evaluation of Indian major carp production with sugarcane bagasse as subatrate for periphyton. *Asian Fisheries Science*, *14*, 367-376. http://dx.doi.org/10.33997/j.afs.2001.14.4.002

Khan, R.A., and Siddiqui, A.Q. (1973). Food selection by *Labeo rohita* and its feeding relationship with other major carps. *Hydrobiologia, 43(3/4)*, 429-442. https://doi.org/10.1007/BF00015361

Lackey, J.B. (1938). The manipulation and counting of river plankton and changes in some organisms due to formalin preservation. *Public Health Report*, *53*, 2080. https://doi.org/10.2307/4582717

Levins, R. (1968). *Evolution in Changing Environments: Some Theoretical Explorations*. Princeton, NJ: Princeton University Press. https://doi.org/10.2307/j.ctvx5wbbh

Majumder, S., and Saikia, S.K. (2020). Ecological intensification for feeding rohu *Labeo rohita* (Hamilton, 1822): A review and proposed steps towards an efficient resource fishery. *Aquaculture Research, 51(8)*, 3072-3078. https://doi.org/10.1111/are.14669

Miah, M.I.U., Dewan, S., and Wahiduzzaman, M. (1984). Studies on the type and amount of food taken by the silver carp *Hypophthalmichthys molitrix* (Val.) and its diet patterns of feeding in a Bangladesh pond. *Bangladesh Journal of Fisheries*, *7*, 49-54

Mishra, S. P. (2020). Analysıs of the gut contents of ındıan major carp rohu (*Labeo rohıta*) from Meeranpur lake of dıstrıct Sultanpur, Uttar Pradesh, Indıa. *International Journal of Zoology and Applied Biosciences*, 5(4), 217–221. https://doi.org/10.5281/zenodo.4015976

Odum, E.P. (1971). Fundamentals of Ecology, Saunders College Publishing, Philadelphia, pp.574.

Pentecost, A. (1984). *Introduction to Freshwater Algae* (1st ed). The Richmond Publishing Co. Ltd. Orchard Road, Richmond, Surrey, England

Perumal, G.M., and Anand, A. (2009). *Manual of Freshwater Algae of Tamil Nadu. Dehradun*: Bishen Singh and Mahendra Pal Singh.

Rahman, M.M., Verdegam, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Milstein, A., and Verreth, J.A.J. (2006). Growth, production and food preference of *Labeo rohita* (H.) in monoculture and in polyculture with Common carp *Cyprinus carpio* (L.) under fed and non-fed ponds. *Aquaculture, 257(1-4),* 359-372. https://doi.org/10.1016/j.aquaculture.2006.03.020

Rahman, M.M., Hossain, Y.M., Jo, Q., Kim, S.K., Ohtomi, J., and Meyer, C. (2008). Ontogenetic shift in dietary preference and low dietary overlap in rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*) in semi-intensive polyculture ponds. *Ichthyological Research, 56*, 28-36. https://doi.org/10.1007/s10228-008-0062-1

Ramesh, M.R., Shankar, K.M., Mohan, C.V., and Varghese, T.J. (1999). Comparison of three plant substrates for enhancing carp growth through bacterial biofilm. *Aquaculture Engineering, 19*, 119-131. https://doi.org/10.1016/S0144-8609(98)00046-6

Rangacharyulu, P.V., Giri, S.S., Paul, N.B., Mukhopadhyay, P.K., Mohanty, S.N. and Yashoda, P.K. (2003) Utilization of fermented silkworm pupae silage in feed for carps. Bioresour Technol. 86 (1): 29-32. https://doi.org/10.1016/s0960-8524(02)00113-x

Saikia, S.K. (2012). Proposal of a new and simple descriptive measure of Diet Breadth. *Ecologia, 2 (3)*, 85-92. http://dx.doi.org/10.3923/ecologia.2012.85.92

Saikia, S.K. (2023). Aquatic resources and feed diversification: Reviewing three case studies from South East Asia with a viewpoint of trophic intensification in rice fish culture. *Aquaculture and Fisheries*. https://doi.org/10.1016/j.aaf.2023.01.006

Saikia, S.K., Majumder, S., Nandi, S., and Saha, S.K. (2013). Feeding ecology of the freshwater fish rohu *Labeo rohita* (Hamilton 1822): A case of intelligent feeding in the periphyton-based environment. *Zoology and Ecology*, 23(4), 266-274. https://doi.org/10.1080/21658005.2013.859849

Saikia, S.K., and Das, D.N. (2009). Feeding Ecology of Common carp (*Cyprinus carpio* L.) in Rice-Fish culture area of Apatani Plateau, Arunachal Pradesh, India. *Aquatic Ecology*, 43, 559-568. https://doi.org/10.1007/s10452-008-9174-y

Majumder, S. Majumdar, N, Ghosh, P, Saikia, S.K., Saha, S.K. (2018) Rohu Labeo rohita (Hamilton, 1822) changes feeding strategy throughout its ontogeny: An explanation from feeding ecology. International Journal of Scientific Research in Biological Sciences, 5 (4), 92-96. https://doi.org/10.26438/ijsrbs/v5i4.9296

Wahab, M.A)., Azim, M.E., Ali, M.H., Beveridge. M.C.M., and Khan, S. (1999). The potential of periphyton-based culture of the native major carp calbaush, *Labeo calbasu* (Hamilton). *Aquaculture Research*, *30*, 409-419. http://dx.doi.org/10.1046/j.1365-2109.1999.00337.x

Sustainable Aquatic Research (2024) 3(1):58-76
DOI: 10.5281/zenodo.11046358
e-ISSN: 2822-4140

SUSTAINABLE AQUATIC RESEARCH
www.saquares.com

RESEARCH PAPER

Assessment of Benthic Macrofauna and Sediment Characteristics of Boracay Island Philippines

Jesseree Joyce B. Naciongayo [1*], **Sheila Mae S. Santander - de Leon** [1]

[1*]*Institute of Marine Fisheries and Oceanology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas Miagao*

Citation

Naciongayo, J.J.B., Santander-de Leon, S.M.S. (2024). Assessment of Benthic Macrofauna and Sediment Characteristics of Boracay Island, Philippines. *Sustainable Aquatic Research, 3*(1), 58-76.

Article History

Received: 12 October 2023
Received in revised form: 28 December 2023
Accepted: 28 December 2023
Available online: 30 April 2024

Corresponding Author

Jesseree Joyce B. Naciongayo
E-mail: jbnaciongayo@up.edu.ph
Tel: +033 3158381

Keywords

Benthic Macrofauna
Abundance
Distribution
Diversity
Sediments

Abstract

Boracay island is a world-famous beach located in the Philippines. Its beaches are economically important due to its unique “white sand” characteristics popular for swimming and other recreational activities. Studies on macrobenthic organisms and sediment characteristics in the area are still lacking. This study assessed the diversity, abundance, and distribution of the benthic macrofauna and the characteristics of the sediments in Stations 1, 2, and 3 of Boracay Island. Tide lines from various times of the day: high tide – morning (HTM), low tide (LT), high tide – evening (HTE); and at 0.5m water depth at high tide morning (0.5) were assessed. Overall, there were 474 individuals belonging to 5 classes, 10 families, and 10 genera collected from the three stations. The major taxa groups found were Bivalvia, Gastropoda, Malacostraca, Polychaeta, and Sipuncula. *Donax* sp. (wedge shell) was the most dominant species in the study area with 266 total individual counts. Among the three stations, Station 1 was the most diverse (H, 1.21) while Station 2 had the most abundant macrofaunal presence (relative abundance, 82.07%). In terms of area, HTE was the most diverse and abundant (H, 0.22; relative abundance, 60.97%). Most of the species found are known to be highly tolerant to wide conditions making it easier for them to adapt with less ideal conditions. The *Armandia* sp., found dominant in the area, is highly sensitive to marine pollution, making it an excellent indicator for future monitoring of Boracay Island and other similar sandy beaches.

Introduction

Sandy beaches is one of the most famous spots for tourist destinations, accommodating various human activities (Maguire et al., 2011). With this, beaches become one of the most exposed spaces to various physicochemical activities (Defeo & McLachlan, 2013). These exposed sandy beaches are categorized along a morphodynamic gradient, from dissipative to reflecting beaches. Reflective beaches are small, have rougher sand, a steeper slope, and have waves that crash along the front of the beach. Meanwhile, dissipative beaches are distinguished by finer sediments, a smooth slope, and a large wave breaking zone (Wright & Short, 1984). The difference in sediment composition of these beaches affects their ability to support particular biological groups (Alongi, 1990). Only a few taxa, predominantly crustaceans, can settle and flourish in harsh reflecting habitats where turbulent hydrodynamic regimes predominate, especially at supralittoral beach levels. As a result, sandy beach settlements are primarily shaped by their physical surroundings (Wright & Short, 1984).

Sandy beaches' biological communities can undergo various spatial and temporal scale changes (Defeo et al., 2009). According to Defeo et al. (2007), species identities vary between and within large geographic regions. The intertidal zone exhibits the widest distribution on a small scale, both vertically and horizontally, leading to a scattered dispersion of organisms especially of benthic macrofauna. Animals in the macrofauna class are categorized by their size. They often live in soft sediments, such as sands and muds, and occasionally gravel. These organisms can be obtained on a 0.5-1.0 mm mesh sieve and typically range from 1 mg to 2 g dry tissue weight. Benthic macrofauna resides on the top of the sediment (epifauna) or in the sediment (infauna). The study of Watling (2019) shows the majority of infauna live in the top few centimeters of the sediment due to their dependence on tiny organic particles that drop from the upper water column to the ocean floor. Furthermore, oxygen concentration is more abundant in the upper layers of the sediment. Their notable trait is a significant level of mobility, which includes the capacity to burrow quickly. The intertidal and surf zones of beaches include populations of these species that change through time. Although polychaete worms, mollusks, and crustaceans typically predominate, all major taxa are represented such as nemerteans, anthozoans, platyhelminthes, sipunculids, echiurans, insects, and echinoderms. Some of these taxa are restricted to protected beaches. In descriptions of sandy beaches, insects are frequently disregarded, but they can occasionally be the most numerous groups found on the shoreline (Cochran et al., 2019; McLachlan & Defeo, 2017).

It has long been understood that sediment properties have a significant impact on the composition and diversity of benthic infaunal populations (Gray, 1974). Numerous habitats and sizes have been investigated while examining the relationships between sediment properties and infaunal communities. It has been demonstrated that salinity, water depth, habitat shape, food abundance, and sediment grain size all affect the composition of infaunal populations (Lindegarth & Hoskin, 2001; Ellingsen, 2002). In the same manner, benthic macrofauna also have various impacts on the sediment's physicochemical properties. The activity of macrofauna is a crucial biological buffer against disturbances like excess organic matter and nutrient loadings, maintaining an oxidized upper sediment layer. In addition to preventing sediment dystrophy and sulfide release to the water column, it promotes mineralization and dissimilatory processes like denitrification. Macrofauna also expedites solute release and promotes benthic-pelagic coupling (Andersen & Pejrup, 2011). Moreover, seabed roughness and sediment buildup are both influenced by macrofauna. Animals usually cause the bed's roughness to intensify due to their own tubes or traces, which results in higher bed shear stress and the potential for bed erosion. Additionally, the macrofauna's eating and movement change how the sediment is accumulated, which has an impact on the sediment's erodibility (Nowell et al., 1981; Grant & Daborn, 1994).

One of the most well-known sandy beaches in the Philippines is Boracay Island, renowned for its crystalline blue ocean and fine white sand. Boracay is one of the top tourist destinations in the Philippines, which increases economic output, generates a large number of employment opportunities, improves infrastructure, and fosters cross-cultural interaction among visitors and locals. Due to its great socioeconomic worth, the island has been the focus of numerous scientific studies that have evaluated its water quality, nutrient intake, mangrove ecosystem, plankton dispersion, and many other factors. However, information about the presence of benthic macrofauna and the assessment of the sand's physicochemical characteristics is rarely available, which should have been given much attention because benthic macrofauna in sandy beaches is a good indicator of ecological status (Schlacher et al., 2008), especially in Boracay where it has become ecologically fragile and susceptible to pollution due to increased developments and tourism activities. With this, the study aims to assess the benthic macrofauna and the sediment characteristics of Boracay Island. Specifically, this aims to compare the diversity, abundance, and distribution of the benthic macrofauna in the three stations of Boracay Island as influenced by tide level, time, and area; and describe the sediment characteristics where the benthic macrofauna was observed.

Materials and Methods

In this study the diversity, abundance, and distribution of benthic macrofauna and sediment characteristics was assessed in the three stations of Boracay Island (Fig. 1). Samples from each station were collected from two areas: within the tide line (TL), and within the 0.5m water depth (0.5). Different sets of samples are also collected during the high tide - morning (HTM), low tide (LT), and high tide - evening (HTE). The macrofaunal samples were subjected for faunal identification, computation of diversity index; and total and relative abundance. Sediment samples were measured based on its salinity, redox potential level, sediment color and smell, total organic matter (TOM), and grain size.

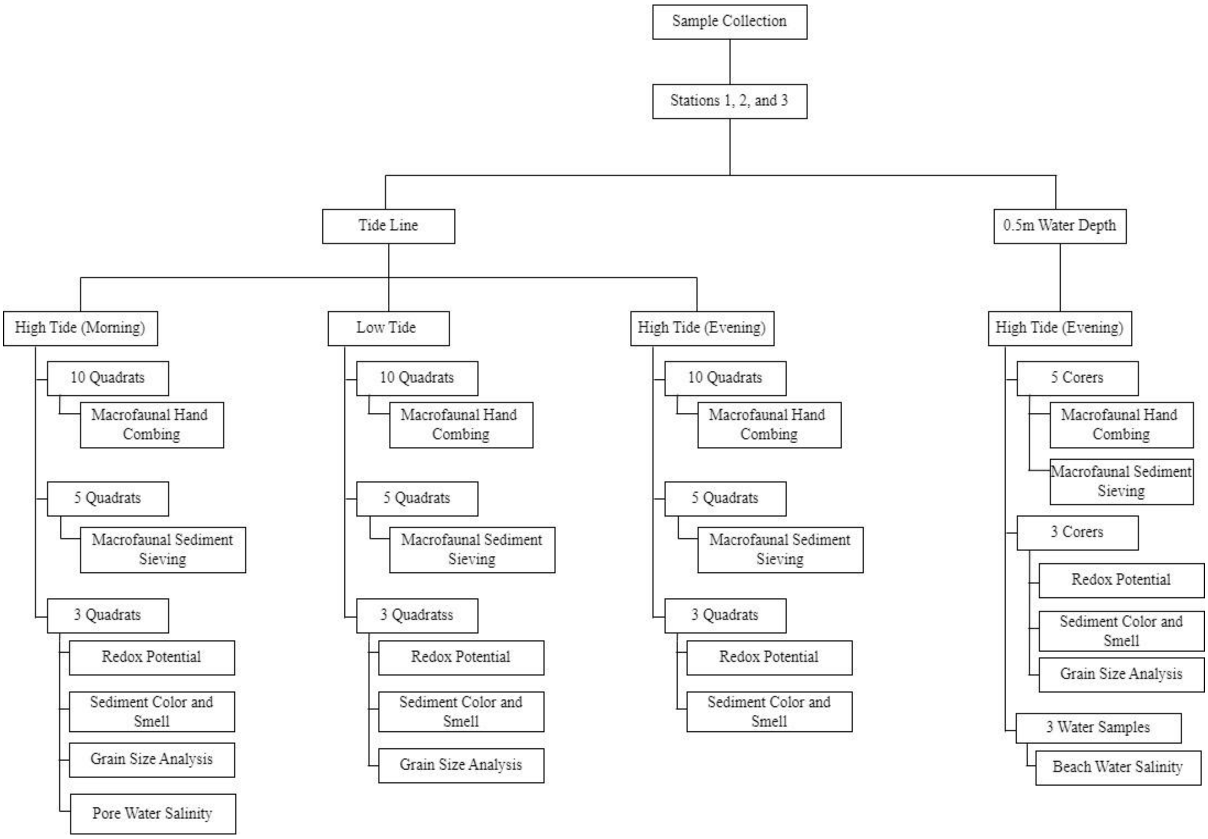


Figure 1. Sampling design for the assessment of benthic macrofauna and sediment characteristics in Stations 1, 2, and 3 of Boracay Island.

Description of Study Area

Boracay Island is situated in the Municipality of Malay, Aklan, northwestern tip of Panay, Western Visayas (Fig.2). The land mass has a maximum width of 3.3 km and a maximum length of 6.8 km, with greatest heights between 50-105 m above mean sea level. The total land area of Boracay Island is 1,006.64 hectares. The island is divided into three barangays - Manoc-Manoc, Balabag, and Yapak - and has over 17 beaches, with White Beach being the most popular (Limates et al., 2016).

The sampling sites of the study were located at the three stations (Figure 2). Station 1, with coordinates of 11° 58 '5.47"N Latitude and 121° 55' 7.08"E Longitude, is the farthest station from the Cagban Port (Boracay Port). According to classification, it has the best and finest stretch of white sands among all stations. This is also the station where the majority of the luxury hotels and resorts are located. Station 2, with coordinates of 11° 57 '39.43"N Latitude and 121° 55' 28.72"E Longitude, is located in the middle area of the beach. It is known as the busiest region of the beach because this is where most shops and restaurants are located. Station 3, with coordinates of 11° 57 '12.11"N Latitude and 121° 55' 44.51"E Longitude, is the nearest station from the port. This station houses mid-range and more affordable hotels and resorts (Morales, 2022).

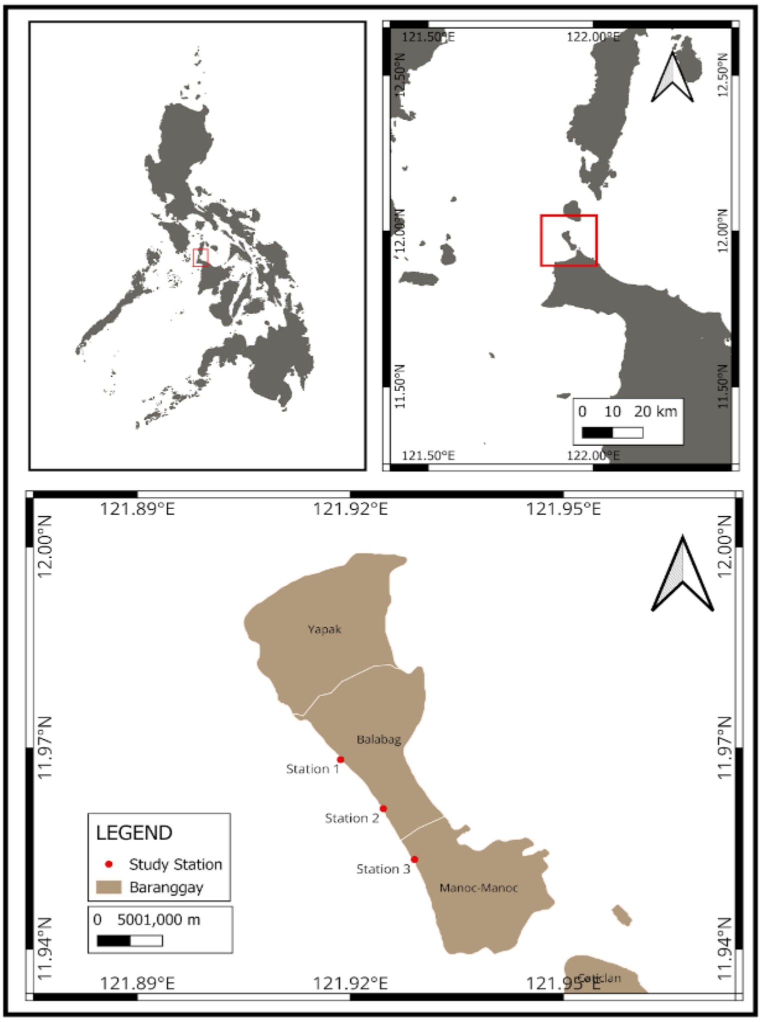


Figure 2. Map of Boracay Island indicating the three-sampling sites Stations 1, 2, and 3

Sampling Method

Sampling was was conducted in October 2022 in the three stations of Boracay Island. In each station, samples were collected in two different areas and at different points of the day to fully cover the occurrence and presence of the benthic organisms. The two different areas were within the tide line (TL) and at 0.5-meter water depth (0.5) while the different points of the day were one during the peak of high tide in the morning (HTM), one on the peak of low tide (LT) in the afternoon, and one on the peak of high tide in the evening (HTE). To collect the samples in the tide line, a 100-meter transect was placed within each station. Then 10 quadrats were equally distributed within the 100-meter distance. Each quadrat has a dimension of 0.5 x 0.5 meter. Samples were then collected within the quadrat using small shovels. To collect samples in the 0.5-meter water depth, a 100-meter transect was placed within the station. Then, 5 transect points were equally distributed within the given distance. In each point, a corer was used to collect the samples.

Macrofauna Collection

To collect the macrofauna in the tide line, two methods were used: hand-combing and sieving. For hand-combing, visible macrofauna within the top 5 cm layer of sediments were hand-picked and were manually counted. Then all collected macrofauna were placed in a container and were preserved with 10% formalin. For sieving, a depth of 5 cm of sediments were collected and were stored in a zip lock. In the laboratory, the sediments were sieved using a stack of 5 mm, 3 mm, 2mm, 1mm, and 0.5 mm sieve mesh. All sieved macrofauna were then preserved with 10% formalin. All the collected samples from two methods, hand-combing & sieving, were combined to get the total individual count per area and station.

To collect the macrofauna at 0.5m water depth, a corer was pushed directly into the sediment at 15 cm depth. Then, the corers were retrieved and observed if visible macrofauna was found. After that the sediments were transferred to a clean basin and were homogenized. A subsample of the homogenized sediment was then kept to be used for macrofaunal sieving. All visible macrofauna in the corer and those collected from the sieve were combined to get the total individual count per area and station.

For the identification of these samples, all collected macrofauna were initially sorted into major taxonomic groups. They were then placed in separate sample vials with identification labels. Taxonomic guides and literatures were used to identify each group at the lowest taxonomic level possible.

Sediment Characteristics

Sediment colors were analyzed using Munsell Soil Color Chart. The depth at where the sediment changed in color was also recorded. Black sediment with rotten egg smell was observed for the presence of hydrogen sulfide. Redox potential was measured using a redox meter (Horiba) where its Ag/AgCl probe was inserted in the top 1 cm layer of the sediments in the quadrat or core. For samples with both white and gray colors, both layers were measured for redox. Values were corrected with +150 mV. The salinity of the sediment was determined by excavating the sediments close to the tide line until pore water was visible. The pore water was filtered in situ using a plankton sieve to remove sediments. Then the filtered pore water was placed in the refractometer to determine salinity.

For grain size analysis, sediments were collected using small shovels or corer. Samples were homogenized before they were kept in airtight plastic bags. Samples were then brought to the laboratory for sieving. For dry sieving, 25 g of the sample was weighed and was dried in the oven for 24 hours at 110 degree Celsius. Samples were then transferred to the desiccator and cooled for 15 minutes. The samples were then added on a sieve stack with mesh sizes of Wentworth Scale classifications as follows: 5.66 mm, 4.76 mm, 3.36 mm, 2.83 mm (Granule); 1.41 mm (very coarse sand); 0.84 mm (coarse sand); 0.350 mm (medium sand); and 0.053 mm and 0.037 mm (coarse silt) (Holme and McIntyre, 1984). The weight for all fractions were recorded.

To measure for TOM, 3g of samples were dried for 24 hours in the oven at 80 degree Celsius then placed in the desiccator to cool. Samples were weighed. Combustion was done using a furnace for eight hours at 500 degree Celsius transferred in desiccator and weighed. The difference in the weight of the sample prior and after combustion was recorded as the total organic matter content of the samples.

Data Analysis

Mean values were calculated for salinity level, redox potential, and TOM in three different stations. Diversity index was computed using the formula of Shannon-Wiener Index (H) and Percent Relative Abundance (R) using the formula below:

$$H = p_i * ln(p_i)$$

where

H = Shannon-Weaver diversity index

p_i = the proportion of individuals (n/N) of one species (n) divided by the total number of individuals found (N)

ln = Natural logarithm

Σ = Summation from the first species to the last

$$R = \frac{n}{N} x100\%$$

where

R = percent relative abundance

n = Number of individuals of one species

N = Total number of individuals found

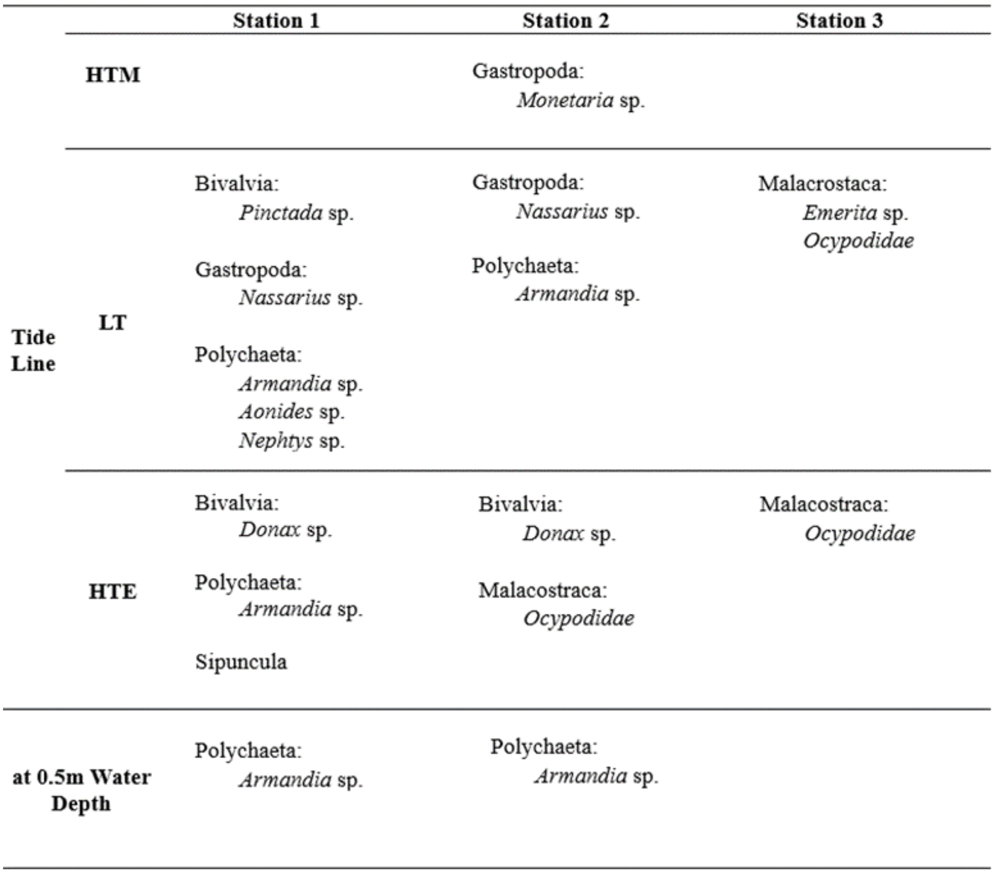
One-way ANOVA and Independent T-test were used to assess significant differences in sediment characteristics including salinity, total organic matter, and redox potential. Post Hoc Test was performed in the results of TOM to further quantify the observed differences.

Results and Discussions

Distribution, Abundance, and Diversity of Benthic Macrofauna

The distribution of the individual species collected from the study area presents the occurrence of the collected macrofauna based on tide level, time, and area (Table 1). It was observed that various taxa reside in each station and certain species are only present in particular locations during a specific time. For instance, during HTM, no macrofauna was observed in both Station 1 and Station 3, but a few gastropoda, specifically, *Monetaria* sp. (Cowrie) were collected in Station 2. During the LT, bivalvia, gastropoda, and polychaeta are observed in Station 1 and Station 2 while malacostracans like *Emerita* sp. (Pacific Mole Crab) and Ocypodidae (Ghost Crab) are seen to be settling in Station 3. It can also be noted that there are certain genera of polychaetes such as *Aonides* sp. and *Nephtys* sp. and bivalves like *Pinctada* sp. (Sea Mussel) that are observed in Station 1 but are not present in other stations. During the HTE, *Donax* sp. (Wedge Shell), *Nassarius* sp. (Nassa Mud Snails), and *Armandia* sp. are abundantly observed in both Station 1 and 2. Meanwhile, in Station 3, only Ocypodidae species are found. Ocypodidae are also found in Station 2 but none have ever been observed in Station 1 over the whole sampling period. A species from the class Sipuncula was also found solely in Station 1. Lastly, only one species of macrofauna was observed at 0.5m and was collected in Stations 1 and 2. This species was identified to be *Armandia* sp. and can also be observed in the tide line area.

Table 1. Distribution and composition of macrofauna in Stations 1, 2, 3 of Boracay Island. HTM = High Tide in the Morning, LT = Low Tide, HTE = High Tide in the Evening.



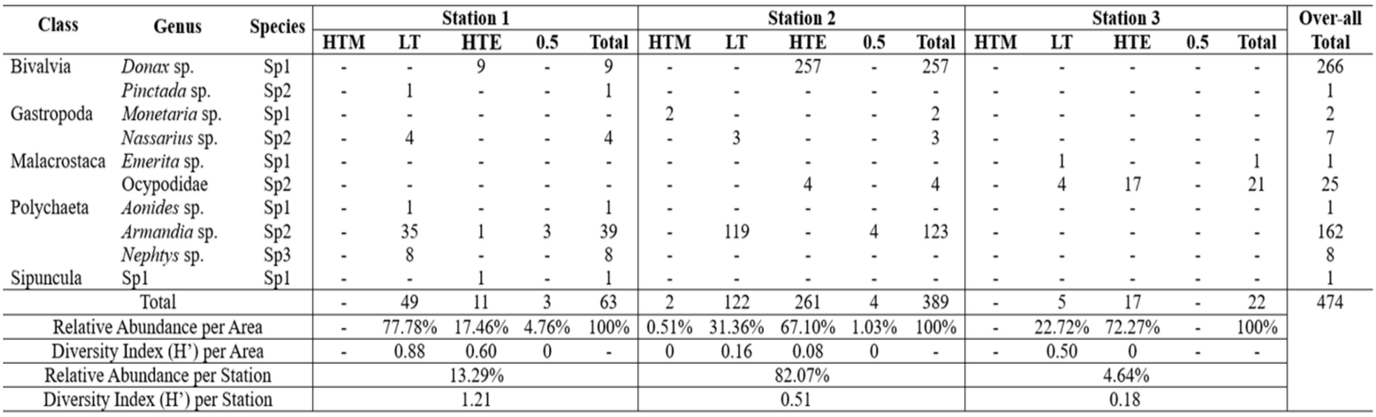
		Station 1	Station 2	Station 3
Tide Line	**HTM**		Gastropoda: *Monetaria* sp.	
	LT	Bivalvia: *Pinctada* sp. Gastropoda: *Nassarius* sp. Polychaeta: *Armandia* sp. *Aonides* sp. *Nephtys* sp.	Gastropoda: *Nassarius* sp. Polychaeta: *Armandia* sp.	Malacrostaca: *Emerita* sp. *Ocypodidae*
	HTE	Bivalvia: *Donax* sp. Polychaeta: *Armandia* sp. Sipuncula	Bivalvia: *Donax* sp. Malacostraca: *Ocypodidae*	Malacostraca: *Ocypodidae*
at 0.5m Water Depth		Polychaeta: *Armandia* sp.	Polychaeta: *Armandia* sp.	

The summary of abundance and diversity index of macrofauna in the three stations are shown in Table 2. In Station 1, most benthic macrofauna appeared in LT with a total of 49 individuals and a relative abundance of 77.89% while the least abundant area was observed in HTM with 0 individual counts and 0 relative abundance. The table also shows that the dominant genera in this station is *Armandia* sp. with a total count of 39 individuals and most of them also appeared during the low tide. Meanwhile, the least dominant species include *Pinctada* sp., *Aonides* sp., and *Sipuncula* sp. which only have 1 total count for each species. In Station 2, it can be observed that most benthic macrofauna appeared during HTE with a total of 261 individuals and a relative abundance of 67.10% while the least abundant was observed to be during HTM with only 2 individuals and a relative abundance of 0.51%. The table also shows that the most dominant species in this station is the *Donax* sp. with a total count of 257 which are only found during the evening. Meanwhile, the least dominant species in this station is the *Monetaria* sp. with a total of 2 individuals. In Station 3, most benthic macrofauna can be found during HTE with a total individual count of 17 and a relative abundance of 77.27 % while no macrofauna was observed during HTM and at the 0.5m. The table also suggests that the dominant species in this Station is Ocypodidae while the least dominant is *Emerita* sp. with a total count of 21 and 1, respectively.

Table 2 also shows the comparison of abundance per station and per area and time. This indicates that in terms of station, Station 2 is the most abundant with a total of 389 individuals and a relative abundance of 82.07% while Station 3 is the least abundant with only 22 individuals and a relative abundance of 4.64%. In terms of area, most macrofauna appeared in the HTE with a total of 289 individuals and a relative abundance of 60.97% meanwhile the least benthic macrofaunal presence was observed in the HTM with a total count of 2 individuals and a relative abundance of 0.42%. Furthermore, *Donax* sp. dominated the three stations with a total count of 266.

In terms of area, all stations show low diversity, with HTE having the highest diversity with H = 0.22, based on the Diversity Index Criteria (Table 3). However, the diversity index per station shows that Station 1 is moderately diverse with a diversity index of 1.21 while Stations 2 and 3 remain to be at low diversity with Station 3 being the least among the three of them with only 0.18 diversity index.

Table 2. Occurrence, Composition, Relative Abundance, and Diversity Index (H) of benthic macrofauna in Stations 1, 2, 3 of Boracay Island



Class	Genus	Species	Station 1					Station 2					Station 3					Over-all
			HTM	LT	HTE	0.5	Total	HTM	LT	HTE	0.5	Total	HTM	LT	HTE	0.5	Total	Total
Bivalvia	*Donax* sp.	Sp1	-	-	9	-	9	-	-	257	-	257	-	-	-	-	-	266
	Pinctada sp.	Sp2	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Gastropoda	*Monetaria* sp.	Sp1	-	-	-	-	-	2	-	-	-	2	-	-	-	-	-	2
	Nassarius sp.	Sp2	-	4	-	-	4	-	3	-	-	3	-	-	-	-	-	7
Malacrostaca	*Emerita* sp.	Sp1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1
	Ocypodidae	Sp2	-	-	-	-	-	-	-	4	-	4	-	4	17	-	21	25
Polychaeta	*Aonides* sp.	Sp1	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1
	Armandia sp.	Sp2	-	35	1	3	39	-	119	-	4	123	-	-	-	-	-	162
	Nephtys sp.	Sp3	-	8	-	-	8	-	-	-	-	-	-	-	-	-	-	8
Sipuncula	Sp1	Sp1	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	1
Total			-	49	11	3	63	2	122	261	4	389	-	5	17	-	22	474
Relative Abundance per Area			-	77.78%	17.46%	4.76%	100%	0.51%	31.36%	67.10%	1.03%	100%	-	22.72%	72.27%	-	100%	
Diversity Index (H') per Area			-	0.88	0.60	0	-	0	0.16	0.08	0	-	-	0.50	0	-	-	
Relative Abundance per Station			13.29%					82.07%					4.64%					
Diversity Index (H') per Station			1.21					0.51					0.18					

Table 3. Diversity Index Criteria by Wibowo et al., (2021) used to assess the diversity status of macrofauna in Stations 1, 2, and 3 of Boracay Island.

Value of H’	Indication
H’≤1	Low Diversity
1≤H’≤3	Moderate Diversity
H’≥3	High Diversity

The abundance per species in each station is also presented in Tables 4-6. As shown, *Armandia* sp. is the most abundant species in Station 1 with 61.90% abundance while *Pinctada* sp., *Aonides* sp., *Nephtys* sp., and Sipuncula are the least abundant with 1.59%. For Station 2, *Donax* sp. is the most abundant species with 66.06% relative abundance while *Monetaria* sp. is the least abundant. For Station 3, Ocypodidae is the most abundant species with 95.45% abundance while *Emerita* sp. is the least abundant with 4.5% abundance.

Table 4. Total abundance of individual species and their relative abundance in Station 1, Boracay Island. Ind=Individual.

Class	Genus	No. of Ind	Relative Abundance (%)
Bivalvia	*Pinctada* sp.	1	1.59
	Donax sp.	9	14.29
Gastropoda	*Nassarius* sp.	4	6.35
Polychaeta	*Armandia* sp.	39	61.90
	Aonides sp.	1	1.59
	Nephtys sp.	8	12.70
Sipuncula	Sp1	1	1.59
Total	**7**	**63**	**100**

Table 5. Total abundance of individual species and their relative abundance in Station 2, Boracay Island. Ind=Individual.

Class	Genus	No. of Ind	Relative Abundance (%)
Bivalvia	*Donax* sp.	257	66.06
Gastropoda	*Monetaria* sp.	2	0.51
	Nassarius sp.	3	0.77
Malacostraca	Ocypodidae	4	1.03
Polychaeta	*Armandia* sp.	123	31.62
Total	**6**	**389**	**100**

Table 6. Total abundance of individual species and their relative abundance in Station 3, Boracay Island. Ind=Individual.

Class	Genus	No. of Ind	Relative Abundance (%)
Malacostraca	*Emerita* sp.	1	4.55
	Ocypodidae	21	95.45
Total	**2**	**22**	**100**

The benthic macrofauna of sandy beaches comprises members of numerous phyla, although crustaceans, mollusks, and polychaetes are typically dominant and can be separated into intertidal and supralittoral forms (Brown & McLachlan, 1990). These macrofauna are influenced by the tidal cycle, wave pattern, and sediment composition (Defeo & McLachlan, 1991). Previous assessment of the benthic macrofaunal assemblages in the southern beaches of Philippines also shows the occurrence of sipuncula, polychaetes, and bivalves in the study area (Leopardas et al., 2014). In a study conducted in west portion beaches of Guimaras, Philippines most macrobenthic invertebrates include hard and soft coral, gastropods like cowrie, bivalves, and malacostracans. These organisms live in sandy-silty types of substrates with salinity range of 28-30 ppt (De La Cruz et al., 2012). These observations are also parallel to the results of this study which shows the occurrence of the same classes of benthic macrofauna.

The benthic macrofauna living in these sandy sediments are essential for the mixing, ventilation, oxygenation, and irrigation of sediments (bioturbation) (Meysman et al., 2005; Snelgrove et al., 2018). The bioturbation activity enhances benthic-pelagic connection, substrate permeability, food resource redistribution, buffering against nutrient enrichment, and nutrient cycling (Kristensen et al., 2012). The chemical reactions in general such as redox potential are positively influenced and the depth of the oxic layer is extended over the anoxic one (Koike & Mukai, 1983; Snelgrove et al., 2018).

The high productivity of certain sandy beaches is mainly due to the density of interstitial fauna. Species diversity is usually low and confined to a few species of polychaetes, molluscs, and crustaceans. Among the invertebrates, molluscs are the most important taxa in terms of biomass (McLachlan, 1983). The ecological dominance of some species depends on various factors such as environmental parameters, competition or the physiological and behavioral adaptations of these species. Bivalves of the genus *Donax* are an important constituent of the fauna of sandy beaches. Because of their physiological makeup, they can stay in the intertidal zone and reduce their risk of stranding and desiccation (Ansell et al., 1980). They tend to be particular about their substrate, therefore they only dig in sand with an appropriate grain size. These bivalves burrow more quickly as grain sizes get smaller thus significant numbers of this species are observed where grain sizes are smaller (De La Huz et al., 2002). Furthermore, *Donax* sp., specifically *D. trunculus*, has resistance to low and high salinity levels (Neuberger-Cywiak et al. 1989). The majority of bivalve species can also endure anoxic environments by lowering metabolic energy expenditures (Yusseppoine et al, 2018). Another feature of bivalve is the presence of alternate oxidase in their mitochondria which renders bivalves tolerant to hydrogen sulfide and low redox potential (Fenchel & Finlay, 1995). A prior study explored the distribution of *Donax* sp. which demonstrates that in response to regular and seasonal environmental fluctuations such as tides, waves, and currents, they typically undergo patchy but aggregated distribution (Neuberger-Cywiak et al., 1989). Interspecific competition with *Emerita* sp. may have contributed to the aggregated pattern in *Donax*. A patchy distribution may also be the result of other interactions with other organisms (Leber 1982).

Patchy distribution is also observed in filter feeders such as polychaete worms for the same reasons. Polychaete worms like *Armandia* sp., *Aonides* sp., and *Nephtys* sp. generally favor fine sands with little to no mud content for their habitats. Since a compacted substrate or low porosity hinders their capacity to burrow, these species, especially the *Armandia* sp., are extremely sensitive to sediment textures (Saes et al., 2018). These marine worms can also tolerate lower salinities up to 20 ppt (McLachlan & Brown 2006; Woodin, 1974). Additionally, the majority of polychaete species probably aren't extremely resistant to hypoxia, but they have life-history traits that make it possible for them to quickly adapt after better oxygen conditions. For instance, family of polychaete worms such as the Spionid reveals that it can endure severe episodes of hypoxia for at least two weeks without suffering any significant mortality (Llansó, 1991). Additionally, in the work done by Saes et al. (2018), species from the genus *Armandia,* notably the *Armandia agilis*, were tested as model organisms for sediment toxicity assessments. The outcome shows that *A. agilis* responses are comparable to other sensitive benthic animals revealing that this polychaete is responsive to contaminated sediments and may not be a pollution tolerant. With this, the abundant presence of *Armandia* species in the study area may indicate the current environmental health status of the island.

Gastropods like *Monetaria* sp. and *Nassarius* sp., are also among the benthic macrofauna that usually resides in the intertidal zones. Generally, gastropods are one of the most competitive scavengers on sandy beaches. Species like *Nassarius* sp. plays a crucial part as a cleaner of an area (Morton, 1994). Like bivalves, these gastropods also reside in finer sand, typically 2mm or less grain sizes, as they don’t burrow well in larger sediment sizes. Previous studies on physiological energetics of *Nassarius* sp. also have shown that this species is tolerant to environmental stresses, including low salinity and hypoxia (Morton, 1997). However, continuous exposure to such stress increases susceptibility of gastropods with diseases (Wittmann & Pörtner, 2013).

A distinct species that was discovered along the tide line of Station 1 can be traced into the class of

Sipuncula. Sipuncula is also considered as a marine worm, however, unlike polychaetes, this species is unsegmented. Many of the sipunculan worms live in soft substrates and are deposit feeders. They frequently reside inside the shells of specific molluscs or the unfilled tubes of polychaetes and mostly play a role as significant bioturbators. Sipuncula are rarely abundant, yet occasionally they can dominate other species. In the study conducted by Ferrero-Vicente et al. (2011), there is a negative link between the quantity of sipuncula and fine sand and a substantial positive correlation between medium sand. There are also few investigations on the reaction of sipunculans to rapid changes in salinity and organic matter. However, some species have been found to have osmoconformers with limited ion regulating abilities in response to salinity change events (Chew et al., 1994).

Unlike the above mentioned macrofauna, malacostracans like *Emerita* sp. and Ocypodidae can withstand coarser sediments because of their burrowing mechanism and locomotory abilities (Lastra et al., 2002). The study of Dugan et al. (2004) also observed that crabs dug faster in coarser sand and slower in finer sand, contrary to the theory that *Emerita analoga* is a generalist in terms of sediment. The study also noted that while grain size affects burrowing speed, it has no immediate impact on crab mortality. A defining ecological trait of this class is its fossorial habit where the crabs excavate deep, voluminous and complex burrows alternating between activity on the beach surface and underground. They also have evolved to a range of physiological, morphological, and behavioral adaptations which results in the increased tolerance in fluctuating environmental parameters. Decapod crabs can endure a wide range of salinity but their survival rate is optimal at salinity level of 30 ppt and higher (Varadharajan et al. 2013). These species also flourish in areas with higher organic matter content as revealed in the study of Frusher et al. (1994). According to Rahim et al. (2021), crabs actively aid in the cycling of organic matter as these crabs bury the organic matter in the sediments.

Sediment Characteristics

The sediments in the three stations of Boracay were subjected to different parametric tests such as color, smell, salinity, redox potential, total organic matter content (TOM), and grain size analysis. The results revealed that sediments in the sampling area have two distinctions: white and odorless sand, and gray and sulfide-smelling sand (Figure 3). The sulfide odor was described to smell like a rotten egg. Generally, the sediments in Boracay are white and odorless. However, in Station 1 and in some parts of Station 2 (at low tide line and 0.5m water depth), sediments start to change in color and smell 5 cm below the surface.

Pipes partly buried in the sand were observed in the sampling areas. Previous reports of inadequate sewage and wastewater management systems is one of the prevalent issues in Boracay island. Wastewater discharge may have a negative impact on the ecosystem, such as reducing aquatic biodiversity, altering the color and smell of sediments (Peng et al., 2021).

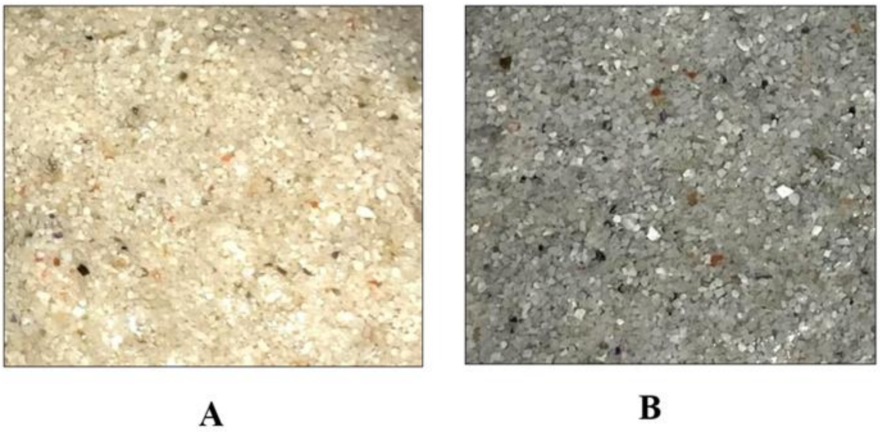


Figure 3. Collected sample of white and odorless sand (A) and gray and sulfide-smelling sand (B) in Boracay Island.

Figure 4A presents the results of the in-situ measurement of the salinity of beach water which was collected from the 0.5m water depth and pore water which was collected from the sediment in the tide line. Among the three stations, Station 1 has the highest beach water salinity level with 32.33 ppt while Station 3 has the lowest salinity with only 30.33 ppt. However, with regards to pore water salinity, Station 2 has the highest salinity level while Station 1 has the lowest salinity level with 24 ppt and 20.33 ppt, respectively. One-way ANOVA was used to determine whether there is a difference between mean values in each station and the p values show no significant difference for all stations. Over-all, beach water salinity has an average of 31.22 ppt and a range from 30.33 ppt - 32.33 ppt. Meanwhile, pore water salinity has an average of 22.55 ppt and a range from 20.33 ppt – 23.33 ppt. These indicate that pore water salinity is slightly lower than beach water salinity. An independent T-test was used to test if there is a significant difference between the salinities in beach water and pore water. The result shows a p-value of 0.002 indicating that there is a significant difference between the two.

The normal range of salinity for sandy beaches is between 30-35 ppt. Waska et al. (2021), also mentioned that salinity level decreases in deeper sediment depth which suggest that seawater has higher salinity level than beach porewater. This particular trend is observed in the result of this study where the salinity level of beach water is higher than that of pore water. Changes in salinity can be observed when there is a change in some ecological parameters such as the fluctuation of climate conditions because of low and high pressure or the runoff of freshwater and sewage (Wear et al., 2021). The study of Lercari & Defeo (2006), shows that with higher salinity, species richness was also evidently higher and it significantly decreased when the salinity range also decreased.

For the redox potential of white sand, results are summarized in Figure 4B. In Station 1 and 2, redox is highest at 0.5m with 938 mV and 974.67 mV, respectively and lowest in LT with 376 mV and 436 mV. For Station 3, redox is highest at HTM and lowest at LT with 896.67 mV and 726 mV. Over-all, Station 3 has the highest redox while Station 1 has the lowest with 826 mV and 704.41 mV, respectively. In terms of area, 0.5m has the highest redox with 923.56 mV while LT has the lowest with 512.67 mV. One way ANOVA was also used to test if there is a significant difference among the areas in each station, the p-values ($p = 0.179$; $p = 0.185$) show no significant difference in HT and 0.5m. However, a low p-value in the LT ($p < 0.001$) suggests that there is a significant difference among the three stations in this area. Thus, to further investigate this difference, a Tukey Post-Hoc test was performed which later revealed that the difference can be observed between Station 1 and 3 and Station 2 and 3.

Figure 4C summarizes the redox potential for gray sand. In Station 1, the highest redox is at 0.5m with 702.67 mV while lowest at LT with 149.33 mV. In Station 2, redox is highest at 0.5m with 855.33 mV and lowest at LT with -9.67 mV. No data was recorded for Station 3 because there was no observed gray sand in the area. Overall, the highest redox potential was observed in Station 1 with 476.58 mV while Station 2 is the lowest with 422.83 mV. In terms of area, 0.5m is the highest and LT is the lowest with 779 mV and 69.83 mV, respectively. White sand redox potential is greater than the gray sand at $p<0.008$.

Positive value of redox potential (+1 mV to 100 mV) indicates aerobic sediment or oxic conditions and larger benthic fauna accumulation while negative values (< -100 mV) indicate anaerobic sediments or anoxic conditions which contributes to the decline in environmental condition thus limiting biological processes (Holmer et al. 2005). High redox potential gradient is taken advantage of by a diverse microbial community in an undisturbed sediment. For instance, the top few millimeters of sediment, where oxygen is abundant and redox potentials are high, is inhabited by aerobic bacteria and other aerobic organisms. As the redox potential drops farther down, the aerobic organisms will no longer be observed and are replaced by fermenters. Anaerobic respiration takes over when the soil gets less oxygenated (McLachlan et al., 1979). Poorly oxygenated sediments can also occur in sandy beaches characterized as gray or black color sediment and emit a rotten egg smell of Hydrogen Sulfide (H_2S) (Burone et al. 2003). Previous study revealed that gray sand with rotten egg smell has lower redox potential (McLachlan et al., 1979). These observations are parallel to the result of the study which revealed that as sediments change from white sand to gray sand, the redox potential decreased with rotten egg smell, characteristics of anoxic conditions.

The TOM results are summarized in Figure 4D. In Station 1, TOM is the highest at 0.5m and lowest in LT with 1.38% and 0.87% respectively. In Station 2, TOM is highest in LT with 1.68% and lowest at 0.5m with 0.98%. Meanwhile in Station 3, TOM is highest in HT and lowest in LT with 3.42% and 2.94%. Overall, TOM is the highest at 0.5m with a mean percentage of 1.92% while lowest in LT with a mean percentage of 1.83%. In terms of station, Station 3 has the highest TOM for all areas with mean percentage of 3.26% while Station 1 has the lowest TOM in all areas with 1.09%. Results show $p < 0.05$ for all areas suggesting that there is a significant difference between the three stations.

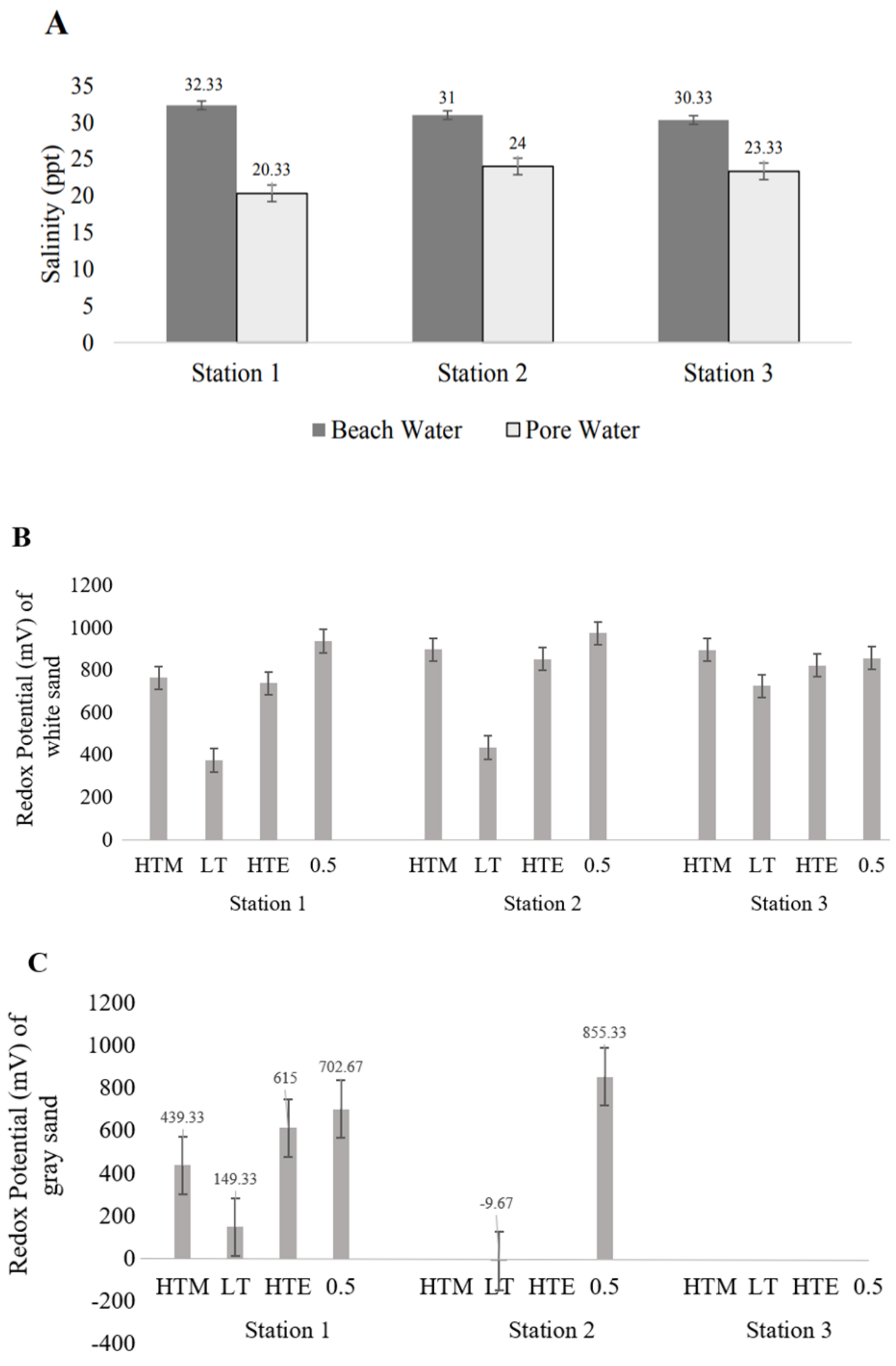
A number of factors, including sedimentary characteristics, the rate of microbial degradation, column water productivity, and terrestrial inputs, affect the amount of organic carbon in surface sediments (Burone et al., 2002). The density of meiobenthic fauna is characterized by organic matter concentrations (Gierre, 2009). An increase in organic matter in sediments promotes the richness and diversity of many organisms (Lercari and Defeo, 2003). However, there is a community shift if there is a presence of high total organic matter especially in sandy beaches where most species are under aerobic processes and prefer oxic conditions. High TOM stimulates anaerobic processes which is less ideal for sandy beach species. Thus, in this context, TOM is inversely related to the abundance of beach organisms (Khan et al., 2012). This is further supported by another study which states that TOM is negatively correlated with redox potential because the aerobic bacteria involved with the absorption of dissolved oxygen in the breakdown of organic matter depletes oxygen which results in lower redox potential level (Xia et al., 2022). It was also observed that the concentrations of organic matter were much higher on protected beaches than on exposed ones, and the protected beaches' low hydrodynamic conditions encouraged the accumulation of sedimentary organic matter (Incera et al., 2003). These findings corroborate the study's result which shows a low diversity and abundance in Station 3 and at 0.5 where TOM was recorded to be the highest.

For grain size analysis, each station shows a varied dominant proportion of sizes. Figure 4E shows that coarse silt has the highest percentage in all areas of Station 1, with a mean percentage of 82.57%, 94.53%, and 72.29% for HT, LT, and 0.5m respectively. Station 1 is mostly covered with coarse silt with a total percentage of 83.13%. Silt is a granular material between sand and clay which means that the sand in Station 1 is very fine.

On the other hand, the grain size analysis in Station 2 suggests a variation in size per area. For instance, in HT and 0.5m, most of the sediments are classified as medium sand with a mean percentage of 52.61% and 43.93%. While in LT, most sediments are coarse silt with a mean percentage of 81.72%. This shows a trend where sediments go from coarse to fine to coarse again. However, overall, Station 2 is still mostly composed of coarse silt with a total percentage of 45.75%.

Lastly, Station 3 has a lot of coarser sand compared to Station 1 and 2. The station is mostly covered with medium sand in HT and LT with a mean percentage of 57.97% and 51.74%. While coarse sand covers most of the area in 0.5m with a mean percentage of 35.33%. Over-all, Station 3 is dominated by medium sand with a total percentage of 47.06%.

Grain size has an influence on the diversity and number of species of macrofauna (Soto et al., 2017). According to the study of Rodil and Lastra (2004), the same pattern appears from exposed to very exposed sandy beaches where biomass of macrofauna decreases as the average grain size increases. These patterns are consistent with earlier research on several coastlines across the globe where coarse sands restrict the benthic macrofauna. Furthermore, the study of Barboza & Defeo (2015), mentioned that reduced sand particle size and wider, flatter beaches led to a large increase in species richness. At a worldwide level, species richness was mostly predicted by grain size and beach slope, which increased from reflective to dissipative beaches. This pattern is in line with the Swash Exclusion Hypothesis, which claims that species richness, abundance, and biomass steadily increase when circumstances shift from reflecting to dissipative. Because of the constancy of these patterns across the globe and the close connection between morpho dynamics and species richness at regional scales, it is possible to forecast the species richness of sandy beaches based on the physical characteristics of beach habitats.


A
Salinity (ppt)
35
30
25
20
15
10
5
0
32.33
20.33
31
24
30.33
23.33
Station 1
Station 2
Station 3
■ Beach Water
□ Pore Water
B
Redox Potential (mV) of white sand
1200
1000
800
600
400
200
0
HTM LT HTE 0.5
Station 1
HTM LT HTE 0.5
Station 2
HTM LT HTE 0.5
Station 3
C
Redox Potential (mV) of gray sand
1200
1000
800
600
400
200
0
-200
-400
439.33
149.33
615
702.67
-9.67
855.33
HTM LT HTE 0.5
Station 1
HTM LT HTE 0.5
Station 2
HTM LT HTE 0.5
Station 3

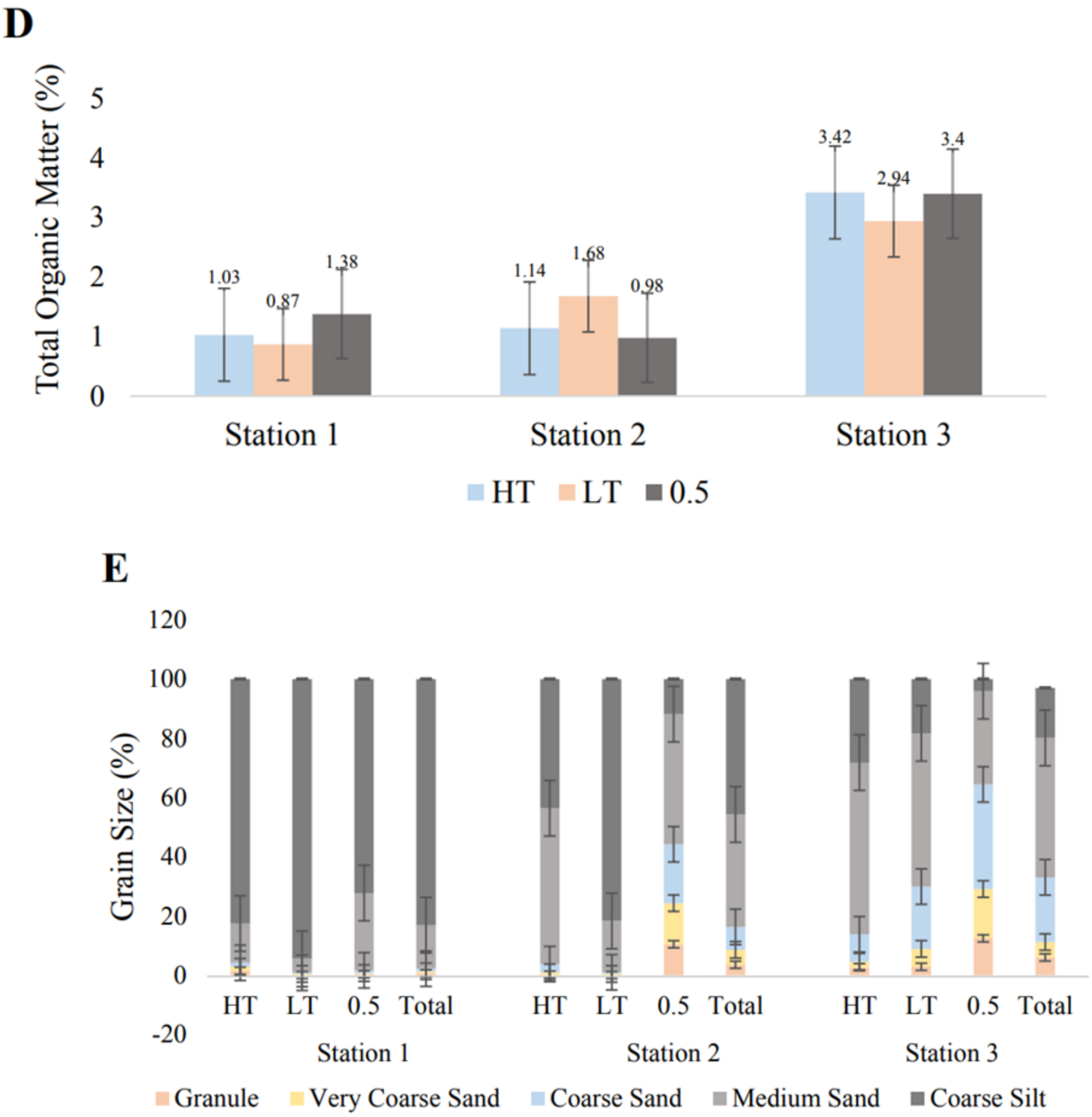


Figure 4. . Sediment Characteristics (A-E) in Stations 1, 2, 3 of Boracay Island. Values are mean and mean percentage (n=3). HT = High Tide, LT = Low Tide, HTM = High Tide in the Morning, HTE = High Tide in the Evening, 0.5 = 0.5m Water Depth

Benthic macrofaunal composition, structure, and distribution are highly influenced by the sediment characteristics. For instance, most of the benthic macrofauna, such as the bivalves, polychaetes, and gastropods, reside in Stations 1 and 2, specifically in the low tide line because these are the areas in the site with smaller sediment sizes. Finer sediments make the burrowing capacity and the locomotion activities of these species faster and more efficient. Meanwhile, malacostracans mostly occurred in station 3 and at 0.5m water depth because their mobility is quicker in bigger grain sizes. Living in a sandy beach where there are fluctuating environmental conditions and high exposure predators and other anthropogenic activities, these species need to develop fast burrowing behavior to protect themselves. Furthermore, diversity and abundance of species is higher in Station 1 and Station 2 where TOM is lesser. TOM stimulates anaerobic processes which are less ideal with beach organisms which live through aerobic processes and in oxic conditions. The result of the redox potential level in this area also shows high positive values which indicates that the sediments are in oxic conditions. However, there are also areas in the sampling site, notably in Station 1 and 2, that contain gray sand with sulfide odor and a comparatively lower redox potential which could indicate anoxic conditions that are still inhabited by some benthic macrofauna, commonly by *Donax* sp. and *Armandia* sp. These two species are also found to be the top two most abundant species in the whole sampling area. The dominance and occurrence of these species may be attributed to their ability to thrive in both anaerobic and aerobic conditions.

Conclusion

The study showed the benthic macrofauna composition and distribution in the sandy beach of Boracay Island; as well as the sediment characteristics where these benthic macrofauna reside. Overall, there are 474 individuals belonging to 5 classes, 10 families, and 10 genera collected from the three stations. The major taxa groups found are Bivalves, Gastropods, Malacostracans, Polychaetes, and Sipuncula. *Donax* sp., under the class of Bivalvia, was the most dominant species in the study area with an overall count of 266. Some taxa were only observed in some stations and areas at a certain period of time. Pore water salinity only measured at 23.33 ppt. Gray and sulfide-smelling sand were present in all areas of Station 1 and in some parts of Station 2, starting from 5 cm below the surface. The dominance of some species can be attributed to their ability to be non-selective and to thrive in anaerobic and aerobic conditions. Some of the macrofauna, specifically the *Armandia* sp., is also studied to be highly sensitive to marine pollution making it a good indicator of the health status of a certain study area.

Acknowledgements

The authors are grateful for the field assistance of Mr Joshua Bacunador.

Ethical approval

The authors declare that this study complies with research and publication ethics.

Informed Consent

Not Applicable

Conflict of interest

There is no conflict of interests for publishing this study.

Data availability statement

The authors declare that the data from this study are available upon request.

Funding organizations

N/A

Author contributions

JNaciongayo- Conceptualization, Sampling, Analyses, Writing, Editing

SSSantander-de Leon - Conceptualization, Sampling, Analyses, Editing

References

Alongi, D. M. (1989). Ecology of Tropical soft-bottom benthos: a review with emphasis on emerging concepts. Review Biology, 37, 85–100.

Andersen, T. J., & Pejrup, M. (2011). Biological influences on sediment behavior and transport. In Elsevier eBooks, 289–309. https://doi.org/10.1016/b978-0-12-374711-2.00217-5

Ansell, A. D., & Lagardère, F. (1980). Observations on the biology of *Donax trunculus* and *D. vittatus at Ile d'Oleron* (French Atlantic coast). Marine Biology, 57(4), 287–300. https://doi.org/10.1007/bf00387572

Barboza, F. R., & Defeo, O. (2015). Global diversity patterns in sandy beach macrofauna: a biogeographic analysis. Scientific Reports, 5(1). https://doi.org/10.1038/srep14515

Brown, A. C., & McLachlan, A. (1990). Ecology of Sandy Shores. 328 pp. Amsterdam: Elsevier.

Burone, L., Muñiz, P., Pires-Vanin, A. M. S., & Rodrigues, M. (2003). Spatial distribution of organic matter in the surface sediments of Ubatuba Bay (Southeastern - Brazil). Anais Da Academia Brasileira De Ciencias, 75(1), 77–80. https://doi.org/10.1590/s0001-37652003000100009

Chew, S. F., Peng, K. W., Low, W. P., & Ip, Y. K. (1994). Differences in the responses between tissues of the body wall and the internal organs of *Phascolosoma arcuatum* (Sipuncula) to changes in salinity. Comparative Biochemistry and Physiology Part A: Physiology. https://doi.org/10.1016/0300-9629(94)90286-0

Cochran, K. J., Bokuniewicz, Henry J., & Yager, P. L. (2019). Encyclopedia of Ocean Sciences: A derivative of the Encyclopedia of Ocean Sciences (3rd ed.). Academic Press.

Defeo, O., Barboza, C. a. M., Barboza, F. R., Aeberhard, W. H., Cabrini, T. M.,Cardoso, R. S., Ortega, L., Skinner, V. B., & Worm, B. (2007). Aggregate patterns of macrofaunal diversity: An interocean comparison. Global Ecology and Biogeography, 26(7), 823–834. https://doi.org/10.1111/geb.12588

Defeo, O., McLachlan, A., Schoeman, D. S., Schlacher, T. A., Dugan, J. E., Jones, A. R., Lastra, M., & Scapini, F. (2009). Threats to sandy beach ecosystems: A review. Estuarine Coastal and Shelf Science, 81(1), 1–12.

https://doi.org/10.1016/j.ecss.2008.09.022

Defeo, O., & McLachlan, A. (2013). Global patterns in sandy beach macrofauna: Species richness, abundance, biomass and body size. Geomorphology, 199, 106–114. https://doi.org/10.1016/j.geomorph.2013.04.013

De La Cruz, M. J. L., Flores, J. R. P. V., Magramo, M., Madas, C., & Terunez, M. (2012). Macrobenthic Composition of Sea Water Associated with Seagrass in East and West Portions of the Igang Bay, Nueva Valencia, Guimaras. JPAIR Multidisciplinary Research, 7(1), 106–118. https://doi.org/10.7719/jpair.v7i1.156

De La Huz, R., Lastra, M., & López, J. (2002). The influence of sediment grain size on burrowing, growth and metabolism of Donax trunculus L. (Bivalvia: Donacidae). Journal of Sea Research, 47(2), 85–95. https://doi.org/10.1016/s1385-1101(02)00108-9

Dugan, J. E., Jaramillo, E., Hubbard, D. A., Contreras, H., & Duarte, C. (2004). Competitive interactions in macroinfaunal animals of exposed sandy beaches. Oecologia, 139(4), 630–640. https://doi.org/10.1007/s00442-004-1547-x

Ellingsen, K. (2002). Soft-sediment benthic biodiversity on the continental shelf in relation to environmental variability. Marine Ecology Progress Series, 232, 15–27. https://doi.org/10.3354/meps232015

Fenchel, T., & Finla, B. J. (1995). Ecology and evolution in anoxic worlds. Oxford Science Publication. Vol 9, 259–260. https://doi.org/10.1046/j.1420-9101.1996.9020259.x

Ferrero-Vicente, L. M., Loya-Fernández, A., Marco-Méndez, C., Martinez-Garcia, E., & Sánchez-Lizaso, J. L. (2011). Soft–bottom sipunculans from San Pedro del Pinatar (Western Mediterranean): influence of anthropogenic impacts and sediment characteristics on their distribution. Animal Biodiversity and Conservation, 34(1), 101–111. https://doi.org/10.32800/abc.2011.34.0101

Frusher, S., Giddins, R. L., & Smith, T. J. (1994). Distribution and Abundance of Grapsid Crabs (Grapsidae) in a Mangrove Estuary: Effects of Sediment Characteristics, Salinity Tolerances, and Osmoregulatory Ability. Estuaries, 17(3), 647. https://doi.org/10.2307/1352412

Grant, J., & Daborn, G. (1994). The effects of bioturbation on sediment transport on an intertidal mudflat. Netherlands Journal of Sea Research 32 (1), 63–72.

Gray, J. S. (1974). Animal-sediment relationships. Oceanography and Marine Biology: An Annual Review, 12: 223–261.

Holme, N. A., & McIntyre, A. D. (1984). Methods for the Study of Marine Benthos. 2nd Editions, 387 pp. Blackwell.

Holmer, M., Wildish, D. J., & Hargrave, B. T. (2005). Organic Enrichment from Marine Finfish Aquaculture and Effects on Sediment Biogeochemical Processes. In Springer eBooks (pp. 181–206). https://doi.org/10.1007/b136010

Incera, M., Cividanes, S., López, J., & Costas, R. C. (2003). Role of hydrodynamic conditions on quantity and biochemical composition of sediment organic matter in sandy intertidal sediments (NW Atlantic coast, Iberian Peninsula). Hydrobiologia, 497(1/3), 39–51. https://doi.org/10.1023/a:1025405519829

Khan, S. a. R., Ansari, K. G. M. T., & Lyla, P. S. (2012). Organic matter content of sediments in continental shelf area of southeast coast of India. Environmental Monitoring and Assessment, 184(12),7247–7256. https://doi.org/10.1007/s10661-011-2494-8

Koike, I., & Mukai, H. (1983). Oxygen and inorganic nitrogen contents and fluxes in burrows of the shrimps *Callianassa japonica* and *Upogebia major*. Marine Ecology Progress Series, 12, 185–190. https://doi.org/10.3354/meps012185

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T. B., Quintana, C. O., & Banta, G.

T. (2012). What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Marine Ecology Progress Series, 446, 285–302. https://doi.org/10.3354/meps09506

Lastra, M., Dugan, J. E., & Hubbard, D. M. (2002). Burrowing and Swash Behavior of the Pacific Mole Crab *Hippa pacifica* (Anomura, Hippidae) in Tropical Sandy Beaches. Journal of Crustacean Biology, 22(1), 53–58. https://doi.org/10.1163/20021975-99990208

Leber, K. M. (1982). Bivalves (Tellinacea: Donacidae) on a North Carolina Beach: Contrasting Population Size Structures and Tidal Migrations. Marine Ecology Progress Series, 7, 297–301. https://doi.org/10.3354/meps007297

Leopardas, V., Uy, W. H., & Nakaoka, M. (2014). Benthic macrofaunal assemblages in multispecific seagrass meadows of the southern Philippines: Variation among vegetation dominated by different seagrass species. Journal of Experimental Marine Biology and Ecology, 457, 71–80. https://doi.org/10.1016/j.jembe.2014.04.006

Lercari, D., & Defeo, O. (2003). Variation of a sandy beach macrobenthic community along a human-induced environmental gradient. Estuarine, Coastal and Shelf Science, 58, 17–24. https://doi.org/10.1016/s0272-7714(03)00043-x

Lercari, D., & Defeo, O. (2006). Large-scale diversity and abundance trends in sandy beach macrofauna along full gradients of salinity and morphodynamics. *Estuarine, Coastal and Shelf Science*, *68*(1–2), 27–35. https://doi.org/10.1016/j.ecss.2005.12.017
Limates, V. G., Cuevas, V. C., & Benigno, E. (2016). Water quality and nutrient loading in the coastal waters of Boracay Island, Malay, Aklan, Central, Philippines. Journal of Environmental Science and Management, 15–29. https://doi.org/10.47125/jesam/2016_sp2/0

Limates, V. G., Cuevas, V. C., Tajolosa, M. a. T., & Benigno, E. (2016). Phytoplankton abundance and distribution in selected sites of Boracay Island, Malay, Aklan, Central Philippines. Journal of Environmental Science and Management, 1–14.https://doi.org/10.47125/jesam/2016_sp2/01

Lindegarth, M., & Hoskin, M. G. (2001). Patterns of distribution of macro-fauna in different types of estuarine, soft sediment habitats adjacent to urban and non-urban areas. Estuarine, Coastal and Shelf Science, 52(2), 237–247. https://doi.org/10.1006/ecss.2000.0741

Llansó, R. J. (1991). Tolerance of low dissolved oxygen and hydrogen sulfide by the polychaete *Streblospio benedicti* (Webster). Journal of Experimental Marine Biology and Ecology, 153(2), 165–178 https://doi.org/10.1016/0022-0981(91)90223-j

Maguire, G. S., Miller, K. K., Weston, M. A., & Young, K. (2011). Being beside the seaside: Beach use and preferences among coastal residents of south-eastern Australia. Ocean & Coastal Management, 54(10), 781–788. https://doi.org/10.1016/j.ocecoaman.2011.07.012

McLachlan, A. (1983). Sandy Beach Ecology - A Review. In Springer eBooks, 321–380. https://doi.org/10.1007/978-94-017-2938-3_25

McLachlan, A., & Brown, A. S. (2006). Sandy-beach Invertebrates. In Elsevier eBooks, 65–90.https://doi.org/10.1016/b978-012372569-1/50005-7

McLachlan, A., & Defeo, O. (2017). *The ecology of sandy Shores* (3rd ed.). Academic Press. https://doi.org/10.1016/C2015-0-04773-6

McLachlan, A., Dye, A., & Van Der Ryst, P. (1979). Vertical gradients in the fauna and oxidation of two exposed sandy beaches. South African Journal of Zoology, 14(1), 43–47. https://doi.org/10.1080/02541858.1979.11447646

Meysman, F. J. R., Galaktionov, O., & Middelburg, J. J. (2005). Irrigation patterns in permeable sediments induced by burrow ventilation: a case study of *Arenicola marina*. Marine Ecology Progress Series, 303, 195–212. https://doi.org/10.3354/meps303195

Morton, B. (1994). Hong Kong's coral communities: Status, threats and management plans. Marine Pollution Bulletin, 29(1–3), 74–83. https://doi.org/10.1016/0025-326x(94)90429-4

Morton, B. (1997). The Marine Flora and Fauna of Hong Kong and Southern China IV. Hong Kong University Press.

Neuberger-Cywiak, L., Achituv, Y., & Mizrahi, L. (1989). The ecology of *Donax trunculus* Linnaeus and *Donax semistriatus* Poli from the Mediterranean coast of Israel. Journal of

Experimental Marine Biology and Ecology, 134(3), 203–220. https://doi.org/10.1016/0022-0981(89)90070-1

Nowell, A. R. M., Jumars, P. A., & Eckman, J. E. (1981). Effects of biological activity on the entrainment of marine sediments. *Marine Geology*, *42*(1–4), 133–153. https://doi.org/10.1016/0025-3227(81)90161-4

Peng, C., Yan, X., Wang, X., Huang, Y., Jiang, L., Yuan, P., & Wu, Q. (2021). Release of odorants from sediments of the largest drinking water reservoir in Shanghai: Influence of pH, temperature, and hydraulic disturbance. Chemosphere, 265, 129068. https://doi.org/10.1016/j.chemosphere.2020.129068

Rahim, H. A., Shuhaida, S., & Yahya, K. (2021). Rapid baseline assessment of crab abundance and species richness in mangroves using a video recording method. IOP Conference Series. https://doi.org/10.1088/1755-1315/736/1/012056

Saes, R. V. S. T., Moreira, L. B., Davanso, M. B., Perina, F. C., & De Souza Abessa, D. M. (2018). Developing a protocol whole sediment toxicity testing with the polychaete Armandia agilis. Ecotoxicology and Environmental Contamination, 13(2), 85–97. https://doi.org/10.5132/eec.2018.02.11

Schlacher, T., Schoeman, D., Dugan, J., Lastra, M., Jones, A., Scapini, F., & McLachlan, A. (2008). Sandy beach ecosystems: key features, sampling issues, management challenges and climate change impacts. Marine Ecology, 70–86. http://obpa-nc.org/DOI-AdminRecord/0067932-0067952.pdf

Snelgrove, P. V. R., Soetaert, K., Solan, M., Thrush, S. F., Wei, C., Danovaro, R., Fulweiler, R. W., Kitazato, H., Ingole, B., Norkko, A., Parkes, R. J, & Volkenborn, N. (2018). Global carbon cycling on a heterogeneous seafloor. Trends in Ecology and Evolution, 33(2), 96–105. https://doi.org/10.1016/j.tree.2017.11.004

Soto, E., Quiroga, E., Ganga, B., & Alarcón, G. (2017). Influence of organic matter inputs and grain size on soft-bottom macrobenthic biodiversity in the upwelling ecosystem of central Chile. Marine Biodiversity. 47. 433-450. 10.1007/s12526-016-0479-0.

Varadharajan, D., Soundarap, P, I., & Pushparajan, N. (2013). Effect of physico-chemical parameters on crabs biodiversity. Marine Science Research and Development, 3(1). https://doi.org/10.4172/2155-9910.1000116

Waska, H., Greskowiak, J., Ahrens, J., Beck, M., Ahmerkamp, S., Böning, P., Brumsack, H., Degenhardt, J., Ehlert, C., Engelen, B., Grünenbaum, N., Holtappels, M., Pahnke, K., Marchant, H. K., Massmann, G., Meier, D., Schnetger, B., Schwalfenberg, K., Simon, H., Dittmar, T. (2019). Spatial and temporal patterns of pore water chemistry in the Intertidal zone of a high energy beach. Frontiers in Marine Science, 6. https://doi.org/10.3389/fmars.2019.00154

Watling, L. (2019). Macrofauna. Encyclopedia of ocean sciences, 728–734. https://doi.org/10.1016/b978-0-12-409548-9.11069-3

Wear, S. L., Acuña, V., McDonald, R. I., & Font, C. (2021). Sewage pollution, declining ecosystem health, and cross-sector collaboration. Biological Conservation, 255, 109010. https://doi.org/10.1016/j.biocon.2021.109010

Wibowo, Y. S., Kesumadewi, A. A. I., & Suwastika, A. A. N. G. (2021). Soil Macrofauna Community Structure and Biodiversity on Organic and Conventional Vegetable Land in Bedugul, Bali Island. International Journal of Education and Research, 9(7). https://www.ijern.com/journal/2021/July-2021/09.pdf

Wittmann, A. C., & Pörtner, H. (2013). Sensitivities of extant animal taxa to ocean acidification. Nature Climate Change, 3(11), 995–1001. https://doi.org/10.1038/nclimate1982

Wright, L. D., & Short, A. D. (1984). Morphodynamic variability of surf zones and beaches: A synthesis. Marine Geology, 56(1–4), 93–118. https://doi.org/10.1016/0025-3227(84)90008-2

Woodin, S. A. (1974). Polychaete Abundance Patterns in a Marine Soft-Sediment Environment: The Importance of Biological Interactions. Ecological Monographs, 44(2), 171–187. https://doi.org/10.2307/1942310

Xia, J., Han, Y., Tan, J., Abarike, G. A., & Song, Z. (2022). The characteristics of organic carbon in the offshore sediments surrounding the Leizhou Peninsula, China. Frontiers in Earth Science, 10. https://doi.org/10.3389/feart.2022.648337

Yusseppone, M. S., Rocchetta, I., Sabatini, S. E., Luquet, C. M., Del Carmen Ríos De Molina, M., Held, C., & Abele, D. (2018). Inducing the Alternative Oxidase Forms Part of the Molecular Strategy of Anoxic Survival in Freshwater Bivalves. Frontiers in Physiology, 9. https://doi.org/10.3389/fphys.2018.00100